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Exploitation of Coles (Brassica oleracea L.) and tomato (Solanum lycopersicum L.*) wild and cultivated germplasm for innovating organic vegetable production and quality*

PhD Thesis

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Riassunto

La presente Tesi di Dottorato ha avuto come obiettivo l'individuazione dei genotipi più promettenti di broccoli (*Brassica oleracea* L. var. *italica* Plenk) e pomodoro (*Solanum lycopersicum* L.) al fine di utilizzarli come base genetica per programmi di miglioramento genetico volti alla realizzazione di individui resilienti, ai fini del loro possibile utilizzo in agricoltura biologica. Lo studio è stato condotto presso l'Università degli Studi di Catania (UNICT), utilizzando un approccio multidisciplinare che ha coinvolto la caratterizzazione morfologica delle diverse cultivar esaminate, in aggiunta alla valutazione delle loro prestazioni agronomiche, alla determinazione delle caratteristiche qualitative e dei tratti genetici di interesse per dei futuri programmi di miglioramento genetico. Il seguente elaborato, inoltre, è stato realizzato nell'ambito del progetto H2020 *Breeding for Resilient, Efficient, Sustainable Organic Vegetable production* (BRESOV), *G.A. n. 774244*.

Per quanto riguarda *Brassicaceae* di interesse orticolo, sono state analizzate diverse varietà, tra cui varietà commerciali, linee genetiche in via di sviluppo e *crop wild relatives* (CWRs). Sono state valutate diverse caratteristiche morfologiche ed agronomiche riguardanti la struttura dell'infiorescenza, la quale è determinata dall'architettura morfologica della pianta. In funzione di tale caratteristica, è possibile distinguere tra i genotipi *sprouting*, i quali sono costituiti da numerose infiorescenze ascellari che si dipartono dall'asse principale costituendo vere e proprie ramificazioni principali, e quelli a dominanza apicale, rappresentati da un'unica, ipertrofica, infiorescenza principale. Oltre ai tratti morfologici, sono stati esaminati anche i tratti genetici relativi all'infiorescenza, comparandoli con quelli relativi alle CWRs, le quali differiscono massicciamente per l'architettura della pianta e nella struttura dell'infiorescenza. In particolare, mediante lo studio del dominio dei MADH box, sono stati identificati diversi alleli che, si suppone, svolgono un ruolo nell'induzione a fiore all'interno della specie esaminata (*B. oleracea* L.), delle sue varietà differenziatesi nell'ambiente mediterraneo come cavolo broccolo (*B. oleracea* L var. *italica* Plenk) e cavolfiore (*B. oleracea* L. var. *botrytis*). Sulla base dei risultati ottenuti, sono stati individuati i genotipi più promettenti ai fini della coltivazione mediante regime colturale biologico oltre all'individuazioni di specifiche variante alleliche coinvolte nello sviluppo dell'infiorescenza ipertrofica.

Un ulteriore studio svolto su un subset varietale composto da due genotipi locali di cavolo broccolo e due popolazioni del parentale selvatico *B. macrocarpa* Guss., ha consentito inoltre di individuare i pattern genici relativi alla resistenza allo stress idrico. La prova è stata svolta al fine di monitorare le variazioni di diversi *pathway* metabolici coinvolti alla risposta dello stress ossidativo indotto da condizioni deficitarie di irrigazione. Entrambe le popolazioni di *B. macrocarpa* esaminate, hanno mostrato una notevole tolleranza allo stress idrico, comprovata dalle analisi di due metaboliti chiave nella risposta allo stress ossidativo (malonilaldeide e perossido di idrogeno). La selezione di genotipi altamente resistenti allo stress idrico, in aggiunta a quelli suscettibili, ha permesso lo svolgimento di un'analisi di espressione genica volta all'individuazione dei geni differenzialmente espressi in condizioni di stress idrico, in relazione ai controlli non stressati e soprattutto in relazione ai genotipi resistenti e suscettibili.

Dai dati emersi, è possibile confermare l'elevata resistenza di *B. macrocarpa* allo stress idrico, il che la rende un parentale ideale per i programmi di miglioramento genetico delle *Brassicacee* di interesse orticolo,

deputati al trasferimento dei geni di resistenza allo stress idrico. Questo obiettivo rappresenta un aspetto cruciale dell'agricoltura biologica.

Per quanto riguarda *S. lycopersicum*, sono state analizzate diverse varietà nell'ambito di due prove sperimentali di cui la prima ha riguardato la caratterizzazione bio-morfometrica, qualitativa e genetica di 34 varietà di pomodoro afferenti alla collezione del germoplasma del Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), dell'Università degli Studi di Catania (UNICT). Per quanto concerne la seconda prova sperimentale svolta su pomodoro, quest'ultima ha avuto come oggetto lo studio delle *performance* agronomiche di tre portinnesti sperimentali, prodotti nell'ambito del sopra citato progetto BRESOV. Tali portinnesti sperimentali, sono stati realizzati mediante un programma di miglioramento genetico svolto dall'Università Politecnica di Valencia (UPV), in cui sono stati introgressi su tali portinnesti inter ed intraspecifici geni di resistenza ad importanti virosi e a malattie radicali causate da patogeni tellurici. L'introgressione di tali geni è avvenuta mediante incrocio tradizionale, confermando la presenza dei geni di resistenza mediante marcatori molecolari, e stabilizzando la progenie.

Sulla base dei risultati ottenuti, sono stati individuati i portinnesti maggiormente promettenti per l'agricoltura biologica i quali sono caratterizzati da una maggiore vigoria delle porzioni epigee ed ipogee, oltre ad una produttività maggiore in termini di produzione di bacche per pianta.

In conclusione, lo studio svolto nell'arco dei tre anni del percorso effettuato in qualità di Dottorando di Ateneo, ha consentito l'individuazione dei genotipi più promettenti di cavolo broccolo e pomodoro per l'agricoltura biologica, fornendo utili informazioni e strumenti per la selezione di varietà resilienti a stress biotici ed abiotici, oltre a soddisfare le specifiche richieste del mercato in termini produttivi e qualitativi, nonché per lo sviluppo di programmi di miglioramento genetico mirati a migliorare la resa, la qualità e l'adattabilità delle colture di cavolo broccolo e pomodoro.

Synthesis

The aim of this Ph.D. thesis was to identify the most promising genotypes of broccoli (*Brassica oleracea* L. var. *italica* Plenk) and tomato (*Solanum lycopersicum* L.) for their potential use as genetic bases in breeding programs to develop resilient individuals for organic farming. The study, conducted at the University of Catania (UNICT), employed a multidisciplinary approach, involving morphological characterization, assessment of agronomic performance, and determination of qualitative traits and genetic markers of interest for future breeding programs. The research was part of the H2020 project Breeding for Resilient, Efficient, Sustainable Organic Vegetable production (BRESOV), Grant Agreement No. 774244.

For Brassicaceae of horticultural interest, various varieties were analysed, including commercial varieties, developing genetic lines, and crop wild relatives (CWRs). Morphological and agronomic characteristics related to inflorescence structure were evaluated, distinguishing between sprouting genotypes with numerous axillary inflorescences forming main branches and those with apical dominance characterized by a single, hypertrophic main inflorescence. Genetic traits related to inflorescence were also examined, comparing them with CWRs, which differ significantly in plant architecture and inflorescence structure. Several alleles involved in flowering induction were identified through the study of the MADH box domain, playing a role in the evolution of the examined species (*B. oleracea* L.), its varieties adapted to the Mediterranean environment like broccoli (*B. oleracea* L. var. *Italica* Plenk), and cauliflower (*B. oleracea* var. *botrytis*). Promising genotypes for organic cultivation and specific allelic variants involved in the development of hypertrophic inflorescence were identified based on the results.

A molecular study on a subset of broccoli varieties and wild parent populations (*B. macrocarpa* Guss.) aimed to identify gene patterns related to water stress resistance. The research monitored variations in metabolic pathways responding to oxidative stress induced by irrigation deficit. Both *B. macrocarpa* populations showed significant water stress tolerance, confirmed by key metabolite analyses. The study facilitated the identification of differentially expressed genes under water stress conditions, especially in resistant and susceptible genotypes.

Regarding *S. lycopersicum*, two experimental trials characterized 34 tomato varieties genetically, bio-morphometrically, and qualitatively. Another trial evaluated the agronomic performance of three experimental rootstocks developed in the BRESOV project. These rootstocks, produced by the Polytechnic University of Valencia (UPV), incorporated resistance genes to important viruses and soilborne diseases through traditional breeding methods. The study identified the most promising rootstocks for organic farming based on their enhanced vigour and berry production per plant.

In conclusion, the three-year study identified promising genotypes of broccoli and tomato for organic farming, providing valuable information and tools for selecting varieties resilient to biotic and abiotic stresses. The research meets market demands for productivity and quality, offering insights for targeted breeding programs to enhance the yield, quality, and adaptability of broccoli and tomato crops.

1. General section

1.1. *Brassica oleracea* L. complex species (n=9) crops

1.1.1. Origin and diversification

Brassica oleracea origin in Mediterranean region is supported by several authors and was developed based on the new genotyping techniques such as the Genotyping – by – Sequencing (GBS) (Maggioni, 2015; Maggioni *et al*., 2018; Stansell *et al*., 2018; Mabry *et al*., 2021; Stansell and Björkman, 2020). *B. oleracea* vegetables include different morphotypes showing enormous phenotypic variation which can be exploited by its different crops like broccoli (*B. oleracea* var. *italica*), cauliflowers (*B. oleracea* var. *botrytis*), cabbage (*B. oleracea* var. *capitata*), kale (*B. oleracea* var. *acephala*), savoy cabbage (*B. oleracea* var. *sabauda*), and Brussel sprouts (*B. oleracea* var. *gemmifera*) (Maggioni *et al*., 2010; Ciancaleoni *et al*., 2014; Branca *et al*., 2018)**.** According to an extension leaflet of Iowa State University, all cole crops are cultivated varieties of the species *Brassica oleracea* (Haynes *et al*., 2009).

The marked morphological diversification of *B. oleracea* L. has determined the interest of geneticists who have used the species as a model in plant genetic studies. *B. oleracea* is an excellent source of vitamins and minerals. These vegetables are low in calories and high in fibre, which makes them an ideal food for weight management. *Brassica* vegetables are rich in bioactive compounds like polyphenols, vitamins and glucosinolates developed by its secondary metabolism which confer high sensorial properties (Picchi *et al*., 2018; Ben Ammar *et al*., 2022). In *B. oleracea* and *B. rapa* genomes 13-17 million years ago the whole genome triplication (WGT) event in the *Brassica* ancestor, followed by an extensive gene loss. For this reasons *B. oleracea* and *B. rapa* genomes present each feature three paleosubgenomes and multiple copies of paralogous genes and several of them, with their presence/absence and copy number variation determine different genes expression and morphotype diversification (Cheng *et al*., 2016; Golicz *et al*., 2016) such as the development of a BeadChip array with about 50.000 SNPs markers for population structure (Pelc *et al*., 2015; Mei *et al*., 2017). During their domestication process, several evolutionary pathways have been traced that established the modern cultivars of both crops.

Over the time, the high level of similarity between the two crops generated confusion about the classification of the landraces and of the related types and forms (Branca and Cartea, 2011; Branca *et al*., 2018). Among the *B. oleracea* L. crops, broccoli (*B. oleracea* L. var. *italica* Plenck) and cauliflower (*B. oleracea* L. var. *botrytis* L.) are the only two varieties that offer a product represented by a hypertrophic reproductive organ, whereas all the others have vegetative organs, quite often modified. The differentiation process of broccoli and cauliflower began centuries ago, however, similar morphological structures of the edible organs of the two crops always generated confusion to find unique descriptions of the plants, as well as the names used for them in the past and for today's crops (Branca *et al*., 2018). *B. oleracea* L. complex species share the same genome (2*n* = 18 chromosomes) with several wild species of Brassica that are usually found growing on rocky limestone cliffs along the Atlantic coasts of Britain, France, and Spain, as well as in the

Mediterranean basin (Snogerup *et al*. 1990). The apparent similarity of broccoli and cauliflower plants and the similar morphology of the inflorescence probably influenced both the scientific and common names, which in some cases are interchangeable (Maggioni *et al*. 2010). Far into the past, Castore Durante (1529–1590) in his opera titled ‘‘*Herbario nuovo*’’ (1585) only mentioned one variety: *cauolo fiore*; however, is difficult to be sure, only on the basis of an assonance (i.e., the resemblance of sounds in words), whether he was referring to the variety botrytis of *B. oleracea* or not, because broccoli seems to be an older crop than cauliflower. Moreover, the Italian word broccoli has a Latin root originating from *brachium*, meaning an arm or branch, and it referred to the young edible flower sprouts before anthesis (Maggioni *et al*. 2010).

Teofrasto (III-IV B.C.) described three types of Brassicas, one of which was wild, characterized by smooth leaves, acrid taste, and utilized for medicinal purposes; the two others were cultivated, and differentiated by the crisp leaves in one of the types and by smooth leaves and reproductive difficulties in the other; the latter type could be related to one of the ancient initial forms of cauliflower which showed less flower sterility. Among these types, forms with sprouts development were also identified. It would seem, at that time, that several types of *B. oleracea* were grown in the west Mediterranean basin. Plinio (I B.C.) mentioned types which seem intermediate forms between cauliflower and broccoli, and which showed variation in terms of curd/head compactness, symmetry, and uniformity. These types, very similar to cauliflower and broccoli, were grown in the Roman age, especially above all on the Italian peninsula, where the differentiation process took place (Nuez *et al*., 1999). During these times, broccoli was grown from the eastern to the western coast of the Mediterranean basin (Gray, 1982). Abu -Zacarìa (Ibn-el-Awan) wrote at Sevilla during the XII century, the gathering of the “*berzas de los cristianos*” (Christian kale) and the cultivation of the “*berza siriacas*” (Syriac kale), which could be similar to both cauliflower and broccoli (Nuez *et al*., 1999). Miller (1731) suggested the cauliflower was introduced from Cyprus and the winter cauliflower (white cauliflower) from Sicily. In Great Britain the cauliflower appeared in London markets in 1619 but it did not adapt well to cultivation till 1660 when the Enfurt type (snowball) was selected in Germany. This type permitted the establishment of Northern European cauliflower seed production, which till that date concentrated in the Mediterranean basin (Cyprus). This was possible with the Dutch method to propagate vegetatively some plant parts in the greenhouse to overcome the winter conditions, which induced flowering, during the second growing year. With that propagation method the selection of types suited to environmental conditions that were different to Mediterranean ones took place (Branca, 2008). During the XIX and XX centuries, trade development and human migration among several continents spread the crop to all sides of the world, because of the new cultivars and hybrids mainly developed in European and Asian countries. In India, the crop was introduced from Great Britain in 1822. The cultivation of the first forms of broccoli along the Italian peninsula determined the selection of several types of broccoli characterized mainly by the aptitude for sprouting and by a wide range of head sizes. The more compact type, like cauliflower, was named Calabrese (from Calabria, a southern Italian region). During the XVII century, broccoli was grown in Great Britain and in the northern European countries and in the XVIII century in Spain (Boutelou and Boutelou, 1801). From this time cultivars were established and named in relation to the colour or to the harvest period (Crisp P., 1982). Broccoli crops were

widespread during the XIX century in North America, introduced by the Italian community emigrating from Calabria and Sicily, and in the XX century throughout the world, above all during the colonial period (Buck, 1956). The absence of cold requirements for summer cauliflower, which was the first to be grown in Europe, supported the hypothesis that the east Mediterranean basin was the area of origin of cauliflower. In any case it is thought that the cauliflower was introduced into Italy by Genova citizens from the west or from Cyprus around 1490 and after this its seed production started in Campania. In 1578 Dodoneus described a type of cauliflower, agamously propagated, from material from Cyprus, called *B. cypria*, which in other countries did not produce seed because it was highly sensitive to the cold. The cauliflower crop, widespread on the Italian peninsula since the XV century, appeared in France and in Great Britain during the XVI century with the name of "Cyprus kale"; in the same period European writers described the crop in Egypt and in Turkey. Cauliflower spread during the XVII and XVIII centuries throughout all Europe (Boutelou and Boutelou, 1801; Hyams, 1971). Several studies on the relationship between cauliflower and broccoli have been performed over recent years based on the bio-morphological, anatomical, biochemical, and molecular traits. Several landraces of broccoli, which are highly valued by locals for their organoleptic qualities, are now grown in both big fields and household gardens (Ciancaleoni *et al*., 2014).

B. oleracea L. (2n = 2 = 18) is an insect-pollinated crop with substantial outcrossing (72-95%) due to a sporophytic self-incompatibility system regulated by a polymorphic S locus (Takayama *et al*. 2001). The species is one of the most striking examples of structural evolution among domesticated plants (Babula *et al*. 2007). For significant agronomic properties, most landraces and synthetics clearly separate themselves from hybrid types. Many morpho-physiological features (such as harvesting time, head size, color, and number of leaves) separate landraces and synthetics, as previously observed in other investigations (Branca, 2008). Their names, on the other hand, already allude to identifiable and/or specific characteristics (Iannarisi and Marzolo, that flower in January and March, respectively; Nero, which has a very dark color, etc.). This is most likely owing to a long history of adaptation to local pedo-climatic circumstances and farmer selection in favor of crucial characteristics like maturity date, corymb form, or flavour. It is also worth noting that detailed characterization aids in the promotion of products derived from local variations, as is now being done for many cultures (Negri, 2012). Furthermore, it is necessary to use the diversity of local races in the selection. The development of varieties from local varieties may also help to keep their diversity alive in the fields. In recent years, considerable progress in understanding the B. oleracea crop group has been made. Specifically, several key objectives have been accomplished: parsing fundamental genomic architecture (Cheng *et al*., 2016), publication of high-quality reference genomes (Belser *et al*., 2018), evaluating diversity and domestication processes (Lazaro *et al*., 1998) (King *et al*., 2003) (Mabry *et al*., 2019), and identifying genomic regions or candidate genes associated with horticultural quality (Stansell *et al*., 2019) and biotic/abiotic stress resistance (Branham *et al*., 2017).

1.1.2. Agronomic and economic importance

Brassica oleracea is a crucial crop species that plays a significant role in both agronomy and economics. Its ability to adapt to diverse climatic conditions and soil types, high yields, and resistance to pests

and diseases make it economically important for growers. Its high nutritional value and unique taste make it a popular and staple food around the world. The global market for *B. oleracea* vegetables is growing, driven by the increasing demand for healthy foods and convenience foods. *B. oleracea* is a valuable crop for farmers and the economy. These crops are grown in many countries around the world, including China, India, Italy, and the United States. Cabbage is the most exploited *B. oleracea* vegetable which contains the largest harvested area and production which is about 2,414,000 ha and about 70,900,000 t respectively, followed by cauliflowers and broccoli cultivated for about 1,357,000 ha in addition to about 25,000,000 t of production, respectively (FAOSTAT, 2020). The global market for *B. oleracea* vegetables is also growing. According to a report by Mordor Intelligence, the global market for broccoli and cauliflower is expected to grow at a CAGR of 3.8% from 2020 to 2025. One of the most important aspects of *B. oleracea* is its ability to adapt to diverse climatic conditions and soil types. This makes it a valuable crop in areas that are not suitable for other crops. For example, broccoli can be grown in areas with cool temperatures and high humidity, while cauliflower can be grown in areas with warm temperatures and low humidity. Another important feature of *B. oleracea* is its relatively high yields per hectare. This makes it an economically important crop for growers. Furthermore, *B. oleracea* is resistant to pests and diseases, which reduces the need for pesticides and other chemical inputs. This not only saves money for growers but also reduces the environmental impact of agriculture. *B. oleracea* is also a valuable crop in crop rotation systems. Growing *B. oleracea* crops can help break the life cycle of soil-borne pests and diseases that affect other crops. This reduces the need for chemical inputs and promotes soil health.

The increasing demand for healthy foods and the growing popularity of plant-based diets are driving this growth. In addition to fresh produce, *B. oleracea* is used in the food processing industry. For example, cabbage is used to make sauerkraut and kimchi, while broccoli and cauliflower are used to make frozen vegetables and vegetable purees. The market for processed *B. oleracea* products is also growing, driven by the increasing demand for convenience and innovative foods. The interest in cauliflower and broccoli cultivation has grown in recent years due to the genetic improvement programs carried out in several countries, mainly in Asia and due to the new opportunities offered by the food industry in exploiting traditional and new phenotypes in new transformation processes (IV and V gamme). *B. oleracea* has been used for centuries as a food source due to its high nutritional value and unique taste. However, in recent years, there has been a surge in popularity of novel *B. oleracea* foods such as sprouts, baby leaf, and microgreens. These new forms of *B. oleracea* have gained popularity due to their convenience and versatility in cooking. Sprouts, for example, are young plants that have just begun to grow. They are packed with nutrients and can be added to salads, sandwiches, or smoothies. Baby leaf, on the other hand, refers to the young leaves of *B. oleracea* plants. They can be eaten raw or cooked and are often used in salads or as a garnish. Microgreens are another novel form of *B. oleracea* that have gained popularity in recent years. They are essentially small plants that are harvested when they are only a few inches tall. They are often used as a garnish or added to salads and sandwiches. Overall, the popularity of novel *B. oleracea* foods such as sprouts, baby leaf, and microgreens is on the rise due to their convenience, versatility, and high nutritional value. They are a great way to incorporate this nutrient-rich

vegetable into your diet in new and exciting ways. According to an extension leaflet of Iowa State University, all cole crops are cultivated varieties of the species *B. oleracea* (Haynes *et al*. 2009). The marked morphological diversification of *B. oleracea* L. has determined the interest of geneticists who have used the species as a model in plant genetics studies.

1.1.3. Biology and requirements

B. oleracea complex species has a biennial/annual life cycle that may be perennial and suffrutescent (Snogerup *et al*., 1990). It has a short stem and forms a rosette of leaves in the first year of growth, followed by a flowering stem in the second year. The leaves are large, green, and have a waxy coating to reduce water loss. The flowers are small and yellow, and the plant produces fruit in the form of a capsule containing numerous seeds. *B. oleracea* is a highly variable species, with many cultivars developed for different purposes, such as cabbage, broccoli, cauliflower, kale, and Brussels sprouts. Broccoli are vigorous and rustic plants usually 50-150 cm tall; they adapt to several environmental conditions. Leaf is lyrate, usually glabrous, green and it can reach considerable size. In some genotypes, leaves may be antocyanic. Young plant first forms a basal rosette of large leaves, later prolonged, but leaves sometimes again aggregate apically in a head-like manner (Snogerup *et al*., 1990). Basal leaves have a long petiole while the upper side one gradually smaller, usually undivided and with a broad amplexicaul base. Usually starting flowering on the main stem. Stem lignified with easily visible year rings, up to 30-50 mm thick; leaf scars prominent, ofter remaining on older stems. First inflorescence is often ½ - 1 m with 100-200 flowers usually with only 2-5 branches, later side inflorescences sometimes almost as large; partial racemes often with 25-50 flowers, buds usually densely placed, flower thus opening close to the top. Pedicels 10-22 mm, at anthesis erecto-patent up to 45° to quite patent. Flower usually cospicuously protandrous, anthers ripening 1-2 days before the stigma (Snogerup *et al*., 1990). Broccoli has all the fertile floral sketches that determine a greater grain of the curd (Branca *et al*., 2018). Optimal germination temperatures are close to 25 ° C while growth temperatures are between 15 and 20 ° C. The minimum growing temperature is about 5 ° C.

Environmental factors influence the reproductive differentiation and the subsequent inflorescence induction after the vernalization process, in addition to the photoperiod of 10 hours which determine the endogenous gibberellins accumulation in broccoli, cauliflower and cabbage (Gauss and Taylor, 1969; Hamano *et al*., 2002; Guo *et al*., 2004). The biennial is correlated to the passage from the vegetative to the reproductive phase, which is strictly correlated to the need in cold (temperatures below 10 ° C). The various cultivars require low temperatures for the induction and differentiation of the reproductive apex that will origins the inflorescence (Branca *et al*., 2018). High temperature causes unevenly sized flower buds on broccoli inflorescence. This deformity limits production of broccoli to areas where summer temperature rarely exceeds 30 °C (Björkman *et al*., 1998). The flower induction determines the period of production of the various cultivars. Temperatures above 25 °C are also harmful because they can cause the production of young leaves on the curd surface (Branca *et al*., 2018). The crop prefers well-drained soils rich in organic matter with a pH range of 6.0 to 7.5. Adequate soil moisture is essential throughout the growing season, especially during the early stages of growth. The crop also requires good aeration and a moderate level of soil fertility for optimal

growth. In terms of environmental factors, *B. oleracea* is sensitive to high temperatures and drought stress, which can lead to stunted growth and reduced yield. Prolonged exposure to high temperatures can also cause the crop to bolt prematurely, resulting in poor quality produce. With regards to the kale (*B. oleracea* var. *acephala*) agronomic requirements, they are like the previous indicated for broccoli. For kale, it is recommended to do rotation with other crops each 3-4 years to avoid radical or basal stem disease. The optimal soil Ph for kale is low acid or neutral (6-7) and the crop has a high demand of nutrient uptake and for this the inoculation of organic manure before the transplant, it is recommended (Šamec *et al*., 2019). Overall, ensuring optimal growing conditions is crucial for the successful cultivation of *B. oleracea*, and farmers must pay close attention to temperature, soil moisture, soil fertility, and other environmental factors to achieve high yields and quality produce.

1.1.4. Growing techniques and harvesting

B. oleracea can be grown through direct seeding or transplanting seedlings. For direct seeding, the seeds are sown at a depth of about 1 cm and a spacing of about 30 cm between rows. Usually, *B. oleracea* crops are sown in cellular trays after the germination testing, to calculate the number of seeds to put in each hole. Transplanting is usually done when the seedlings are about 4-6 weeks old. The plants require well-drained soils with a pH of 6.0-7.5 and cool temperatures of around 15-20°C. Adequate soil moisture is also important for proper growth and development (Branca, 2018). The selection of fertilizers and minerals for *B. oleracea* cultivation, it's important to consider the specific needs of the plant and the soil conditions. A soil test can help determine which nutrients are lacking in the soil and which fertilizers and minerals will be most beneficial. Nitrogen is essential for plant growth, and brassicas require a lot of it. A nitrogen-rich fertilizer can help promote healthy foliage and root development. Phosphorus is important for root development and helps with flower and fruit production. Adding a phosphorus-rich fertilizer can help improve yield. Potassium is crucial for plant growth and helps with overall stress tolerance. A potassium-rich fertilizer can help improve the plant's ability to withstand environmental stresses. Calcium is important for cell wall development and can help prevent diseases like blossom end rot. Adding calcium to the soil can help promote healthy plant growth. Magnesium is important for photosynthesis and helps with chlorophyll production. Adding magnesium to the soil can help improve the plant's ability to produce energy. Sulphur is important for protein synthesis and can help improve plant growth and development. Adding sulphur to the soil can help promote healthy foliage and root development.

The inorganic fertilizers and the micro elements play an important role in the plant growth and development process. For example, in the work of Ouda and Mahadeen (2008) carried out in cabbage crop grown under different nutrition protocols, the use of inorganic fertilizers significantly enhances the plant growth parameters such as the plant height, main root length, the stem length, the number of leaves per plant and the leaf length.

The use of inorganic fertilizers, in broccoli, allows to obtain higher yield in comparison to the organic fertilizers also because most of the broccoli varietal breeding programs have been focused on the hight input plant

cultivation instead, the use of organic products, is spread for organic farming and they are often used for it (Sakhonwasee *et al*., 2015).

The harvesting of *B. oleracea* depends on the specific type of vegetable being grown. For example, broccoli heads should be harvested when they are tight and firm, while kale can be harvested throughout the growing season by picking individual leaves. It is important to harvest the vegetables at the right time to ensure good quality and flavour. Dellacecca (1994), developed a technique based on the cut of the plant terminal shoot at the transplant moment, resulting in an earlier and simultaneous yield in inflorescences which allowed an important cost reduction. As reported by the work of Casajús *et al*. (2020), the secondary metabolites of broccoli such as the glucosinolates, are influenced by the cutting point and by the time of cutting in the same day. Furthermore, the postharvest storage affects differently aliphatic - indolic glucosinolate metabolisms changing their relative gene expression which are influenced by the light (Casajús *et al*., 2020). For the broccoli harvesting, a future perspective could be represented by the 3D view performed by the robotic system, which assesses the correct size and maturity of broccoli head, determining an important cost reduction for the harvest. The machine vision also allows the selection of the broccoli head, cutting them in their proper time (Blok *et al*., 2016; Kusumam *et al*., 2017). Nowadays, broccoli is only hand harvested and the following process requires the visual analysis, experience and the skill of the farm workers (Ramirez, 2006). Concerning the harvesting of cabbage, Hachiya *et al*. (2004) developed a mechanical trailer supported harvesting system which requires three people to operate. The above-mentioned system allows the cabbage harvesting with high work efficiency and ergonomic, permitting the produce processing at the harvest moment. With regards to the kale (*B. oleracea* var. *acephala*) cultivation, a significant amount of nitrogen is necessary for its growth (Haile and Ayalew 2018). However, the concentration of glucosinolates, a beneficial compound found in kale, can be influenced by the amount of nitrogen applied, the method of application, and the type of fertilizer used. Similarly, the level of glucosinolate in *B. oleracea*, can be increased by applying sulphur during fertilization. Groenbaek *et al*. (2014) found that as the amount of sulphur applied during fertilization increased, the concentration of glucosinolate in *B. oleracea* also increased.

In Brassica crops, seed production is a difficult procedure due to the specific flower biology and for the environmental conditions needed. Several years ago, some genotypes were selected to improve seed production and to give a better cauliflower production; as regards this crop the sowing date for seed production is related to the biological materials utilized (population, breeding lines, etc.) and to their requirements according to environmental conditions which permit flower induction (vernalization is required for over-winter cultivars). Usually, cauliflower and broccoli seed producers sow the seeds into containers and transplant the plantlets at the five-seven leaf stage in the greenhouse, discarding off types and sick plants. Plant density is strictly related to the vigour of the plants of the cultivars or lines utilized for allowing plant inspection. The starting material consists of the mother plants which must be grown in their optimal season to facilitate selection based on plant characteristics, mainly curd/head quality. Concerning summer cultivars, plants are cultivated *in situ* in open fields but for over-winter ones in continental climate conditions, the plants need to be transferred to the greenhouse; Mediterranean areas allow winter cultivars in open fields.

The growing technique could also be differentiated in the frame of organic agriculture, for example using cover crops among the rows of cultivation, as was performed by Thavarajah *et al*. (2019) showing positive effects for the yield and in the nutrient concentration. With regards to the plant nutrition and the fertilizers management, was observed that the organic matter in the soil promoted by different combination of humic acids, in addition to the application of biofertilizers and dripping irrigation system, influence positively the broccoli yield enhancing the soil fertility (Thompson *et al*., 2002; Al-Taey *et al*., 2019).

1.1.5. Produce quality

Broccoli is a highly nutritious vegetable containing a range of essential vitamins and minerals. It is particularly high in vitamin C, with fresh broccoli containing almost twice as much as cauliflower. Per pound of edible portion, broccoli also contains protein, fat, carbohydrates, calcium, phosphorus, iron, vitamin-A, riboflavin, and thiamine. According to analytical data, broccoli is more nutritious than other cole crops like cabbage and cauliflower (Roni *et al*., 2014). Eating broccoli enriched in antioxidants can help reduce the risk of certain cancers and heart disease by the isothiocyanates belonging to the glucosinolates degradation carried out by the myrosinase enzyme (Herr *et al*., 2010; Nandini *et al*., 2020). Therefore, it is recommended to include broccoli in one's diet for overall health benefits. Broccoli contains many bioactives, including vitamins C and E, quercetin and kaempferol glycosides and, like other members of the *Brassicaceae*, several glucosinolates, including glucobrassicin (3-indolylmethyl glucosinolate) and glucoraphanin (4-methylsulphinylbutyl glucosinolate) (Jeffery and Araya, 2009). Dietary broccoli can upregulate detoxification enzymes, GSHsynthesis and several antioxidant enzymes. These may all play a role in prevention of initiation of cancer (Jeffery and Araya, 2009). The United States Department of Agriculture (USDA) database reports the nutritional values of broccoli and they contain, in 100 g of fresh product, 90 g of water, 0.41 g of nitrogen, 2.57 g of proteins, 0.34 grams of total lipids, 0.83 g of ash, 6.27 g of carbohydrates, 2.4 g of total dietary fibre and 1.4 g of total sugars. Concerning the microelements, broccoli contain about 91.3 mg of total ascorbic acid, 0.077 mg of thiamine, 0.114 mg of riboflavin, 0.639 mg of niacin, 0.61 mg of pantothenic acid, 0.191 mg of vitamin B6, 65 µg of folate, 93 µg of beta carotene 745 µg of lutein and zeaxanthin, and important amino acids like 0.033 g of tryptophan, 0.088 g of threonine, 0.079 g of isoleucine, 0.129 g of leucine, 0.135 g of lysine, 0.191 g of arginine, 0.325 g of aspartic acid, and 0.525 g of glutamic acid (USDA, 2022). The above-mentioned information regarding the broccoli nutritional values can differ from the different typology of broccoli or from the growing technique utilised. For example, the biofortification can enrich the final product of vitamin C or selenium, as was reported by several authors (Bañuelos *et al*., 2015; Muñoz *et al*., 2021; Kathi *et al*., 2023).

Broccoli quality and nutritional values change in post-harvest conditions during the cold storage in plastic packaging. Without the modified atmosphere packaging (MAP), can we observe a rapid decrease of quality manifested by weight loss, yellowing, chlorophyll degradation and stem hardening. In terms of biochemical compounds concentration, the ascorbic acid, the antioxidant activity and the total phenolic compounds quickly decrease in normal conditions (Serrano *et al.*, 2006). In post-harvest conditions, the effect of elicitors can enlarge the conservation period of the broccoli florets. In this regard, in the work of Miao *et al*. (2020), a post-harvest melatonin treatment was performed, and it resulted in the significative delay of the

broccoli senescence which maintained high levels of antioxidants, such as carotenoids, vitamin C and total phenols. Furthermore, the 1 μM melatonin treatment sustained the higher content in terms of glucosinolates, in particular of the most anticarcinogenic one which was the glucoraphanin (Miao *et al*., 2020). Concerning the exogenous post-harvest treatments, also the methyl jasmonate, the calcium chloride and the salicylic acid, show important effects in terms of the storage conservation of the broccoli florets (Xu *et al*., 2020; El-Beltagi *et al*., 2022).

1.1.6. Genetic improvement

Broccoli and cauliflower are important vegetable crops that are consumed worldwide for their high nutritional value, including vitamins, minerals, and fiber. However, these crops are also susceptible to various biotic and abiotic stresses, such as pests, diseases, and adverse climatic conditions, which can significantly reduce their yield and quality. To address these challenges, breeding programs have been established to develop improved cultivars with desirable traits, such as high yield, disease resistance, and adaptability to different environments (Giudice *et al*., 2021; Nerva *et al*., 2022). Traditional breeding methods, such as selection of superior genotypes and hybridization, have been used to develop new cultivars. For example, the "green revolution" in the 1960s and 1970s resulted in the development of high-yielding broccoli and cauliflower cultivars that were adapted to intensive production systems.

Recent advances in molecular biotechnology have opened a new avenue for genetic improvement of vegetable crops, including broccoli. Vegetable crop productivity and quality are often negatively affected by various biotic and abiotic stresses, leading to significant losses in yield and quality (Farnham and Bjorkman, 2011).

Despite the significant improvements made through conventional breeding in the past decades, there are still many limitations that can only be overcome through modern biology. Broccoli is a valuable vegetable crop that suffers from various stresses during cultivation, leading to significant yield losses.

The development of tools like the molecular markers represented a crucial purpose for the investigation of the molecular patterns linked to economic traits. Additionally, it can be beneficial to assist the breeding oriented to the improvement of plant production, resistance to abiotic and biotic stresses and more recently the plant-microbiome interaction (Makukha and Dubina, 2021; Treccarichi *et al*., 2021; Treccarichi *et al*., 2023; Malgioglio *et al*., 2022). The marker assisted selection (MAS) involves the use of molecular markers, such as DNA sequences, to identify and select plants with desirable traits at an early stage of development, without the need for costly and time-consuming phenotypic screening. For example, MAS has been used to develop broccoli cultivars with resistance to downy mildew, a fungal disease that can cause significant yield losses. Genetic engineering, on the other hand, involves the manipulation of the genetic material of plants to introduce or enhance specific traits. For example, genetic engineering has been used to develop cauliflower cultivars with enhanced levels of glucosinolates, compounds that have been linked to anti-cancer properties.

The next generation sequencing (NGS) technologies and the publishing of the draft genome sequence of cauliflower allow to search, discover, map and clone new QTLs such as the downy mildew resistance loci

(Shaw *et al*., 2021). Cauliflower's genetic resource can represent an important source of biochemical traits positively related to nutraceutical properties (Picchi *et al*., 2020) and can it also represent a functional food (Di Bella *et al*., 2021): in this frame, the orthologous gene of cauliflower *Orange* (*or*), involved in the beta-carotene accumulation, was cloned in rice (*Oryza sativa*) (Endo *et al*., 2019). Or gene plays an important role in plant development, petiole elongation induces the formation of chromoplasts, which provide a metabolic sink to sequester and deposit carotenoids; it also regulates carotenoid homeostasis increasing plant tolerance to environmental stress (Kim *et al.,* 2018) and was cloned by several authors (Zhou *et al*., 2011) and mutants were characterised (Welsch *et al*., 2020). Genetic engineering can be used as a tool to insert specific added value traits in the existing cultivars, improving them by exploiting the different transformation and regeneration techniques (Kumar *et al*., 2016). Genetic engineering can be used to introduce specific traits into existing cultivars, but efficient regeneration and transformation techniques are necessary for the successful transfer of genes. With recent advances in plant genetic engineering, there is a great opportunity to improve broccoli in multiple aspects, including yield, quality, and stress resistance.

Genetic manipulation is becoming increasingly important for improving broccoli, as classical breeding techniques have limitations. Inter-specific hybridization has been used by plant breeders to transfer genes between species in the past decades, but sexual incompatibility barriers make this difficult. Genetic engineering can be used to add specific traits to existing cultivars, but efficient regeneration and transformation methods are necessary (Bhalla and Singh, 2008; Gaur 2015; Treccarichi *et al*., 2022).

. Various studies have reported plant regeneration in broccoli using different explants, such as peduncles, anthers, protoplasts, hypocotyls, leaf tissues, cotyledons, and petioles. *Agrobacterium*-mediated gene transfer studies have also been conducted in broccoli. However, plant regeneration efficiency and transformation success depend on the genotype and need to be established for each cultivar (Henzi *et al*., 2000; Shen *et al*., 2011; Ravanfar *et al*., 2017).

Overall, breeding programs for broccoli and cauliflower have resulted in the development of many improved cultivars that have contributed to the increased production and quality of these crops. However, there is still a need for continued research and development to address emerging challenges, such as climate change and evolving pest and disease pressures.

1.1.7. Pests and diseases

B. oleracea crops are susceptible to several pests and diseases that can impact the plant growth and development in addition to the yield and quality of the harvested products. Herein, the most destructive and dangerous ones are resumed and described, highlining on the strategies that can be followed against them in organic farming conditions. Among the several pests that affect the different *B. oleracea* crops, we can count the several species of aphids that causes economically important loss of yield, due to their nutrition activity in the apical meristematic shoots of Brassica and in the siliquas, also affecting the seed production (Broekgaarden *et al*., 2008; Zorempuii and Kumar, 2019). Additionally, aphids can cause indirect damage to plants by transmitting viral diseases through their feeding (Alvarez *et al*., 2007). When probing, aphids move their stylets between plant cells and make short punctures in the epidermal, mesophyll, and parenchymal cells as they

search for phloem cells. It is believed that the plant's response to aphids is mainly triggered by the stylet penetration of plant tissues, as well as the injection of saliva (Goggin 2007; Will *et al*., 2007). Among the different aphids' strains, that usually are polyphagous pests, we can cite *Brevicoryne brassicae*, which is the most common one in *B. oleracea*, *Myzus persicae* and *Lipaphis erysimi* which is most common in mustard plants, but it can also affect the *B. oleracea* crops (Broekgaarden *et al*., 2008; Munthali *et al*., 2014). Several authors highlighted on the variation of several nutraceutical compounds belonging to the secondary metabolism, as results of the aphids' infections (Moyes *et al*., 2000; Khan *et al*., 2010; Newton *et al*., 2010).

Among the several pests that affect the cole crops, are noteworthy the several *Lepidoptera* which causes important losses by their feeding activity. In the above-mentioned insects order, we can cite *Trichoplusia ni* which is a medium sized moth belonging to the *Noctuidae* family representing the common cabbage looper (Cameron *et al*., 2007). The cabbage looper can causes, under its optimal seasonal conditions, important defoliation affecting loss of the yield in terms of vegetative organs in addition to important reduction in the inflorescence weight. Its ovipositional activity is carried out in the cole leaves (Cameron *et al*., 2007).

Among the *Lepidoptera* order, we can also cite *Plutella xylostella* which has the common name of Diamondback moths and it belongs to the family of *Plutellidae*. The diamondback moth is a major pest responsible for the destruction of cabbage and other Brassica vegetable crops in the United States of America (USA) (Srinivasan *et al*., 2011). The insect has a brief life cycle and thrives in warm temperatures but struggles to survive in colder conditions. As a result, its year-round cultivation is significantly impacted in regions with semitropic and temperate climates. Pests' reproductive capabilities are being impacted by climate changes such as global warming, leading to the emergence of new or increased insect infestations (Marchioro and Foerster, 2012). These moths lay their eggs on the leaves of *B. oleracea* crops, and the resulting larvae can cause significant damage by feeding on the leaves (Marchioro and Foerster, 2012). For controlling the above-mentioned pest in organic farming in cabbage crops, the intercropping with non-host crops is recommended (Warwick *et al*., 2010). Another important *Lepidoptera* that causes serious damage in *B. oleracea* crops by the feeding activity of its larvae is *Pieris brassicae* which belongs to the *Pieridae* family, and its common name is the large white butterfly.

P. brassicae (L.) is a harmful pest that is found worldwide on cruciferous crops. It is ubiquitous wherever its host plants grow and is among the most extensively dispersed of all *Lepidoptera* species (Feltwell, 2012). As reported by several works, each single larva usually feeds of about 80 cm^2 of leaf area (Younas *et al*., 2004). As substitute of the chemical products the bacteria *Bacillus thuringensis* can be used for controlling the feeding activity of *P. brassicae*, but several works, reports also the effect of several plant extracts (Sharma and Gupta, 2009).

Among the *Coleoptera* order, are worth mentioning the flea beetles which belong to the *Chrysomelidae* family. Primarily *Phyllotreta nemorum* and *P. undulata* are the most spread species that carry out their feeding activity in the *B. oleracea* crops. The beetles usually consume cotyledons, young leaves in the process of developing, and seedling stems. This leads to a reduction in the plant's ability to photosynthesize, and in severe cases, the death of the plant. Beetle feeding begins within the initial fortnight after emergence, resulting in a

distinct pattern of holes and cell death. On the other hand, when the larvae feed on the root hairs, it has little or no impact on the survival of the plant (Metspalu *et al*., 2014; Kovalikova *et al*., 2019).

Herbivorous insects are a common threat to plants, and as a result, plants have evolved several defensive strategies. In addition to physical features such as trichomes, thorns, and waxy leaf coatings, plants can alter their metabolic processes to generate and store chemical compounds that have insect-repellent or even lethal effects. For example, in the work of Kovalikova *et al*. (2019), they studied the effect on the secondary metabolism as consequence of the beetle's infection and there was a significant increase in the flavonoid, quercetin, the levels of certain phenolic acids and flavonoids exhibited greater variability. Additionally, the leaves that were attacked by the flea beetle showed an increase in ascorbic acid, which is an essential nutrient for cabbage.

With regards to the several diseases that affect the *B. oleracea* grown, it's worth to be mentioned the clubroot, which is a typical soil-borne *Brassica* diseases caused by the obligate fungal parasite *Plasmodiophora brassicae*, which survives inside the soils by three different life cycles which includes the survival into the soil, the root hair infection, and the cortical infection (Dixon *et al*., 2009; Donald and Porter, 2009; Kageyama and Asano, 2009).

Another important disease that causes several economic losses, is represented by the black rot is the most destructive and widespread disease which infects all cultivated variety of worldwide *Brassica* and several authors identified different genes against the race one and four (Vicente and Holub, 2013; Singh *et al*., 2018; Bella *et al*., 2019). The causal agent of the previous mentioned disease is *Xanthomonas campestris* pathovar. *campestris* which causes severe losses in agricultural yield worldwide (Qian *et al*., 2005; Gupta *et al*., 2013). Against the above-mentioned disease, the use of disease-free seeds and implementing good cultural practices, such as crop rotation and sanitation, can allow to reduce the incidence of disease. For example, rotating crops can help break the disease cycle by preventing the build-up of pathogens in the soil. Removing and destroying infected crop debris and weeds can also help reduce the amount of inoculum available to infect future crops. Chemical control can also be part of an integrated disease management approach, but it should not be the only method used. Fungicides or bactericides can be effective at controlling certain diseases, but they can also be costly and potentially harmful to the environment and non-target organisms. As such, it's important to use chemical control judiciously and in conjunction with other disease management strategies. Overall, managing plant diseases requires a multifaceted approach that considers the specific pathogen, the crop being grown, and the local growing conditions. By using a combination of disease-resistant varieties, good cultural practices, and targeted chemical control, growers can help protect their crops and minimize the impact of diseases (Mason *et al*., 2000). To achieve the following goals, the individuation of resistance major genes or QTLs is mandatory, and several researchers are working on this topic (Izzah *et al*., 2014; Sharma *et al*., 2016; Iglesias-Bernabé *et al*. 2019).

With regards to the fungal disease that cause serious economic losses, we can mention the downy mildew that can infect the *B. oleracea* crops from their earliest stages to when they produce seeds. The fungus responsible for this disease is called *Hyaloperonospora parasitica*, also known as *Peronospora parasitica*. It's

a soil-borne disease that can also infect other plants. The fungus thrives in mild daytime temperatures of 20º to 24ºC, high humidity over 80%, and frequent rain or dew. These conditions are common during the main cauliflower growing period from October to December on the Indian sub-continent. When infected, seedlings develop purple or yellow-brown spots on the upper surface of their leaves, while a cotton-like growth appears on the lower surface. The cauliflower curd becomes discoloured and deformed, and the seed stalk and pods can develop black patches. Downy mildew can result in significant crop losses from the seedling stage to the marketing stage and can also affect the storage quality of the several *B. oleracea* crops (Singh *et al*., 2013).

To contrast all the above-mentioned disease in organic farming several strategies must be applied to avoid the extensive use of chemical pesticides, for example it's worth mentioning the use of resistant *B. oleracea* cultivars which have to be developed for properly for the organic farming. Among the different strategies must be applied, there are also the crop rotation, the use of bioproducts such as the *B. thuringensis*, against the *Lepidoptera* larvae. There are also several products which can be used for organic agriculture, for example the copper oxychloride against the fungus, or the azhadirachtin against the several fungi. The most important aspect to take in consideration is to carefully monitor the crops for signs of pests and diseases and to take appropriate action to prevent their spread.

1.2. *Solanum lycopersicum* L. crop

1.2.1. Origin and diversification

Tomato cultivation diversified in Central and Latin America, and its domestication began 7,000 years ago, managing to establish itself as a crop in a wide area of Latin America, including territories such as Chile, Colombia, Bolivia, Ecuador, Peru, and the Galapagos Islands, already in pre-colonial times, colonizing habitats ranging from sea level to over 2,500 meters above sea level (Razifard *et al*., 2020). The geographical origin of tomato domestication is based on two distinct theories, one pointing to southern Mexico and the other to Peru as the places where the first domestication may have occurred. Although domestication in both cases is supported by cultural, linguistic, historical, and genetic-molecular evidence, the most used botanical origin is that derived from the wild forms of *S. lycopersicum* L. var. cerasiforme (small, round berry, also known as cherry), to which introgressions from *S. pimpinellifolium* have contributed. Despite the still unresolved presumed area of origin of the tomato, it seems that the spread of the tomato in Europe started from Mexico to Spain (Bergougnoux, 2014; Razifard *et al*., 2020). During the domestication process, mass selection played a primary role in the variation of seed colour and size, as these traits were correlated with greater resistance and higher germinative power compared to wild tomato species. However, in tomato as well as in other cultivated species, a strong reduction in the genetic base of the species has been observed, due to the diffusion in other geographical contexts, morpho-physiological variations of the plant, and genetic improvement, resulting in a significant loss of genetic variability of the cultivated tomato (Soressi and Mazzuccato, 2010).

The current tomato cultivars origin from the crop wild relatives (CWRs) which belong to the *Solanum* genus and to the *Lycopersicon* group which includes the species *S. pimpinellifolium* L. and the two endemism from Galapàgos Island, *S. cheesmaniae* (L. Riley) Fosberg and *S. galapagense* S.C. Darwin & Peralta (Peralta *et al*., 2008). The pursuit of new tomato cultivars having suitable traits for organic agriculture such as the new sources of resistance against pests and pathogens represents a key point for organic farming (Avdikos *et al*., 2021). The roles of *S. pimpinellifolium* and *S. cerasiforme* during the domestication of tomato are still under debate making nowadays its origin unclear. Some authors consider *S. cerasiforme* to be the ancestor, whereas others think that *S. l. cerasiforme* is an admixture of *S. pimpinellifolium* and the cultivated *S. lycopersicum*. It is also not clear whether the domestication occurred in the Andean region or in Mesoamerica. Most authors agree that *S. pimpinellifolium* is the closest wild species to the cultivated tomato, *S. lycopersicum* var. *lycopersicum* and that *S. lycopersicum* var. *cerasiforme* a variety that usually grows in disturbed lands, is the ancestor of the cultivated variety. However, important aspects of the relationships between these species and varieties have yet to be completely clarified. Tomato belongs to the *Solanaceae* family, *Solanum* L. genus, *Lycopersicon* section (Blanca *et al*., 2012). The wild relatives of the cultivated tomato are native to western South America, from northern Ecuador through Peru to northern Chile, including the Galápagos Islands. They are spread throughout diverse habitats that include the desert of the Pacific coast at sea level, the green inter-Andean valleys and mountainous Andean regions at an altitude of 3,300 meters. This peculiar ecological diversity in the Andean region has contributed to the variability of the tomato related

wild species. Several methods have been performed to assess the genetic diversity of the tomato gene pools such as the genotyping by molecular markers (Shi *et al*., 2011; Foolad and Panthee, 2012; Zhou *et al*., 2015), the genome wide association by linkage maps (Tripodi *et al*., 2021), the construction of physical chip equipped with thousands of molecular markers such as the DNA microarray (Lievens *et al*., 2003; Sim *et al*., 2012; Tranchida-Lombardo *et al*., 2019), and the genotyping by sequencing (Carbonell *et al*., 2018; Xie *et al*., 2019). Various genetic markers have been utilized to comprehensively investigate the phylogenetic connections among the species incorporated into the genus. These markers include cpDNA, mtDNA, nuclear RFLPs, and AFLPs (Palmer and Zamir, 1982; McClean and Hanson, 1986; Miller and Tanksley, 1990; Marshall *et al*., 2001; Peralta and Spooner, 2001; Spooner *et al*., 2005). Additionally, sequence data has been utilized, including ITS rDNA, the GBSSI gene sequence by Peralta and Spooner, and two nuclear genes by Zuriaga *et al*. (2009) In all these studies, *S. pimpinellifolium*, *S. cerasiforme*, and the domesticated tomato have consistently grouped together, revealing their intimate genetic relationship.

S. lycopersicum represents a major ingredient in many cuisines and are used in a variety of dishes, from salads and sandwiches to sauces and soups. However, the tomato was not always a common food item. In fact, it was once thought to be poisonous and was primarily grown as an ornamental plant. The origin of the tomato can be traced back to South America, where it was first domesticated by the indigenous people of the region. Over time, the tomato has undergone significant diversification, resulting in the wide variety of shapes, sizes, and colours that are nowadays available.

1.2.2. Agronomic and economic importance

S. lycopersicum L. represents one of the most economically important crops due to the worldwide distribution of its fruits. In Europe, during the last decades, tomato cultivation is showing an increasing trend in terms of production and area harvested, and Italy and Spain represent the sixth and the eighth world producers, respectively (FAOSTAT, 2021). In the present thesis of PhD, several parameters of agricultural and economic importance for the tomato cultivation have been examined, considering the cultivated area and the quantity of tomato produced, regardless of its intended use, both worldwide and in Europe and Italy, considering the range of time from 1994 to 2021 (FAOSTAT, 2021). Additionally, data were provided also by the Italian National Institute of Statistics (ISTAT) dealing the production of field-grown tomato in Italy, divided by geographical areas and with a focus on the Sicilian region, considering the range of time from 2006 to 2019. There are several countries that produce tomato, and they determine a diversified supply, but nonetheless, three main areas can be identified where most of the supply is concentrated. The first is the Asiatic continent East, whose supply is strongly concentrated in China, which is the world's top tomato producer, with a production of about 40 million tons, and a small share of the continent's surface and product is located in India (FAOSTAT, 2021).

The second production basin consists of the United States, Brazil, and Mexico, with the United States being the largest producer in the Americas. The third area of economic interest is the Mediterranean basin, where the crop is distributed along its coasts in countries such as Turkey, with about 10 million tons produced, thus becoming the largest producer in the Mediterranean region, followed by Egypt; on the European side, the

largest producer is Italy, with just over 6 million tons produced, followed by Spain, which has a production of about 4 million tons.

Tomato are one of the most important vegetable crops in the world, both economically and nutritionally. They are grown in a wide range of climates and soil types, making them a versatile crop for farmers. Tomatoes are also known for their high nutritional value, containing vitamins A and C, potassium, and lycopene, a powerful antioxidant. From an economic standpoint, *S. lycopersicum* are a major cash crop for many farmers around the world. They are used in a variety of food products, such as sauces, soups, and condiments, and are also sold fresh in supermarkets and farmers' markets. In addition, the tomato processing industry is a significant contributor to many national economies.

The agronomic importance of the tomato crop cannot be overstated. Tomato is relatively easy to grow and can be cultivated using a variety of methods, including greenhouse production, hydroponics, and open-field farming. They are also relatively resistant to pests and diseases, making them a popular choice for farmers.

Overall, the tomato crop is a vital component of global agriculture and food systems. Its economic and nutritional importance is matched by its versatility and ease of cultivation, making it a key crop for farmers and consumers alike. Tomatoes are a rich source of vitamins A and C, potassium, and antioxidants such as lycopene, which has been associated with a decreased risk of cancer and heart disease. They are also low in calories, making them an excellent addition to a healthy diet.

Due to their high nutritional value and versatility in the kitchen, tomatoes are a staple ingredient in many food products, including sauces, soups, salads, and sandwiches. The tomato processing industry, which includes canning, drying, and freezing, is a significant contributor to the global food economy. In addition to their economic importance, tomatoes are relatively easy to grow and can be cultivated in a variety of environments, from small backyard gardens to large-scale commercial farms. This makes them a vital component of global agriculture and food systems.

1.2.3. Biology and requirements

S. lycopersicum is herbaceous perennial which its native habitat is located in South America, but it is usually grown as an annual in other regions. The tomato plant typically grows to a height of 90-300 cm, according to the cultivar and the growing conditions, and has a sprawling, branching habit. Its leaves are pinnately compound, with 5-9 leaflets, and are covered in fine, sticky hairs. The plant produces yellow flowers that are 1-2 centimetres in diameter and are followed by fruit that varies in size, shape, and colour depending on the cultivar. Overall, the tomato plant is a highly adaptable crop that can thrive in a range of environmental conditions and is prized for its nutritional and economic value. *S. lycopersicum* are one of the most important vegetable crops in the world, both economically and nutritionally. They are grown in a wide range of climates and soil types, making them a versatile crop for farmers.

S. lycopersicum are a warm season crop that require specific environmental conditions for optimal growth and development. Soil, moisture, and temperature are important factors that affect tomato growth and yield, both in open field and greenhouse production systems. In terms of soil, tomatoes prefer well-drained soils with a pH range of 6.0 to 7.0. The main *Solanaceae* crops are considered moderately sensitive to salinity,

with thresholds ranging from 1.1 dS/m for eggplant to 2.5 dS/m for tomato. This sensitivity has various effects, generally resulting in smaller fruit or tuber size in the case of potato. Only in the case of tomato, the least sensitive crop, negative effects on yields are accompanied by a significant increase in product quality (Leonardi *et al*., 2008).

They also require adequate nutrient availability, particularly nitrogen, phosphorus, and potassium. The use of organic matter and proper fertilization practices can help to maintain soil fertility and promote healthy plant growth (Hochmuth and Hanlon, 2020). Tomato crop requires adequate moisture throughout the growing season, but overwatering can lead to root rot and other diseases. In open field production, irrigation is often required during periods of drought, while in greenhouse production, drip irrigation systems can be used to provide precise control over water application (Shock and Saunders, 2018).

Temperature is a critical factor that affects tomato growth and development. Optimal temperatures for growth and fruit production range from 20-30°C (68-86°F). High temperatures above 35°C (95°F) can cause flower and fruit drop, reduce pollen vitality, and negatively impact fruit quality. In greenhouse production, temperature control systems can be used to maintain optimal growing conditions (Kadir and Ling, 2017).

Pollen vitality is an important factor that affects tomato fruit set and yield. Pollen viability can be affected by a variety of factors, including temperature, humidity, and exposure to pesticides. Pollination efficiency can also be improved by selecting pollinator-friendly plants or using managed pollination techniques, such as bumblebee hives (Delaplane, 2000).

In conclusion, understanding the requirements of tomato biology and environmental factors is essential for achieving optimal growth and yield in both open field and greenhouse production systems. Proper soil management, irrigation, temperature control, and pollination practices can help to promote healthy plant growth and improve fruit quality and yield.

1.2.4. Growing technique and harvesting

The tomato cultivation always starts with the sowing, which is usually carried out in cellular trays, placing one/two seeds per hole, depending for the percentage of germination which can be tested by a previous germination test. The tomato sowing is usually done in a nursery, controlling all the climatic conditions. Seed germinates in 9/15 days, and the plantlets must be irrigated and managed under controlled conditions until the emission of the third pair of true leaves. The sowing is usually carried out two times per year, basing on the growing cycle which must be carried one. For the winter cycle, which in Mediterranean regions is usually performed into cold greenhouse, the sowing usually start in the middle of August. Furthermore, for the summer cultivation in greenhouse or in open field, the sowing is usually done at the end of February or at the beginning of March. Before the transplanting, grafting technique is often performed to ensure a vigorous plant growth and development and to minimize the harmful effects of the soilborne pathogens, which are very common and widespread in tomato growing area. Tomato grafting is a technique that involves fusing the root system of one tomato plant (the rootstock) with the stem of another tomato plant (the scion) to create a single, hybrid plant. Grafting can be used to improve the resistance of tomato plants to various soil-borne diseases, as well as to improve the vigour and yield of the plants (Prohens *et al*., 2021). Tomato grafting involves carefully selecting

and preparing the rootstock and scion plants, making a precise cut in the stem of each plant, and fusing the two plants together using a grafting clip or other device. The grafted plant is grown under controlled conditions until it is ready for transplanting into the field or greenhouse. Grafting can help improve the resistance of tomato plants to soil-borne diseases such as fusarium wilt, verticillium wilt, and bacterial wilt. It can also increase the vigour and yield of the plants, as well as improve their tolerance to environmental stresses such as drought and high temperatures (King *et al*., 2010; Kyriacou *et al*., 2017). There are several types of tomato rootstocks that are commonly used in grafting, each with its own set of characteristics. Some of the most popular rootstocks include Maxifort, Beaufort, and Multifort. The selection of rootstock will depend on a number of factors, including the soil conditions, the desired disease resistance, and the specific variety of tomato being grown.

For the transplant of the plantlets, tomato seedlings should be handled carefully to avoid damaging the fragile stems and roots. Dig a hole that is deep enough to accommodate the entire root system of the seedling, and gently remove the seedling from its container. Place the seedling in the hole and fill in the soil around it, pressing gently to ensure good soil-to-root contact. It's worth the mentioning to irrigate well the seedling immediately after transplanting. Plant density, therefore, the number of tomato plants per unit area, is an important consideration when transplanting tomato seedlings. The appropriate plant density will depend on several factors, including the variety of tomato, the size of the plants at transplanting, and the growing conditions. For determinate varieties of tomatoes, which have a more compact growth habit, a spacing of 45-60 cm between plants and 90-150 cm between rows is often recommended. For indeterminate varieties, which have a more sprawling growth habit, a spacing of 60-100 cm between plants and 120-180 cm between rows is often used. However, these are general guidelines, and the spacing may need to be adjusted based on the specific variety and growing conditions. Plant density can have a significant impact on tomato yield. In general, higher plant densities can lead to higher yields, but there is a point of diminishing returns beyond which increasing plant density does not result in higher yields. The optimal plant density will depend on several factors, including the variety, the growing conditions, and the desired yield.

After transplanting, tomato seedlings should be monitored closely for the first few days to ensure they are adjusting well to their new location. Water the seedlings regularly, keeping the soil moist but not waterlogged. Provide support for the seedlings as they grow, using stakes or cages to prevent them from falling over. Mulching around the base of the plants can help conserve moisture and reduce weed growth, and it is recommended mostly for the greenhouse cultivation.

S. lycopersicum is usually grown in a variety of settings, including greenhouses, open fields, and hydroponic systems. In all cases, the soil should be well-drained and nutrient-rich, with a pH between 6.0 and 7.0. Tomato prefers full sun and warm temperatures, so they should be planted in an area that receives at least 6 hours of direct sunlight per day.

In a greenhouse, tomatoes can be grown year-round, but they require careful monitoring of temperature, humidity, and light levels. The plants are typically trained to grow vertically using stakes or trellises, which helps to maximize space and sunlight exposure. Tomatoes can also be grown in hydroponic

systems, which use nutrient-rich water instead of soil. This can result in faster growth and higher yields but requires careful attention to nutrient levels and water quality. In open fields, tomatoes are usually grown in rows with plenty of space between plants to allow for good air circulation and sunlight exposure. The plants are often trained to grow on trellises or cages to prevent them from sprawling on the ground, which can lead to disease and lower yields. Mulching can help to retain moisture and control weeds, while regular fertilization and irrigation are essential for healthy growth.

Tomato pruning is another management that tomato requires, mostly for the plants having indeterminate growing habitus; it can help improve the fruit yield and its quality. The suckers that grow in the crotch between the stem and the branches should be removed to focus the plant's energy on fruit production. Indeterminate varieties of tomatoes may require staking or trellising to support the weight of the fruit.

When it comes time to harvest, tomatoes should be picked when they are fully ripe but still firm. This ensures that they will have maximum flavour and nutrition. Depending on the variety and growing conditions, tomatoes can be harvested anywhere from 60 to 100 days after planting. To avoid bruising and damage, the fruit should be carefully picked by hand and handled gently. Tomatoes can be stored at room temperature for several days, or in the refrigerator for up to a week.

Overall, growing tomatoes requires careful attention to soil quality, sunlight exposure, and temperature and humidity levels. With the right techniques and care, however, tomatoes can be a highly rewarding crop, providing a nutritious and versatile ingredient for a wide range of dishes.

1.2.5. Produce quality

S. lycopersicum grape is considered a popular fruit that are consumed globally, due to their unique flavour and nutritional value. They are rich in a variety of biochemical compounds, including carotenoids, flavonoids, phenolic acids, and vitamins. The quality of tomatoes can be evaluated based on the concentration of these compounds, as well as their bioavailability and antioxidant activity.

One of the most important biochemical compounds in tomatoes are the carotenoids, which are responsible for the fruit's characteristic red color. The most abundant carotenoid found in *S. lycopersicum* is lycopene, which has been extensively studied for its potential health benefits. Lycopene is a potent antioxidant that has been linked to a reduced risk of certain cancers, cardiovascular disease, and age-related macular degeneration (Agarwal and Rao, 2000). Other carotenoids found in tomatoes include β-carotene, lutein, and zeaxanthin, which also have antioxidant properties and may contribute to the fruit's nutritional value.

In addition to carotenoids, tomatoes contain a variety of flavonoids and phenolic acids, which are also potent antioxidants. These compounds have been shown to have anti-inflammatory, anti-cancer, and anti-diabetic properties (Giampieri *et al*., 2019). Some of the most abundant flavonoids in tomatoes include quercetin and kaempferol, while the most abundant phenolic acid is chlorogenic acid. These compounds are thought to contribute to the fruit's flavor, as well as its potential health benefits.

Tomato fruits represent also a good source of vitamins, particularly vitamin C and vitamin A. Vitamin C is an important antioxidant that plays a role in collagen synthesis, while vitamin A is essential for vision and

immune function. Tomatoes also contain significant amounts of potassium, which is important for maintaining electrolyte balance and regulating blood pressure.

The quality of tomatoes can be influenced by a variety of factors, including genetics, environmental conditions, and post-harvest handling. For example, the concentration of lycopene and other carotenoids can vary depending on the tomato variety and growing conditions (Fraser *et al*., 2009). Post-harvest handling practices, such as storage temperature and duration, can also affect the concentration of biochemical compounds in tomatoes (Sánchez-Moreno *et al*., 2016).

The United States Department of Agriculture (USDA) database reports the nutritional values of the tomato grapes, and they contain, in 100 g of the fresh product, 92.5 g of water, 31 kcal of energy, 0.13 g of nitrogen, 0.83 g of protein, 0.63 g of total fatty acids, 0.56 g of ash, 5.51 g of carbohydrates and 2.1 g of dietary fibre (USDA, 2016). In terms of microelements, are worth to be mentioned the average values of 11 mg of calcium, 0.33 mg of iron, 11.9 g of magnesium, 28 mg of phosphorous, 260 mg of potassium, 6 mg of sodium, 0.2 mg of zinc, 0.058 mg of copper and 0.121 mg of manganese (USDA, 2016). With regards to the vitamin and other components amount, we can mentioned the 27.2 mg of ascorbic acid, 0.075 mg of thiamine, 0.065 mg of riboflavin, 0.805 mg of niacin, 0.06 mg of vitamin B-6, 10 μg of folate, 9.8 mg of choline, 49 μg of cis-beta-carotene, 393 μg of trans-beta-carotene, 4100 μg of lycopene, 95 μg of lutein, 0.98 mg of vitamin E (alpha-tocopherol) and 4.2 μg of vitamin K (phylloquinone) (USDA, 2016).

There are several tomato varieties cultivated worldwide, but some of the most common ones include cherry tomatoes, beefsteak tomatoes, Roma tomatoes, and heirloom tomatoes. These varieties differ in size, shape, colour, texture, and flavour, and they may also differ in their biochemical composition.

Cherry tomato variety produce small, round, fruits and often they are red or yellow in colour. They are commonly used in salads and as a snack. Cherry tomatoes tend to be sweeter than other tomato varieties and may have a higher sugar content. They are also a good source of vitamin C and carotenoids, particularly lycopene (Sánchez-Moreno *et al*., 2006). With regards to the beefsteak tomatoes, they are large and round, with a meaty texture and a mild flavour. They are often used for sandwiches, burgers, and grilling. Beefsteak tomatoes tend to be lower in sugar and higher in acidity than cherry tomatoes and may have a higher concentration of ascorbic acid (vitamin C) (Fraser *et al*., 2009). Roma tomatoes, also known as plum tomatoes, are oblong in shape and have a firm, meaty texture. They are commonly used in sauces and canning. Roma tomatoes tend to be lower in sugar and higher in acidity than other tomato varieties and may have a higher concentration of phenolic compounds, particularly hydroxycinnamic acids (Kader, 2008). Heirloom tomatoes are open-pollinated varieties that have been passed down through generations of farmers and gardeners. They come in a variety of shapes, sizes, and colours and are known for their unique flavours. Heirloom tomatoes may have a higher concentration of certain biochemical compounds, such as anthocyanins, which give them their distinctive colours (Tieman *et al*., 2012).

It is important to note that the biochemical composition of tomatoes can be influenced by a variety of factors, including genetics, growing conditions, and post-harvest handling. Therefore, the exact composition of each tomato variety can vary depending on these factors.

Tomato fruit quality is influenced and closely related by the stage of ripening. Dry matter content, which does not vary significantly among tomato varieties, increases from the green stage (6.66 mg 100 g^{-1}) to the ripened stage (8.17 mg 100 g^{-1}), as reported in previous studies (Suarez *et al*., 2008; Guil-Guerrero and Rebolloso-Fuentes, 2009). The smallest increase in dry matter occurs between the green and breakers stages. Total soluble solids (TSS), an important quality factor for fruits, indicate tomato fruit quality when the Brix degree is between 4.8-8.8. TSS content increases during maturation and ripening, with the highest increase observed between the pink and red stages for all analysed varieties. The soluble solids content is a reliable parameter for evaluating tomato fruit ripening, and it is used to determine if the harvested product has ripened. As the fruit ripens, acidity tends to decrease. These findings have been confirmed by several studies (Raffo *et al*., 2002; Suarez *et al*., 2008; Duma *et al*., 2015)

No significant differences were found among tomato varieties and stages of maturity for the mean values of titratable acidity expressed as g 100 g^{-1} of citric acid, although a slight increase was observed at the breakers or pink stage, as reported in previous studies (Suarez *et al*., 2008; Duma *et al*., 2015). The taste index and maturity of tomatoes are determined using Brix degree and total acidity values, which are used to assess the quality and taste of the fruit. The taste index mean values of all analysed tomato varieties at all ripening stages were above 0.85. Maturity is a better predictor of the acid flavour impact than the Brix degree or acidity alone, according to previous research. Soluble solid content increases during ripening, and therefore, maturity also increases and reaches its highest value at the red stage. The variety of tomato and ripening stage have a significant influence on the maturity value.

In summary, tomatoes are a rich source of a variety of biochemical compounds, including carotenoids, flavonoids, phenolic acids, and vitamins. These compounds have been linked to a variety of potential health benefits, including reduced risk of cancer, cardiovascular disease, and age-related macular degeneration. The quality of tomatoes can be influenced by a variety of factors, and understanding these factors is important for maximizing the nutritional value of this popular fruit.

1.2.6. Genetic improvement

Nowadays there are several techniques used for the genetic improvement of *S. lycopersicum*, including traditional breeding, mutation breeding, genetic engineering, and marker-assisted selection.

Traditional breeding: Traditional breeding involves crossing different tomato plants to produce offspring with desirable traits. This technique has been used for centuries and involves selecting plants with desirable traits and crossing them with other plants to create offspring with a combination of desired traits. The offspring are then evaluated for the desired traits and selected for further breeding. Recent advancements in molecular techniques such as metabolic genetic engineering and genome editing have enabled the development of tomatoes with improved and commercially significant traits. These approaches have overcome previous limitations and have been widely utilized for enhancing agronomic traits such as tolerance to biotic and abiotic stress, as well as improving fruit quality attributes such as antioxidant content and shelf-life extension (Wai *et al*., 2020).

This process can take many years to produce a new variety with the desired traits. The mutation breeding involves exposing tomato seeds to radiation or chemicals to induce mutations in the DNA. The mutated seeds are then grown and evaluated for desirable traits. This technique has been used for many years to produce new tomato varieties with improved traits, such as disease resistance, and has been shown to be safe and effective.

The genetic engineering involves manipulating the DNA of tomato plants to introduce new traits or modify existing ones. This technique can allow for the introduction of desirable traits more quickly than traditional breeding, but it is also more controversial due to concerns over the safety and environmental impact of genetically modified organisms (GMOs) (Kalloo *et al*., 2012).

As concern the marker-assisted selection involves using molecular markers to identify desirable traits in tomato plants and selecting plants with those markers for further breeding. This technique can help speed up the breeding process and is particularly useful for traits that are difficult to select for using traditional breeding methods.

With regards to the genetic improvement of crops for organic farming aims to develop plant varieties with desirable traits such as resistance to diseases and weeds, adaptability to organic farming practices, tolerance to adverse environmental conditions, high yield and superior nutritional quality, and the preservation of biodiversity. The tomato has a wide range of varieties due to significant genetic improvement efforts, and the search for new tomato cultivars with traits suitable for organic farming, such as new sources of resistance against pests and pathogens, is a key point for organic agriculture. In tomato breeding, specific objectives depend on the agronomic purposes that usually coincide with the destination of the product, such as tomatoes for fresh consumption, industry, and storage. Productivity has always been one of the main objectives in tomato cultivation, and it is believed that about 50% of the yield improvements in industry cultivars in the US between 1920 and 1990 were due to genetic improvement. Early flowering and ripening are important characteristics for both fresh consumption and industry cultivars, and there are genetic differences that allow for early, mid-early, and late culture. Another crucial characteristic introduced in modern varieties is the consistency of the fruit, which has a polygenic control and has been introduced into cultivated tomatoes mainly using *S. pimpinellifolium* since the 1940. Quality improvement, diversification of the fruit, and the incorporation of resistance and tolerance to major adversities are two areas where genetic improvement has progressed towards the same objectives for both fresh consumption and industry cultivars. Although the tomato is universally recognized for its red colour at maturity due to the accumulation of lycopene in the pulp and flavonoids in the skin, there are numerous mutations (or genes derived from related wild species) that confer different colours and different pools of pigments and other metabolites of nutritional and/or aesthetic importance (Soressi and Mazzuccato 2010). The objectives of genetic improvement may vary depending on specific crops and the needs of different regions. Furthermore, research in the field of genetic improvement for organic farming is continuously evolving, with new approaches and technologies developed to address the specific challenges of organic agriculture.

4.2.7. Pest and disease

Tomato crop is susceptible to a wide range of pests and diseases that can cause significant yield losses and reduce fruit quality. Therefore, effective pest and disease management strategies are crucial for maintaining high yields and quality of tomato crops. Tomato plants are susceptible to various pathogens, including viruses, fungi, bacteria, oomycetes, and nematodes, which can decrease yield and lower product quality. The study of the tomato-pathogen system has provided insight into the molecular mechanisms underlying disease resistance, offering the potential for improving yield and quality of tomato crops. Functional genomics techniques have played a significant role in identifying key functional genes involved in susceptible and resistant responses, as well as understanding the molecular basis of plant-pathogen interactions. Next-generation sequencing technologies, such as high-throughput RNA-seq, have facilitated the discovery of transcriptome changes in tomato plants responding to different pathogens, providing valuable information for genetic engineering, and developing new sources of resistance for sustainable plant-disease management. This review summarizes the latest research that has used RNA-seq technology to study the transcriptome changes in tomato plants in response to a range of pathogens, including viruses, fungi, bacteria, oomycetes, and nematodes. This information will aid in the development of new strategies for protecting tomato crops against pathogens and advancing plant breeding efforts towards sustainable plant-disease management (Campos *et al*., 2021).

Tomato crops are susceptible to a range of insect pests, including aphids, whiteflies, thrips, mites, and caterpillars. These pests can cause direct damage to the plants by feeding on the leaves, stems, and fruit, leading to reduced yields and fruit quality. Moreover, they can also transmit viral diseases, such as tomato yellow leaf curl virus (TYLCV) and tomato spotted wilt virus (TSWV), which can have devastating effects on tomato crops. Integrated pest management (IPM) strategies are recommended for the management of insect pests in tomato crops (Biondi *et al*., 2018). IPM involves the use of multiple control tactics, including cultural, physical, biological, and chemical control methods, to reduce pest populations and prevent damage to the crop. For example, cultural practices such as crop rotation, use of resistant varieties, and sanitation can reduce pest populations and prevent the spread of diseases. Physical control methods such as sticky traps and netting can also be used to capture and exclude insects from the crop. Biological control methods such as the release of natural enemies, such as parasitoids and predators, can also be effective in reducing pest populations (Desneux et al., 2010). Finally, chemical control methods such as insecticides should be used as a last resort, and their use should be based on careful monitoring of pest populations and application of the least toxic products.

Tomato crops are also susceptible to a wide range of fungal, bacterial, and viral diseases, which can cause significant yield losses and reduce fruit quality. The most common diseases of tomato crops include early blight, late blight, *Verticillium* wilt, *Fusarium* wilt, bacterial spot, and viral diseases such as tomato mosaic virus (ToMV), tomato yellow leaf curl virus (TYLCV), tomato spotted wilt virus (TSWV), and tomato brown rugose fruit virus (ToBRFV) (Singh et al., 2017; Ong et al., 2020; Zhang et al., 2022).

Disease management strategies for tomato crops include the use of resistant cultivars, cultural practices, and chemical control methods (Kunwar *et al*., 2018). Resistant cultivars are the most effective means of disease control, as they provide long-term protection against diseases and reduce the need for chemical

control methods. Cultural practices such as crop rotation, sanitation, and pruning can also reduce disease incidence and severity. For example, removing diseased plant material and avoiding overhead irrigation can prevent the spread of fungal and bacterial diseases. Finally, chemical control methods such as fungicides and bactericides can be used to control diseases when other methods are ineffective. However, the use of chemical control methods should be minimized to reduce the risk of resistance development and environmental contamination.

Additionally, *S. lycopersicum* has been widely used as a model plant to study plant-pathogen interactions and its potential for future research is promising. The International Solanaceae Genomics Project (SOL) is currently conducting whole genome sequencing of tomato, highlighting the importance of accumulating knowledge on tomato-pathogen interactions in the post-genomic era (Arie *et al*., 2007).

As is well known, tomato undergo substantial morphological changes and alter secondary metabolites as a response of the abiotic stresses (Djidonou et al., 2020). In fact, abiotic stressors can provoke shifts in the plant's adaptive traits and biochemical composition, influencing the overall resilience and productivity of tomato crops (Lee et al., 2023). Whitin this context, heat stress negatively affects optimal growth and fruit development, adversely affecting yields. Elevated temperatures can disrupt pollination and lead to sunscald, ultimately diminishing the overall quality of the harvest (Bhattarai et al., 2021). Conversely, water deficit represents a challenge for tomato growing, triggering adaptive responses such as reduced leaf area and altered root architecture. In response to water scarcity, tomato often undergo physiological adjustments, impacting both growth patterns and the composition of secondary metabolites. The adoption of strategic rootstock and scion combinations serves as an effective solution to confront the challenges of water deficit. This synergistic approach facilitates an adaptive response to drought stress, enhancing the resilience and water-use efficiency of the tomato plants (Alves et al., 2021; Argento et al., 2023).

1.3. Organic plant breeding: ethic and methods

Since the 20th century, the popularity of organic farming has increased significantly as it emphasizes a comprehensive and eco-friendly approach to cultivating crops. Nevertheless, organic farming falls short in terms of producing large amounts of biomass, managing diseases, ensuring nutritional quality, and carrying out postharvest treatments, when compared to conventional farming methods. These limitations are also evident in the production of *B. oleracea* and its crops, which is mostly grown using organic farming practices (Reda *et al*., 2021). Organic breeding of *B. oleracea* is an important area of research for organic farmers and breeders due to the rapid growing of organic farming in the world (Willer *et al*., 2018).

The aim of organic breeding is represented by the development of new crops that are well adapted to organic growing systems, which typically rely on natural inputs and management practices to maintain soil health and control pests and diseases.

One of the main goals of organic breeding in *B. oleracea* crops is to develop varieties that are resistant to common pests and diseases, such as cabbage worms, aphids, and clubroot. Breeding for resistance to these pests and diseases can reduce the need for synthetic pesticides and fungicides, which are typically not allowed in organic production (Myers *et al*., 2012). The organic breeding programs of *B. oleracea* crops also highlight on the development of new cultivars that are able to be adapted to specific growing conditions, such as low-input or no-till systems. These varieties may have traits such as deep root systems, drought tolerance, and resistance to soil-borne diseases, which can help them thrive in organic systems (Renaud *et al*., 2014). Organic breeding also aims to develop varieties that are high yielding and have good quality traits, such as flavour, texture, and nutritional content. These traits are important for both farmers and consumers, as they can impact the marketability and profitability of the crop.

Several organizations and institutions are involved in organic breeding research in Brassica oleracea, including the Organic Seed Alliance, the Organic Agriculture Centre of Canada, and the USDA Agricultural Research Service. These organizations work to develop and promote the use of organic breeding techniques, such as participatory breeding and marker-assisted selection, to improve the sustainability and profitability of organic farming (Seed Alliance, 2023).

B. oleracea crops could be exploited for organic farming due to their resilience but several traits like the adaptation to the low soil fertility, the capacity to compete against weeds and the response against pest and pathogen, must be improved with specific breeding programs (Myers *et al*., 2012). New research has indicated that the new cultivars developed from the breeding programs do not possess the proper characteristics needed for organic and low-input farming. The main reason for this is that conventional breeding programs have been developed with a focus on using large amounts of synthetic fertilizers and crop protection products. Additionally, certain traits (such as semi-dwarf genes) were originally introduced to solve specific issues but may not be suitable for organic or low-input farming practices (Van Bueren *et al*., 2011).

Furthermore, the genotypes that are chosen for their excellent performance under ideal and high-input conditions are unlikely to maintain the same level of productivity when exposed to low-input or stressful conditions (Murphy *et al*., 2005).

Domestication and breeding are typically linked with large changes in genetic diversity for a subset of target genes or genomic areas as a result of linkage drag (Suzuki, 2010). This has resulted in the development of novel genome-wide techniques that expose the pattern of genetic alterations and allow for the discovery and dissection of areas that contribute to complicated trait variation without the requirement for previous trait assessment. These methods include genome-wide scans of nucleotide diversity, LD decay patterns, and outlier genomic selection areas based on genetic divergence across populations (Motsinger *et al*. 2006). Organic plant breeding requires several conditions related to the integrity of the crop cultivations and several authors focused on the relationship among the ethical aspects related to genetic engineering approaches and plant breeding (Van Bueren *et al*., 2003; Nuijten *et al*., 2017).

In the work of Torricelli *et al*., 2014, they've assessed the performance and stability of low input lines of broccoli which have been bred for organic farming, in comparison to a F1 hybrid. The two evaluation cycles which have been established were in Italy, having different management and pedo-climatic conditions (Torricelli *et al*., 2014).

The aim of the crop breeding programs is the improvement of the most important agronomic traits and several of them show complex quantitative inheritance, and their analysis and mapping allow the identification of the quantitative trait loci (QTLs) of the candidate gene. Advances in molecular biology and in the knowledge of the genetics of complex traits allowed the development of several methodologies to associate traits to their proper genomic regions through the molecular markers which can be exploited in the marker assisted selection (MAS) (Jaganathan *et al*., 2020). The individuation of *B. oleracea* crops and cultivars suitable for organic farming can be helped by the participatory plant breeding approach, as was reported by Chable *et al*. (2008) for cauliflower, in Brittany.

1.4. Traits of interest for organic breeding

Organic farming requires suitable plant traits which differ from the conventional agriculture ones because they must help the plant to thrive in low-input environments, without relying on synthetic fertilizers or pesticides. In this way, organic farming can produce healthy, nutritious, and sustainable food while also promoting environmental conservation (Rader *et al*., 2014). In this context, the identification and selection of the superior alleles useful for organic farming, plays an important role for the further development of new varieties. Genetic improvement for organic farming aims to develop plant varieties with desirable characteristics in terms of: i) resistance to diseases and weeds, to reduce reliance on synthetic pesticides; ii) adaptability to organic farming practices such as soil management, crop rotation, and the use of organic fertilizers; iii) tolerance to adverse environmental conditions, to ensure crop productivity even in difficult environments without the excessive use of water and other natural resources; iv) high yield and superior nutritional quality, such as increased ability to fix atmospheric nitrogen, improved photosynthetic efficiency, or higher concentration of essential nutrients; v) maintenance and conservation of biodiversity, through crop diversification and the valorization of traditional varieties useful for preserving genetic diversity and protecting the agricultural environment, in accordance with the principles and standards of organic agriculture (Prohens *et al*., 2021).

The evolution and the selection techniques of the new superior alleles in *B. oleracea* crops were examined and reviewed highlighting the new biotechnological methods allowing the identification of candidate genes for the elite traits. The allele identification in *B. oleracea* is usually developed based on the candidate genes previously identified on *Arabidopsis* or *B. rapa* genome and transcriptomic analysis mediates the process of detecting the differential expressed genes in plants grown in opposite conditions. In this paragraph of mine PhD thesis, the methods performed in *B. oleracea* for superior alleles identification were detailed specifying the validated QTLs and candidate genes, useful for organic breeding programs by the exploitation of the *B. oleracea* genetic diversity.

Broccoli and cauliflower produced both in conventional and/or organic farming, are essentially prized for their edible florets. These florets are the commercial organ of the above-mentioned *B. oleracea* crops, meaning they are the part that is harvested and sold. The development of these florets is controlled by a group of genes known as flowering genes and they represent target key genes also for the organic crop improvement. Flowering genes play a crucial role in the development of the reproductive structures of plants, including the flowers and the fruit or seeds that they produce. In the case of broccoli and cauliflower, the flowering genes are responsible for the formation and growth of the florets, in addition they are also involved in the fruit's development, and they can also determine the siliqua shatter resistance (Raman *et al*., 2011; Hossain *et al*., 2012). The timing and duration of the expression of these genes is critical to the quality and yield of the crop. If the genes are not expressed properly, the florets may not form or may not develop to their full size, which can result in a lower yield and lower quality product. Therefore, understanding the role of flowering genes in broccoli and cauliflower is essential for growers to produce high-quality crops with maximum yield, both in organic and conventional agriculture. By manipulating the expression of these genes through breeding

or genetic engineering, growers can optimize the growth and development of the florets, ensuring that they are of the desired size, shape, and quality for the market.

Though some QTLs have been identified in cabbage, research is still in its early stages because QTL cloning has yet to be reported and functional analysis studies are uncommon. To date, cabbage QTLs have primarily been studied for their effects on fertility (Wang *et al*. 2000), plant size (Lan and Paterson 2001), plant regeneration (Holme *et al*. 2004), flowering time (Bohuon *et al*. 1998; Okazaki *et al*. 2007; Uptmoor *et al*. 2008), clubroot resistance (Nagaoka *et al*. 2010), and black rot resistance (Kifuji *et al*. 2013).

Previous research has identified QTL for flowering time in *Brassica oleracea* (Kennard *et al*., 1994), discovered significant QTL using single-factor ANOVA on an F2 from a cabbage broccoli cross, whereas Camargo & Osborn (1996) used F3 families from a different cabbage broccoli cross. Indeed, Rae *et al*., (1996) and over the course of five years, 79 recombinant backcross substitution lines from a cross between *B. oleracea* var. *italica* and *B. oleracea* var. *alboglabra* were grown in field trials alongside the *alboglabra* recurrent parent.

B. oleracea has several classes of genes involved in the floral development and several of them belongs to mutation events which determined the induction of hypertrophic inflorescence in *Brassica* crop and most of these genes are related to the MADS – box family and were studied by several authors (Bowman *et al*., 2003; Smith and King, 2000; Duclos and Björkman, 2008; Irish, 2010; Wils and Kaufmann, 2017; Sheng *et al*., 2019). The MADS-box family includes key regulators genes involved in reproductive development and plays an important role in the floral organ's differentiation, flower's development, flowering time, inflorescence architecture and seed development (Castelán-Muñoz *et al*., 2019). This large family of genes is widely distributed in eukaryotes, and it encodes several known transcription factors having 58-60 conserved amino acids determining the MADS domain which was discovered by founding its first four genes encoded in *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Antirrhinum majus* and *Homo sapiens*, respectively (Passmore *et al*., 1988; Yanofsky *et al*., 1990; Honma *et al*., 2001). Flower development genes were studied by Bowman *et al*. who found several genes involved such as *apetala* 1 and *cauliflower* in *Arabidopsis*. These genes are closely related to members of the MADH-box genes family and a mutant copy of them is present in the *B. oleracea* genome. Irish and Sussex (1990) characterized several floral phenotypes and identified an *Arabidopsis* mutant expressing the recessive homeotic *apetala* 1 (*ap1*) mutation, and the homozygous for this mutation showed weak inflorescence affecting floral primordia formation. Several genes such as *apetala* 1 (**AP1)** have been demonstrated to be involved in floral structures that control meristematic inflorescence development (Wils and Kaufmann, 2017). CAULIFLOWER gene (**CAL**) was identified by Bowman *et al.* (1993) showing the same *ap1* or *CAL* phenotype in *Arabidopsis* exhibiting curd resembling cauliflower. The orthologue of this gene was found in cauliflower by Kempin *et al*. (1995) and was called *BoCAL*. The following genes, in different genotypes, showed different alleles and one of them named *Bocal a* was responsible for the premature stop codon in exon 5, arresting the inflorescence primordia proliferation (Schilling *et al*., 2018). The variation and selection at the *CAULIFLOWER* floral homeotic gene accompanying the evolution of domesticated *B. oleracea*. Sicilian Purple type is considered an intermediate of Calabrese Broccoli and

cauliflower genotype in fact, it shows heterozygosity at the *BoAP1* and *BoCAL loci* exhibiting the following genes at the status AAcc or aaCC with the alleles *BoAP1*-a and *Bocal-a*, or *Boap*1-a and *BoCAL*-a, respectively allowing the study of the following alleles distribution (Smith and King, 2000; Purugganan *et al*., 2000). The allelic variation in MADS – box genes show various developmental processes that generate several different morphotypes in *Brassica* species (Schilling *et al*., 2018). These genes are related to development of reproductive organs and belong to the MADS-box genes family (Wils and Kaufmann, 2017). Another gene named *BoTFL1* was identified as strong repressor of the inflorescence development in *Arabidopsis* but did not arrest its induction in *B. oleracea* confirming that the floral development in broccoli and cauliflower is not managed exclusively by the homologues of the genes of *Arabidopsis* (Duclos and Björkman, 2008).

The development of the floral primordia in *Arabidopsis* is supported by the suppression of *BoCAL*, *BoAP1-a* or *BoLFY* genes or a failure in the suppression of *BoTFL1* gene which is a strong repressor of flowering in *Arabidopsis* which doesn't suppress the development of floral primordia in *B. oleracea* (An *et al*., 2007). AP1 paralogue gene like *LEAFY* (LFY) and *CAL*, interact among their transcription factors to encode proteins to control the onset of flower development and in this regulatory model are also present key regulators like *TERMINAL FLOWER1* (*TFL1*) which plays against the *AP1* and *CAL* genes (An *et al*., 2007; Goslin *et al*., 2017). To resume, *B. oleracea* differs from the *Arabidopsis thaliana* floral genetic model for the different genetic pathways involved.

Flower development in cauliflower is also linked to the expression of the *FLC* gene (flowering *locus*) and its paralogues like *FLC2* which in a segregating F2 population of late flowering cauliflower with the allele *BoFLC2* to an early flowering cauliflower with the allele *Boflc2* accounted about 65% of flowering time variation (Ridge *et al*., 2015). FLC gene is linked to the flowering vernalization regulating flowering time regulation and it was mapped by Okazaki *et al*. detecting several gene's homologues (2007) and its activity in flowering regulation was confirmed also for *B. rapa* (Okazaki *et al*., 2007; Yuan *et al*., 2009; Ridge *et al*., 2015). The isolation of genomic *BoFLC* genes and identification of QTL controlling flowering time using a linkage map and flowering data from an F2 population derived from a broccoli DH line by a cabbage DH line to better understand the genetic control of flowering time in *B. oleracea*, also the role of *BoFLC2* in flowering through vernalization is discussed by Okazaki *et al*., (2007). In fact, four *FLC* homologs (*BoFLCs*) from *B. oleracea* were cloned. Three of these, *BoFLC1*, *BoFLC3*, and *BoFLC5*, have previously been identified. The fourth novel sequence shared 98% sequence homology with the previously identified gene *BoFLC4*, but also 91% homology with *B. rapa*'s *BrFLC2*. Phylogenetic analysis revealed that this clone is a member of the *FLC2* clade. As a result, they named this gene BoFLC2. A detailed linkage map of *B. oleracea* was constructed in the F2 progeny obtained from a cross of *B. oleracea* non-vernalization and vernalization types which covered 540 cM and 9 major linkage groups. Six quantitative trait *loci* (QTL) that control flowering time were discovered. The QTLs controlling flowering time were not linked to *BoFLC1*, *BoFLC3*, or *BoFLC5*. The largest QTL effect, however, was found in the region where BoFLC2 was mapped. Due to a frameshift in exon 4, the *BoFLC2* homologs found in non-vernalization plants were non-functional. Furthermore, BoFLC2

duplications and deletions were found in broccoli and a rapid cycling line, respectively. These findings suggest that *BoFLC2* plays a role in the regulation of flowering time in *B. oleracea*.

Single nucleotide polymorphism can affect the expression of the allelic patterns involved in the flower induction and this can occur in *BoFLC4* for *B. oleracea* showing different responses in the vernalization process (Irwin *et al*., 2016). The functional gene *FRIGIDA* (*FRI*) in *Arabidopsis thaliana* was studied and it encodes for the requirements useful for the vernalization process through the overexpression of the MADS box transcriptional repressor *FLOWERING LOCUS C* (*FLC*). Functional alleles of the FRIGIDA genes act as flowering time regulators in the *Brassica oleracea* genotypes (Irwin *et al*., 2012). FRI orthologues were mapped in *B. oleracea* detecting *BolC.FRI.a* and *BolC.FRI.b* alleles showing an abundant ammino acid concentration in C-terminal and divergences than *A. thaliana* for the ammino acid in N-terminal (Sun *et al*., 2013). FRI allelic orthologues were mapped also in *B. rapa* and they differ from *B. oleracea* for the chromosome region, which is A3 and A4 and C3 and C9, in A and C genomes, respectively (Sun *et al*., 2013; Takada *et al*., 2019). *FRI* paralogues are highly conserved in the subgenomes A and C of tetraploid *B. napus* suggesting the *loci* duplication in the speciation of the *Brassica* genus (Takada *et al*., 2019).

MADS box domain includes two genes' groups named type I and type II which comprise the *STRUBBELIG RECEPTOR* family (SRF) and MEF2-like, respectively (De Bodt *et al*., 2003). Sheng *et al*. (2019) characterized and mapped 91 MADS-box transcription factors distinguishing from the type I (Mα, Mβ, Mγ) and type II ($MIKC^C$, MIKC*) groups as consequence of phylogeny and gene structure analysis: 59 genes randomly distributed on 9 chromosomes and 23 were in 19 scaffolds and 9 of them were not located due to lack of information on NCBI database (Sheng *et al*., 2019). SSRs developed by the MADS box genes can represent a useful tool to detect polymorphisms inducing a hypertrophic curd induction (Treccarichi *et al*., 2021).

Basic helix-loop-helix genes represent the second largest plants' family genes which are involved in the pistil development and in the abiotic and biotic stresses response and belong to a family of transcriptional regulators present in three eukaryotic kingdoms (Heim *et al*., 2003; Skinner *et al*., 2010). The study of Miao *et al*., (2020) allowed to identify 268 *bHLH* genes in *B. oleracea*, 440 genes in *B. napus*, and 251 genes in *B. rapa*, including 21 new *bHLH* members and the gene structures, phylogeny threes and the conservative motif analysis were carried showing similar expression patterns between *B. rapa* and *B. napus* in roots and contrasting ones in stems, leaves and in reproductive organs (Miao *et al*., 2020). Genome wide analysis of the *bHLH* transcription factor was studied for chinese-cabbage showing the interaction network associated to the *Arabidopsis bHLH* genes and in cabbage which revealed several chilling response patterns (Song *et al*., 2014; Shan *et al*., 2019). The divergence of the S-Haplotypes between *B. oleracea* and *B. napus* was also studied to investigate about the recognition specificity of self-incompatibility which is determinate by the SRK alleles encoded in the stigma and by the SP11 ones in the pollen (Kusaba *et al*., 1999; Kimura *et al*., 2002; Sato *et al*., 2003).

As concern the tomato crop, it has a wide range of varieties due to significant genetic improvement activities, which the crop is still subject to (La Malfa, 1990). The search for new tomato cultivars with traits

suitable for organic farming, such as new sources of resistance against pests and pathogens, represents a key point for organic agriculture (Avdikos *et al*., 2021).

For tomato, specific breeding objectives depend on the agronomic goals that usually coincide with the destination of the product. For example, among field-grown tomatoes for the industry, fresh-consumption tomatoes, mostly grown in protected cultivation, and storage tomatoes. In tomatoes, crop productivity has always been one of the main objectives and one of the traits that have seen the greatest successes. For example, it is believed that the average productivity of industrial cultivars in the United States between 1920 and 1990 increased from 10 to over 70 t ha^{-1}. It is estimated that about 50% of these increases are due to genetic improvement (Soressi and Mazzuccato, 2010).

The earliness of flowering and fruit ripening is an important characteristic for both fresh-consumption and industrial cultivars. There are genetic differences that allow early, mid-early, and late cultivation, with which it is possible to extend the harvest period, both in the case of fresh-consumption and industrial tomatoes, to which also staggered planting contributes. Another fundamental trait introduced in modern cultivars, both for fresh consumption and industry, concerns the consistency of the fruit. This trait has a polygenic control and was introduced into cultivated tomatoes mainly using *S. pimpinellifolium* from the 1940s onwards. In Italy, the trait entered the first cultivars (Gimar, Picenum) around the 1960s. In fresh-consumption tomatoes, consistency is one of the components of shelf life, i.e., the fruit's ability to remain turgid even after detachment from the plant (Soressi and Mazzuccato, 2010). Two crucial topics for which the genetic improvement has progressed towards the same objectives for both fresh-consumption and industrial cultivars concern qualitative improvement, diversification of the fruit, and the insertion of resistance and tolerance to the main adversities. Although tomatoes are universally recognized for their red color at ripening, due to the accumulation of lycopene in the pulp and flavonoids in the skin, there are numerous mutations (or genes derived from related wild species) that confer colors different from the classic and different pools of pigments and other metabolites of nutritional and/or aesthetic importance (Soressi and Mazzuccato, 2010).

The goals of genetic improvement may vary depending on specific crops and the needs of different regions. Furthermore, research in the field of genetic improvement for organic farming is continuously evolving, with new approaches and technologies developed to address the specific challenges of organic agriculture.

2. Experimental activities

2.1. General premise and objectives

Brassica oleracea L. and *Solanum lycopersicum* L. are two important species, domesticated for establishing several crops, commonly grown in the Mediterranean basin where they have been domesticated and/or cultivated for centuries. These crops play a critical role for improving sustainable agriculture, providing high-quality food while promoting soil health and biodiversity in alignment the FAO strategic framework 2022-2031 and the goals foreseen for the sustainable development. In addition, the Mediterranean basin represents a hot spot for the diversity of these species, such as it is the center of origin and diversification for *B. oleracea* and the secondary center of diversification for *S. lycopersicum* after its introduction from the new world's by Columbus, with several morphotypes and landraces widespread in different regions provide products requested by local consumers, markets and food industries.

Broccoli (*B. oleracea* var. *italica*), cauliflower (*B. oleracea* var. *botrytis*), cabbage (*B. oleracea* var. *capitata*), kale (*B. oleracea* var. *acephala*), and kohlrabi (*B. oleracea* var. *gongylodes*) are vegetable crops derived from the intraspecific diversification of *B. oleracea* L., each of them has its unique morphological and nutritional characteristics. The *B. oleracea* complex species (n=9) has a fascinating history, with several crop wild relatives (CWRs) have contributed to the deep diversification of the species. This diversification has led to the development of numerous morpho-types and landraces, each with its unique morphology, nutritional profile, and culinary uses. In addition to the well-known vegetable crops, such as broccoli, cauliflower, cabbage, kale, and kohlrabi, the *B. oleracea* complex species (n=9), includes *B. oleracea* wild relatives sources of resistance against several biotic and abiotic stresses, which belonging to the primary gene pool of *B. oleracea,* and several minor and/or neglected *Brassicaceae* species, belonging to the secondary and tertiary gene pools, which have been collected and occasionally used in Italy and in some other Mediterranean countries for centuries. The *B. oleracea* complex species (n=9), some of which are sporadically utilized by local communities, in Sicily and in some other Mediterranean countries, for their traditional gastronomical uses. This important source of genetic diversity for *B. oleracea* can be exploit for improving the resilience of the crops and for improving several organoleptic and nutraceutical traits of the related products. The conservation and the exploitation of the previously cited CWRs, and of the minor and neglected species, is of great interest for developing new resilient cultivars, and to introduce new crops, with improved quality of the products, disease resistance, and stress tolerance, and at the same time for promoting the cultural heritage and traditional knowledge of local communities. Moreover, the conservation and utilization of these genetic resources can help to increase the genetic diversity of *Brassica* crops, which is essential for ensuring the long-term sustainability and resilience of agricultural farming systems in the face of climate change and other environmental challenges.

Conversely, *Solanum lycopersicum* is the most growing vegetable crop in the world and well diversified in the Mediterranean basin. Tomato crop is widespread all over the world due to its richness of vitamins and minerals and it is widely used in Mediterranean cuisine and for food industries. Moreover, tomato

is an ideal crop for organic farming, as it has a relatively short growing cycle, and it can be cultivated in a wide range of soil typologies. The diversity of tomato fruits is also an important aspect in the Mediterranean basin. There are numerous landraces of tomato, each with its unique morphology, flavor, and culinary uses. Some of the most common tomato cultivars grown in the Mediterranean basin include beefsteak, cherry, and the midi-plum (datterino), Sammarzano, mini-Sammarzano, Cuore di bue, Canestrino, Sorrentino, Insalataro and Riccio types. These landrace cultivars vary in size, shape, and color, ranging from small and sweet cherry tomatoes to large and meaty beefsteak tomatoes. This diversity of fruit types and cultivars allows for a wide range of culinary applications, making tomatoes a versatile and essential ingredient in Mediterranean cuisine. Moreover, the diversity of tomato cultivars also provides an important source of genetic diversity that can be used to improve the resilience and productivity of tomato crops in the face of environmental challenges such as climate change and emerging pests and diseases.

These crops offer several benefits, including high nutritional value due to their biochemical compounds, adaptability to different growing conditions, and of great potential to improve soil health through the crop rotation. In this context, the improvement of the genetic traits related to the production is essential for increasing importance to the organic farming.

The aim of the organic system is to produce food by sustainable and environmentally friendly methods, without the use of synthetic pesticides, fertilizers, or genetically modified organisms (GMOs). Therefore, organic farmers rely on natural methods, such as crop rotation, intercropping, and the use of natural pest control methods, to maintain soil fertility and control pests and diseases. To ensure that organic farming can meet the increasing demand for organic food while maintaining these principles, it is necessary to improve genetic traits could improve the resilience, efficiency, and sustainability of vegetable crops. Additionally, this genetic improvement should enhance the nutritive, organoleptic and nutraceutical traits of the products improving the related food supply chains “from farm to fork”.

One of the main challenges faced by organic farmers is the increment of the yield in organic methods and techniques without any use of synthetic fertilizers and pesticides. Therefore, it is important to develop new varieties with improved nutrient/water use efficiency, which can better absorb and utilize the available nutrients and water into the soil. By selecting for these traits, it is possible to develop cultivars can provide good yields even under limited nutrient/water availability, improving the economic viability of organic farming. The development of new varieties with improved disease and pest resistance is critical for organic farming. Organic farmers rely on natural pest control methods, such as the use of beneficial insects, microrganisms, natural compounds, and crop rotation, for controlling pests and diseases. Therefore, developing new varieties with natural resistance to pests and diseases can reduce the need for synthetic pesticides and fungicides, promoting a more sustainable and environmentally friendly farming system.

2.2. Framework of the research lines of activity

During the three years of the XXXVI PhD cycle, the research activities focused on the identification, studies, and analysis of a wide range of bibliographic references related to the *Brassica oleracea* complex species (n=9) and to Solanum lycopersicum L. The research aimed to investigate the diversity of the main bio-morphological, biochemical and genetic traits suitable for organic farming for these important vegetable species, and related crops.

Through an extensive review of the available research databases which was carried out in the first year of my PhD course, we collected and studied several articles, chapters and books, dealing with the genetic diversity, evolution, and adaptation of the *B. oleracea* landraces and crops, of the *B. oleracea* complex species (n=9), and of the *S. lycopersicum* types and landraces, to different environmental conditions and farming practices.

Since the first year and during all my PhD course, the following study was addressed on the evaluation of inter and intraspecific diversity of the aforementioned crops by the detection of the bio-morphometric, biochemical and genetic traits of interest, for the genetic improvement of the coles and tomato crops for the organic farming. The main objective of the research was the identification of genetic traits associated with pest and disease resistance, nutrient and water use efficiency, and abiotic stress tolerance that are suitable for organic farming. The findings of these research are expected to contribute to the development of more resilient and sustainable organic farming systems for providing a better understanding about the genetic diversity and of the main agronomic traits of these important crops. Moreover, the research activity also highlighted the importance of conserving and utilizing the genetic resources of *B. oleracea* and *S. lycopersicum* to improve the productivity and the sustainability of the agricultural systems for the future agroecological transition.

Another perspective of the present PhD thesis is to identify the most promising genotypes of broccoli and tomato to use as genetic bases for breeding programs aimed at producing resilient individuals for possible use in organic agriculture. The study was conducted at the University of Catania (UNICT) using a multidisciplinary approach that involved the morphological characterization of different landraces and wild relatives cultivars, as well as the evaluation of their agronomic performance, determination of qualitative characteristics, and genetic traits of interest for future breeding programs. The present work was carried out within the framework of the H2020 Breeding for Resilient, Efficient, Sustainable Organic Vegetable production (BRESOV) project (GA no. 774244; www.bresov.eu).

The experimental activities carried out during the three years of the PhD course involved three main research lines each of which comprised two trials (Trial A and Trial B), one for each crop.

1) Phenotyping and genotyping of Mediterranean landraces, heirlooms and crop wild relatives of *B. oleracea* and *S. lycopersicum* L., for assessing their bio-morphological, biochemical, and genetic diversity.

2) Individuation of the source of resistance in *B. oleracea* L. complex species (n = 9) and *S. lycopersicum* L. crops.

3) Development of different agronomic and genetic tools for improving the resilience of *B. oleracea* L. and *S. lycopersicum* L. for organic farming.

The first research line was focused on the phenotypical and genotypical characterization of nine species within the *B. oleracea* complex species (n=9) and various accessions of *S. lycopersicum* (tomato). The investigation encompassed a diverse range of *B. oleracea* complex species (n=9), including crop wild relatives (CWRs), traditional landraces, and heirloom varieties. The primary objective of this research line was to delve into the extensive genetic and phenotypic diversity present in these species to identify genetic traits suitable for organic farming practices. To achieve this goal, we conducted a genome-wide association study (GWAS) where we associated molecular markers with specific phenotypic traits. Our research employed a variety of techniques, including genotyping by sequencing (GBS), single sequence repeats (SSRs) assays, and the Single Primer Enrichment Technique (SPET). Regarding Genotyping by Sequencing (GBS), the results are currently in progress and will be provided as soon as they become available.

The second research line was focused on evaluating drought stress tolerance in a diverse collection of Cole Crops, specifically the *B. oleracea* complex species (n=9). The aim of this study was to identify genotypes with resilient traits suitable for organic farming, with a view to enhancing water use efficiency (WUE), a critical factor for future agricultural sustainability. To reach this objective, it was intentionally imposed a drought stress conditions on a total of 89 accessions within the *B. oleracea* complex species (n=9). Subsequently, based on their phenotypic response to drought stress, a subset of four accessions were selected, comprising two sensitive and two tolerant ones. This selected genetic material included two accessions of *B. macrocarpa*, known for their drought tolerance, as well as two landraces of broccoli. Within this subset, it was performed a transcriptomic analysis to identify differentially expressed genes (DEGs) associated with drought stress tolerance. This analysis revealed various molecular patterns characterized by either overexpression or downregulation between the sensitive and tolerant accessions.

The third research line delved the phenotypic and genetic traits associated with disease resistance in *S. lycopersicum* (tomato), targeting both soil-borne and air-borne diseases. To achieve this, it was employed a set of experimental rootstock varieties, including both inter and intraspecific hybrids, which were developed through traditional breeding in the frame of the BRESOV project. These rootstocks were meticulously screened using molecular markers to identify specific resistance genes, particularly against diseases caused by Verticillium and Fusarium pathogens. The study also assessed the agronomic performance and behavior of these rootstocks in various grafting combinations. For this purpose, the rootstock employed were grafted with three F1 hybrids (Cherry, Vittorio, Barbarela), utilized as scion. Additionally, in a separate study using a different set of genetic material of tomato, it was conducted a genotyping assay using molecular markers, including Single Sequence Repeats (SSRs) and Single Nucleotide Polymorphisms (SNPs). These markers were employed to assess resistance against two highly significant air-borne diseases that have a substantial impact on global crop yields. Specifically, it was evaluated the resistance to Tomato Mosaic Virus (ToMV) using the SNP marker *Tm2* and resistance to Tomato Spotted Wilt Virus (TSWV) utilizing the SSR marker *Sw5*.

In conclusion, the present Doctoral Thesis provided valuable insights into the characterization of different genotypes of the Cole and tomato crops, with the aim of identifying promising genetic bases for breeding programs aimed at producing resilient individuals for organic agriculture. The multidisciplinary approach

adopted allowed the evaluation of several morphological, agronomic, qualitative, and genetic traits, providing a comprehensive overview of the potential of the analyzed genotypes. The results obtained can contribute to the development of more efficient and sustainable organic vegetable production systems, which are crucial for addressing the challenges of modern agriculture, such as climate change, soil degradation, and loss of biodiversity.

2.3. Research line I

2.3.1. Introduction

EXPERIMENTAL TRIAL A

Brassicaceae family includes herbaceous crops spread on all the continents, which can easily adapt to several environmental conditions, and they can grow from the extratropical to the artic regions (Warwick et al., 2011; Branca et al., 2018). The highest center of biodiversity for this family was the Mediterranean basin and Sicily represented a hot spot for the diversification of Brassica oleracea complex species (n=9), in terms of the number of species and of their morphological diversity (Cartea et al., 2020). These processes allowed the evolution of different crops also within individual species (Branca and Cartea, 2011; Maggioni et al., 2018).

The enormous bio-morphological diversity exhibited by the different B. oleracea morphotypes belongs to the different selection of the edible organs developed by the different European populations and is confirmed by the different morphotypes which are defined also by the different polymorphisms in the chromosomic regions (Treccarichi et al., 2021; Treccarichi et al., 2023). Another crucial aspect is represented by the richness in healthy compounds such as glucosinolates and polyphenols present in B. oleracea complex species (n=9) organs, which nowadays is exploited by the development of their novel foods (Ragusa et al. 2017; Di Bella et al., 2019; Di Bella et al., 2021). Brassica genre belongs to the asymmetrical evolution of the polyploid genomes which is strictly connect to the triangle of U's, including the three primary diploids Brassica gene pools (B. rapa, B. nigra and B. oleracea: AA, BB and CC, respectively) from which belong the amphiploids B. napus, B. carinata and B. juncea having the AACC, BBCC and AABB, respectively (Nagaru, 1935; Liu et al., 2014; Koh *et al*., 2017; Bayer *et al*., 2020; Li *et al*., 2021). *B. oleracea* vegetables include different morphotypes showing high phenotypic variation which can be exploited by its different crops like broccoli (B. oleracea var. *italica*), cauliflowers (*B. oleracea* var. *botrytis*), cabbage (*B. oleracea* var. *capitata*), kale (*B. oleracea* var. acephala), savoy cabbage (B. oleracea var. *sabauda*), and Brussel sprouts (*B. oleracea* var. *gemmifera*) (Maggioni et *al*., 2010; Ciancaleoni *et al*., 2014; Branca *et al*., 2018). The morphological differences among the above-mentioned crops, are mainly based on the edible parts of the plant deriving by the different human selection occurred. For instance, broccoli is characterized by its large, fleshy, and green edible florets, while cauliflower for its large and compact head. Cabbage has a dense rosette of leaves, which can be green or red, Brussels sprouts are characterized by small, compact buds that grow along the stem, moreover kohlrabi by an enlarged short non branching stem supporting leaves and savoy cabbage it appears similar to cabbage, but it has the crinkled leaves. These crops allow an improvement of the soil fertility for their rustic features that allow even marginal land to be used for their growing. In various home and suburban gardens in Sicily, they are often cultivated also along the slopes and on sloping lands to limit landslides thanks to the persistence over time of the robust root system that allows the state of the places. The introduction of new F1 hybrids of B. oleracea crops poses a challenge to preserving the species diversity due to its peculiar floral biology, which facilitates cross-fertilization.

Next-generation sequencing (NGS) is a groundbreaking technology that rapidly and cost-effectively generates millions of short DNA sequence reads, typically ranging from 25 to 400 base pairs in length. This technology is gaining increasing popularity in a wide range of applications, including sequencing entire genomes, studying gene expression (transcriptomes), investigating epigenetic modifications (epigenomes), examining small RNA molecules, identifying molecular markers and genes, exploring comparative and evolutionary genomics, and conducting association studies.

As previously mentioned, several *B. oleracea* complex species (n=9) have undergone a complex genetic process such as polyploidization, which has resulted in genomes that are exceptionally intricate. This complexity poses significant challenges for genomics research. NGS allowed to deepen the study of Brassica species, but it also faces specific hurdles when it comes to analyzing the genomes and traits of these complex crops (Wei *et al*., 2013).

Within this framework, the aim of the present study is to characterize by the international descriptors, a panel set of the 182 *B. oleracea* complex species (n=9) belonging to the H2020 BRESOV project. The goal of the following study is to group and diversify the different *B. oleracea* crops and CWRs throughout the bio-morphometric characterization and the statistical analysis performed. Furthermore, genotyping by sequencing was employed to establish connections between the bio-morphometric traits and the numerous nucleotide variants discovered during the analysis. This was done in order to gain deeper insights into genotype distribution, particularly concerning their distinct origins and morphotypes.

EXPERIMENTAL TRIAL B

One of the challenges of the modern agriculture is to contrast the intensive growing systems which impact the soil fertility causing significative reductions in terms of crops production. The organic farming has been proposed as alternative production system in comparison to the conventional agriculture and it represents a new model which impacted the consumers environmental attitudes and behaviours (Lazaroiu *et al*., 2019; Sivaranjani and Rakshit 2019; Saffeullah *et al*., 2021). The development of new cultivars able to contrast the issue of the burdens of malnutrition as the ammino acids and bioactive compounds deficiency, represents a new challenge and frontier of the organic farming mostly in the frame of the climatic changing and global warming conditions (Dwivedi *et al*., 2019; Navarro-Pedreño *et al*., 2021).

Solanum lycopersicum represents one of the most economically important crops due to the worldwide distribution of its fruits. In Europe, during the last decades, tomato cultivation is showing an increasing trend in terms of production and area harvested, and Italy and Spain represent the sixth and the eighth world producers, respectively (FAOSTAT, 2021). The current tomato cultivars origin from the crop wild relatives (CWRs) which belong to the *Solanum* genus and to the *Lycopersicon* group which includes the species *S. pimpinellifolium* L. and the two endemism from Galapàgos Island, *S. cheesmaniae* (L. Riley) Fosberg and *S. galapagense* S.C. Darwin & Peralta (Peralta *et al*., 2008). The pursuit of new tomato cultivars having suitable traits for organic agriculture such as the new sources of resistance against pests and pathogens represents a key point for organic farming (Avdikos *et al*., 2021).

The exploitation of new genetic resources by next generation sequencing (NGS) techniques could be assessed for discovering new resistance traits suitable for more sustainable productions (Ashraf *et al*., 2022). The new technologies can enhance and shorten the process of selection of new genotypes in comparison to the normal, conventional breeding techniques by the marker assisted selection (MAS), quantitative traits loci (QTLs) mapping, and genome sequencing and assembly. All the above-mentioned techniques require the application of bioinformatic tools and molecular techniques to be performed.

Single primers enrichment technology (SPET) is a new frontier for hight -throughput genotyping in tomato and it is based on the targeted genotyping n specific region flanking primers which are selected basing on Tomato SolGenome database (Barchi *et al*., 2019a). The above-mentioned technique was developed by Nugen® (United States Patent 9,650,628) and it offers an innovative approach based on the integration of *a priori* information regarding known single nucleotide polymorphisms (SNPs) in addition to the random discovery of new markers, surrounding the targeted ones. The probes used for the following techniques are designed around to the regions containing the targeted SNP and their size is about 40-bases (Barchi *et al*., 2019b). SPET technology was used for used for human's medical use (Scolnick *et al*., 2015; Nairismägi *et al*., 2016) and its application for plants was reported for maize (Scaglione *et al*., 2019), black poplar (Scaglione *et al*., 2019), recently on apricot (Baccichet *et al*., 2022), and for tomato and eggplant (Barchi *et al*., 2019a,b; Portis *et al*., 2019).

2.3.2. Materials and methods

EXPERIMENTAL TIAL A

A total panel of 182 *B. oleracea* complex species (n = 9) accessions (Table 1), belonging to the H2020 BRESOV (Breeding for Resilient, Efficient and Sustainable Vegetable Production) project, was examined for their bio-morphometric traits, listed in Table 2. The selected set includes six *B. oleracea* crops (60 accessions of *B. oleracea* var. *capitata*, 37 of *B. oleracea* var. *italica*, 26 of *B. oleracea* var. *botrytis*, 18 of *B. oleracea* var. *acephala*, 18 of *B. oleracea* var. *alboglabra* and 17 of *B. oleracea* var. *gongylodes*) in addition to six accessions representing four crop wild relatives (2 accessions of *B. incana*, 2 of *B. villosa*, 1 of *B. rupestris* and 1 *B. drepanensis*). The material belongs from three different germplasm active collections, which were the one of the Department of Food and Agriculture (Di3A) of University of Catania (UNICT), the second one of the University of Liverpool (UNILIV), while the third one was provided by the Crop Research Institute of Czech Republic (VURV).

Sowing was carried out in cellular trays in a cold greenhouse under natural light (4.6 to 9.2 $MJ.m^{-2}d^{-2}$) and temperature (15.4 ± 5.8 ± C°), from October to December 2018 in the Experimental Agricultural Institute "IAS" (Istituto Agrario Sperimentale) located in Catania (37°31010" N 15°04018" E; 105 m above sea level) using organic growing practices, utilizing the growing substrate Brill® Semina bio (Geotech, Italy). During the nursery stage plantlets were treated by BTK® 32 WG (Xeda, Italy) based on *Bacillus thuringiensis* sub. Kurstaki for controlling *Pieris brassicae*. Some treatments have been carried out with organic fertilizers based on macro-minerals and microelements such as copper, nitrogen, and iron. All the organic products were

provided by ITAKA crop solutions S.R.L. The gowing cycle started with the transplanting carried out on December 2018 and finished in June 2019. The plantlets were transplanted in a cold greenhouse (36°51'13.3" N 14°29'32.0" E, Contrada Randello, Ragusa). For each accession three biological replicates were analyzed for their bio-morphometric traits, following the International Board for Plant Genetic Resources (IBPGR) descriptors.

With regards to the phenological descriptor related to the inflorescence appearance it was calculated registering the date in correspondence of the inflorescence induction and converting it into days from transplanting (Table 2). Concerning the morphometric descriptors selected (Table 2), they were qualitative and quantitative; the first ones were assigned by the visual analysis while the second ones by ruler for the PSL, by caliber for BRD, and for the RFW and RDM by the analytical scale. The leaf parameter LA, LL, LW, LPL and LPW, in addition to the RA, were calculated by the software ImageJ (National Institutes of Health, USA). With regards to the root's traits MRD, MRL, LRD, and MSA, they were calculated by the shovelomics high-throughput phenotyping approach.

Statistical analysis was performed by IBM SPSS software version 27 (IBM, Armonk, USA) performing Pearson's correlation among the examined traits. Furthermore, the principal component analysis (PCA) was conducted for clustering genotypes into different morphotypes based on their bio-morphometric traits. The hierarchical clustering was performed by DARwin software with Euclidean distance was also carried out for measuring the similarity and ward's linkage in the different genotypes, to elucidate the relationship for each group of *B. oleracea* crops and CWR, using the Jaccard's dissimilarity coefficient.

Table 1. List of the accessions used for the trial. The crop code refers to the morphotype, which were BH: kale, BR: broccoli, CWR: crop wild relatives, CC: cabbage, CK: Chinese kale, CR: kohlrabi and CV: cauliflower.

Accession ID	Code	Species	Crop code	Local Name	Status	Origin
UNICT361	BH1	*B. ol.* var. *acephala*	BH	Vecchio	LR	ITA
UNICT364	BH2	*B. ol.* var. *acephala*	BH	Cavolo Nero	LR	ITA
UNICT375	BH3	*B. ol.* var. *acephala*	BH	Couve Galega	LR	PRT
UNICT3381	BH4	*B. ol.* var. *acephala*	BH	Rizzo	LR	ITA
UNICT4591	BH5	*B. ol.* var. *acephala*	BH	Cavolo Da Foglia	LR	ITA
UNICT379	BH6	*B. ol.* var. *acephala*	BH	Cavolo Da Foglia	LR	PRT
UNICT360	BH7	*B. ol.* var. *acephala*	BH	Cavolo Riccio	LR	ITA
UNICT4538	BH8	*B. ol.* var. *acephala*	BH	-	LR	ITA
UNICT4601	BH9	*B. ol.* var. *acephala*	BH	Cavolo Da Foglia	LR	ITA
UNICT 4853	BH10	*B. ol.* var. *acephala*	BH	Cavolo Nero	LR	ITA
HRIGRU9936	BH11	*B. ol.* var. *acephala*	BH	Berza Amarilla	LR	ESP
HRIGRU13102	BH12	*B. ol.* var. *acephala*	BH	Butzo	LR	ESP
HRIGRU9527	BH13	*B. ol.* var. *acephala*	BH	Couve Galega	LR	PRT
UNICT4448	BH14	*B. ol.* var. *acephala*	BH	Cavolo Nero	LR	ITA
HRIGRU9993	BH15	*B. ol.* var. *acephala*	BH	Berza Entrepascua	LR	ESP
HRIGRU7545	BH16	*B. ol.* var. *acephala*	BH	Littmawns	LR	DEU
UNICT353	BH17	*B. ol.* var. *acephala*	BH	D'estate	LR	ITA
HRIGRU9847	BH18	*B. ol.* var. *acephala*	BH	White On Green	LR	JPN
UNICT659	BR1	*B. ol.* var. *italica*	BR	Natalino	LR	ITA
UNICT4369	BR2	*B. ol.* var. *italica*	BR	Broccolo D'aci	LR	ITA
UNICT568	BR3	*B. ol.* var. *italica*	BR	Verde Romanesco	LR	ITA
UNICT4939	BR4	*B. ol.* var. *italica*	BR	Broccolo nero	LR	ITA
UNICT4620	BR5	*B. ol.* var. *italica*	BR	Natalisi	LR	ITA

UNICT656	BR6	*B. ol.* var. *italica*	BR	Bastard.	LR	ITA
UNICT3122	BR7	*B. ol.* var. *italica*	BR	Cavolfiore	LR	ITA
HRIGRU10660	BR8	*B. ol.* var. *italica*	BR	Christmas Purple	LR	GBR
HRIGRU7432	BR9	*B. ol.* var. *italica*	BR	De Cicco	LR	JPN
HRIGRU8624	BR10	*B. ol.* var. *italica*	BR	Early Autumn	LR	GBR
HRIGRU10769	BR11	*B. ol.* var. *italica*	BR	Broccoletti Neri	LR	Di3A
HRIGRU3521	BR12	*B. ol.* var. *italica*	BR	Purple Early	LR	GBR
HRIGRU3558	BR13	*B. ol.* var. *italica*	BR	White Late	LR	GBR
HRIGRU3570	BR14	*B. ol.* var. *italica*	BR	White Sprouting	LR	GBR
HRIGRU9963	BR15	*B. ol.* var. *italica*	BR	Broculi Tardio	LR	ESP
HRIGRU8668	BR16	*B. ol.* var. *italica*	BR	-	LR	USA
HRIGRU8665	BR17	*B. ol.* var. *italica*	BR	Sperlings Sparko	LR	DEU
HRIGRU4710	BR18	*B. ol.* var. *italica*	BR	Ramoso Calabrese	LR	ITA
HRIGRU5256	BR19	*B. ol.* var. *italica*	BR	Precoce Violetto	LR	ITA
HRIGRU12799	BR20	*B. ol.* var. *italica*	BR	Royal Purple	LR	GBR
UNICT660	BR21	*B. ol.* var. *italica*	BR	Muzzatura	LR	ITA
UNICT2711	BR22	*B. ol.* var. *italica*	BR	Spigariello	LR	ITA
UNICT613	BR23	*B. ol.* var. *italica*	BR	-	LR	ITA
UNICT4286	BR24	*B. ol.* var. *italica*	BR	Sparaceddi	LR	ITA
HRIGRU8656	BR25	*B. ol.* var. *italica*	BR	Rapine	LR	USA
UNICT4322	BR26	*B. ol.* var. *italica*	BR	'Marathon'	LR	ITA
CRI2400004	BR27	*B. ol.* var. *italica*	BR	Limba	AL	CZE
HRIGRU6702	BR28	*B. ol.* var. *italica*	BR	Charteuse	LR	CAN
HRIGRU6703	BR29	*B. ol.* var. *italica*	BR	Early Purple Head	LR	CAN
HRIGRU4704	BR30	*B. ol.* var. *italica*	BR	Ramoso Calabrese	AL	ITA
HRIGRU5416	BR31	*B. ol.* var. *italica*	BR	Cavolina Rizza	LR	ITA
CRI09H2400006	BR32	*B. ol.* var. *italica*	BR	Miranda	LR	CZE
CRI09H2400005	BR33	*B. ol.* var. *italica*	BR	Leonorwaya	LR	CZE
HRIGRU2405	BR34	*B. ol.* var. *italica*	BR	Roccolo Apriloto	LR	ITA
HRIGRU8680	BR35	*B. ol.* var. *italica*	BR	Couve Roxo	LR	PRT
HRIGRU4716	BR36	*B. ol.* var. *italica*	BR		LR	ITA
CRI09H2400001	BR37	*B. ol.* var. *italica*	BR	Vitamina	AL	CZE
UNICT4796	BS1	*B. drepanensis*	CWR	-	CWR	ITA
UNICT3406	BS2	*B. rupestris*	CWR	-	CWR	ITA
UNICT3513	BS3	*B. incana*	CWR	-	CWR	ITA
UNICT4803	BS4	*B. incana*	CWR	-	CWR	ITA
UNICT3944	BS5	*B. villosa*	CWR	-	CWR	ITA
UNICT 5035	BS6	*B. villosa*	CWR	-	CWR	ITA
CRI1800004	CC1	*B. ol.* var. *capitata*	CC	-	AL	ITA
CRI1800287	CC2	*B. ol.* var. *capitata*	CC	Vysocke (Frydstejn)	LR	CZE
CRI1800283	CC3	*B. ol.* var. *capitata*	CC	-	LR	ITA
CRI1800276	CC4	*B. ol.* var. *capitata*	CC	-	LR	ITA
CRI1800277	CC5	*B. ol.* var. *capitata*	CC	-	LR	ITA
CRI1800274	CC6	*B. ol.* var. *capitata*	CC	-	LR	ITA
HRIGRU12966	CC7	*B. ol.* var. *capitata*	CC	Kvislar	LR	NOR
HRIGRU12965	CC8	*B. ol.* var. *capitata*	CC	Blatopp Kvithamar	LR	NOR
HRIGRU6215	CC9	*B. ol.* var. *capitata*	CC	Cappuccio Rosso	LR	ITA
CRI1800144	CC10	*B. ol.* var. *capitata*	CC	-	LR	SVK
HRIGRU7835	CC11	*B. ol.* var. *capitata*	CC	Lumbarda Morada	LR	ESP
CRI180011	CC12	*B. ol.* var. *capitata*	CC	-	AL	ITA
CRI1800005	CC13	*B. ol.* var. *capitata*	CC	-	LR	ITA
CRI1800015	CC14	*B. ol.* var. *capitata*	CC	-	LR	SVK
CRI1800147	CC15	*B. ol.* var. *capitata*	CC	Breza	LR	SVK
CRI1800268	CC16	*B. ol.* var. *capitata*	CC	-	LR	ITA
HRIGRU6690	CC17	*B. ol.* var. *capitata*	CC	Cape Splits	LR	ZAF
HRIGRU4505	CC18	*B. ol.* var. *capitata*	CC	Flat Dutch	LR	IRL
HRIGRU4886	CC19	*B. ol.* var. *capitata*	CC	Miscuglio	LR	ITA
HRIGRU7798	CC20	*B. ol.* var. *capitata*	CC	-	LR	NLD
HRIGRU5567	CC21	*B. ol.* var. *capitata*	CC	Bewaar Lares Rs	LR	NLD

HRIGRU2700	CC22	*B. ol.* var. *capitata*	CC	Offenham Sel B G 283	LR	FRA
UNICT4636	CC23	*B. ol.* var. *capitata*	CC	Cavolo Cappuccio	LR	ITA
UNICT4408	CC24	*B. ol.* var. *capitata*	CC	Cavolo Cappuccio	LR	ROU
HRIGRU3681	CC25	*B. ol.* var. *capitata*	CC	April	LR	GBR
HRIGRU1975	CC26	*B. ol.* var. *capitata*	CC	Tipburn Resistant	LR	USA
HRIGRU4320	CC27	*B. ol.* var. *capitata*	CC	Kantorjanosi	LR	HUN
HRIGRU12479	CC28	*B. ol.* var. *capitata*	CC	Yalova	LR	TUR
HRIGRU7826	CC29	*B. ol.* var. *capitata*	CC	Ljubljanskim Belo	LR	SRB
HRIGRU12994	CC30	*B. ol.* var. *capitata*	CC	Rodynda G S	LR	DEU
HRIGRU8431	CC31	*B. ol.* var. *capitata*	CC	King Sel Cergy	LR	FRA
CRI1800269	CC32	*B. ol.* var. *capitata*	CC	-	LR	ITA
HRIGRU2648	CC33	*B. ol.* var. *capitata*	CC	Ostara	LR	PRT
HRIGRU6178	CC34	*B. ol.* var. *capitata*	CC	Large Flat Head	LR	CHN
UNICT4633	CC35	*B. ol.* var. *sabauda*	CC	Cavolo Verza	LR	ITA
HRIGRU10591	CC36	*B. ol.* var. *capitata*	CC	Spring Cross	LR	JPN
UNICT4854	CC37	*B. ol.* var. *sabauda*	CC	Cavolo Verza	LR	Di3A
HRIGRU2607	CC38	*B. ol.* var. *capitata*	CC	Finesse	LR	DNK
CRI09H1800263	CC39	*B. ol.* var. *capitata*	CC	Pluto	LR	CZE
CRI09H1800009	CC40	*B. ol.* var. *capitata*	CC	Pozdní	LR	CZE
HRIGRU5703	CC41	*B. ol.* var. *capitata*	CC	Dieners Fruhrotkohl	LR	DEU
HRIGRU9977	CC42	*B. ol.* var. *capitata*	CC	Rizada	LR	ESP
HRIGRU5696	CC43	*B. ol.* var. *capitata*	CC	-	LR	TUR
CRI09H1800151	CC44	*B. ol.* var. *capitata*	CC	Parnica	LR	SVK
CRI09H1800152	CC45	*B. ol.* var. *capitata*	CC	Kralovany	LR	SVK
CRI09H1800013	CC46	*B. ol.* var. *capitata*	CC	Polar	AL	CZE
CRI09H1800333	CC47	*B. ol.* var. *capitata*	CC	Inter	AL	CZE
CRI09H1800003	CC48	*B. ol.* var. *capitata*	CC	Zora	LR	CZE
CRI09H1800007	CC49	*B. ol.* var. *capitata*	CC	Mars	LR	CZE
CRI09H1800332	CC50	*B. ol.* var. *capitata*	CC	-	LR	CZE
CRI09H1800319	CC51	*B. ol.* var. *capitata*	CC	Jenisovice	LR	CZE
CRI09H1800146	CC52	*B. ol.* var. *capitata*	CC	Zakamenne	LR	SVK
CRI09H1800142	CC53	*B. ol.* var. *capitata*	CC	-	LR	SVK
CRI09H1800148	CC54	*B. ol.* var. *capitata*	CC	Babin	LR	SVK
HRIGRU6562	CC55	*B. ol.* var. *capitata*	CC	White Cabbage	LR	EGY
HRIGRU5697	CC56	*B. ol.* var. *capitata*	CC	Red Cabbage	LR	TUR
CRI09H1800008	CC57	*B. ol.* var. *capitata*	CC	Polopozdní	LR	CZE
CRI09H1800083	CC58	*B. ol.* var. *capitata*	CC	Krimicke	LR	CZE
CRI09H1800149	CC59	*B. ol.* var. *capitata*	CC	Velicna	LR	SVK
CRI09H180010	CC60	*B. ol.* var. *capitata*	CC	Pourova Červené	AL	CZE
HRIGRU6226	CK1	*B. ol.* var. *alboglabra*	CK	Giant Jersey Kale	LR	GBR
HRIGRU006812	CK2	*B. ol.* var. *alboglabra*	CK	Curly Kale	LR	GBR
HRIGRU4302	CK3	*B. ol.* var. *alboglabra*	CK	Covo	LR	ZWE
HRIGRU7546	CK4	*B. ol.* var. *alboglabra*	CK	Stabil	LR	DEU
HRIGRU9932	CK5	*B. ol.* var. *alboglabra*	CK	Berzacas	LR	ESP
HRIGRU5140	CK6	*B. ol.* var. *alboglabra*	CK	Canson	LR	GBR
HRIGRU7544	CK7	*B. ol.* var. *alboglabra*	CK	Late	LR	CHN
HRIGRU6421	CK8	*B. ol.* var. *alboglabra*	CK	Surmoel	LR	USA
HRIGRU7547	CK9	*B. ol.* var. *alboglabra*	CK	Furchenkohl	LR	DEU
HRIGRU12992	CK10	*B. ol.* var. *alboglabra*	CK	Vitessa	LR	DEU
HRIGRU3592	CK11	*B. ol.* var. *alboglabra*	CK	Dwarf Curled	LR	GBR
HRIGRU9428	CK12	*B. ol.* var. *alboglabra*	CK	Couve Galega	LR	PRT
HRIGRU5108	CK13	*B. ol.* var. *alboglabra*	CK	CHINESE Kale	LR	CHN
HRIGRU5693	CK14	*B. ol.* var. *alboglabra*	CK	Curly Kale	LR	GBR
HRIGRU5688	CK15	*B. ol.* var. *alboglabra*	CK	Scotch Kale 1008	LR	GBR
HRIGRU3316	CK16	*B. ol.* var. *alboglabra*	CK	-	LR	GBR
HRIGRU4887	CK17	*B. ol.* var. *alboglabra*	CK	Black Kale	LR	ITA
HRIGRU3597	CK18	*B. ol.* var. *alboglabra*	CK	Westland Autumn	LR	GBR
UNICT4447	CR1	*B. ol.* var. *gongylodes*	CR	Bianco	LR	ITA
HRIGRU5389	CR2	*B. ol.* var. *gongylodes*	CR	Cavolo	LR	NLD

HRIGRU2388	CR3	*B. ol.* var. *gongylodes*	CR	Gigante Violetto	LR	ITA
CRI2200005	CR4	*B. ol.* var. *gongylodes*	CR	Moravia	AL	CZE
CRI2200009	CR5	*B. ol.* var. *gongylodes*	CR	Kozmanova Modra	AL	CZE
CRI2200002	CR6	*B. ol.* var. *gongylodes*	CR	Blankyt	AL	CZE
CRI2200023	CR7	*B. ol.* var. *gongylodes*	CR	Luna	AL	CZE
HRIGRU5428	CR8	*B. ol.* var. *gongylodes*	CR	September	LR	ITA
HRIGRU5427	CR9	*B. ol.* var. *gongylodes*	CR	August	LR	ITA
CRI2200001	CR10	*B. ol.* var. *gongylodes*	CR	Azur	AL	CZE
HRIGRU5612	CR11	*B. ol.* var. *gongylodes*	CR	Pollux Rs	LR	NLD
UNICT2945	CR12	*B. ol.* var. *gongylodes*	CR	-	LR	ITA
HRIGRU2483	CR13	*B. ol.* var. *gongylodes*	CR	Goliat Local	LR	SRB
HRIGRU5265	CR14	*B. ol.* var. *gongylodes*	CR	Rosso	LR	ITA
HRIGRU8723	CR15	*B. ol.* var. *gongylodes*	CR	Goliath	LR	NZL
HRIGRU8620	CR16	*B. ol.* var. *gongylodes*	CR	King Of Market	LR	IND
HRIGRU8916	CR17	*B. ol.* var. *gongylodes*	CR	Purple Top	LR	GBR
HRIGRU006797	CV1	*B. ol.* var. *botrytis*	CV	Sofia	LR	ESP
HRIGRU2840	CV2	*B. ol.* var. *botrytis*	CV	April Prince	LR	GBR
HRIGRU6253	CV3	*B. ol.* var. *botrytis*	CV	Emu	LR	AUS
CRI2300023	CV4	*B. ol.* var. *botrytis*	CV	Octavian	AL	CZE
CRI2300022	CV5	*B. ol.* var. *botrytis*	CV	Gameta	AL	CZE
CRI2300021	CV6	*B. ol.* var. *botrytis*	CV	Fontana	AL	CZE
CRI2300020	CV7	*B. ol.* var. *botrytis*	CV	Beta	AL	CZE
UNICT4449	CV8	*B. ol.* var. *botrytis*	CV	Cavolfiore	LR	ITA
HRIGRU10572	CV9	*B. ol.* var. *botrytis*	CV	Strong	LR	DNK
UNICT4855	CV10	*B. ol.* var. *botrytis*	CV	Cavolfiore	LR	ITA
UNICT3879	CV11	*B. ol.* var. *botrytis*	CV	Cavolfiore	LR	ITA
HRIGRU2838	CV12	*B. ol.* var. *botrytis*	CV	Algromajo	LR	NLD
HRIGRU4812	CV13	*B. ol.* var. *botrytis*	CV	Febbrarese Napoletano	LR	ITA
HRIGRU11728	CV14	*B. ol.* var. *botrytis*	CV	Tardivo Di Verona	LR	ITA
HRIGRU4225	CV15	*B. ol.* var. *botrytis*	CV	Primus	LR	DEU
HRIGRU4237	CV16	*B. ol.* var. *botrytis*	CV	Yapraklar Kapali	LR	TUR
UNICT3605	CV17	*B. ol.* var. *botrytis*	CV	Violetto Di Sicilia	LR	ITA
UNICT909	CV18	*B. ol.* var. *botrytis*	CV	-	LR	ITA
HRIGRU9383	CV19	*B. ol.* var. *botrytis*	CV	Mishmar Ha'emek 314	LR	ISR
HRIGRU7521	CV20	*B. ol.* var. *botrytis*	CV	Zimniaja	LR	RUS
HRIGRU7367	CV21	*B. ol.* var. *botrytis*	CV	Beladi	LR	SYR
HRIGRU8263	CV22	*B. ol.* var. *botrytis*	CV	Hagar	LR	ISR
HRIGRU7522	CV23	*B. ol.* var. *botrytis*	CV	Vesenniaja 20	LR	RUS
HRIGRU2894	CV24	*B. ol.* var. *botrytis*	CV	Summer Wonder	LR	NLD
UNICT4451	CV25	*B. ol.* var. *botrytis*	CV	Cavolfiore	LR	ITA
UNICT3121	CV26	*B. ol.* var. *botrytis*	CV		LR	ITA

Table 2. List of the bio-morphometric descriptors used for the trial.

Code	**Descriptor**
IA	Inflorescence appearance (d)
PBR	Plant branches (n)
PLS	Plant leaf shape (1-7)
PGH	Plant growth habit (1-9)
PLN	Plant leaves number (n)
PSL	Plant stem length (cm)
LHR	Leaf hairiness (0-7)
LA	Leaf area (cm^2)
LL	Leaf length (cm)
LW	Leaf width (cm)
LD	Leaf division (incision)
LWN	Leaf wings (0-1)

LPL	Leaf petiole length (cm)
LPW	Leaf petiole width (cm)
MSA	Main secondary root angle (°)
BRD	Basal root diameter (mm)
MRD	Main root diameter (mm)
MRL	Main root length (cm)
LRD	Lateral root diameter (mm)
RA	Roots area (cm^2)
RFW	Roots Fresh weight (g)
RDM	Roots dry matter (g)

GBS libraries were constructed reducing genome complexity with restriction enzymes (REs), as described by (Elshire *et al*., 2011). To build a new map, the population studied were aligned with the reference genome TO1000DH3, available on NCBI database. For the sequencing, was used NovaSeq6000 (Illumina, San Diego, USA), digesting the DNA fragments with the *ApekI*. Each read was about 200-700 bp. VCF data were filtered for removing the P5 and P7 adapters and for reads quality. The bio-morphometric data from the core collection analyzed were used for the linkage association and for the QTL mapping. A new linkage map was estimated using JoinMap v5 (Van Ooijen, 2006). The new map has 2486 loci with an average inter-marker distance of 0.76cM and is 1890.932cM long.

EXPERIMENTAL TRIAL B

Plant materials includes 34 accession of *Solanum lycopersicum* belonging to gene bank of the Department of Agriculture, Food and Environment (Di3A) of the University of Catania (UNICT), as shown in Table 3.

Table 3. List of the genotypes tested for the trial with the origin or the common name.

Accession	**Crop code**	**Working code**	**Origin**
	A-RC	1	Reggio Calabria
	LINEA 17	2	Borghese Sluis
UNICT2009	LINEA ITALIANA COR B	3	Corbarese
UNICT1912	PO163	4	California
UNICT1913	PO164	5	California
UNICT1915	PO166	6	California
UNICT1960	PO211	7	S. Stefano di Camastra.-Azzolina
UNICT1975	PO226	8	Trapani
UNICT3301	PO264	9	T-47
UNICT3371	PO269	10	P4 - COIS94
UNICT1754	PO5	11	La rosa
UNICT2028	PS LA ROSA 12	12	La rosa
UNICT2021	PS05	13	Melfi
UNICT2017	PS1/18	14	Italsementi
UNICT2028	PS12	15	Francavilla
UNICT2029	PS13	16	Ponderosa
UNICT2031	PS15/8	17	Milazzo 1
UNICT2036	PS20	18	Montechiaro
UNICT2040	PS24	19	Zorzi
UNICT2042	PS26	20	Vibo Valentia
UNICT2043	PS27	21	Palmi
UNICT2044	PS28	22	S. Stefano in Aspromonte
UNICT2051	PS35	23	Piccolo rosso a punta
UNICT2020	PS4/20	24	Basico'

UNICT2060	PS44	25	La Rosa
UNICT2063	PS47	26	Trapani
UNICT2067	PS51	27	F2 Q53*EE12M Irene - Dr. Acciardi
UNICT2069	PS53	28	F2 20*126 Irene - Dr. Acciardi
UNICT2075	PS59	29	Enna - Prof. Noto
UNICT2022	PS6	30	S. Giorgio – Calabria
UNICT2023	PS7	31	Rizziconi – Calabria
UNICT2023	PS7/10	32	Rizziconi – Calabria
UNICT2024	PS8	33	Pizzoni – Calabria
	SAL	34	Lipari
BT05000	TDP	35	Tomate de Penjar, Valencia
BT04140	RDA	36	Rosada de Ademuz
BT05010	CT	37	Tomate comercial
ALD1	TVA	38	Tomata Valenciana

Plants were sown in March 2022 in cellular trays, and they grow until the phenological phase of four true leaves inside growth chambers at the Universitat Politècnica of València (UPV), controlling light intensity, temperature, and humidity. Plants were transplanted in April 2022 in open field, in an organic farming in Valencia (ES) with the experimental design of three randomized blocks (Figure). Plants were grown at single stem by the pruning of the lateral shoots.

Figure 1. *S. lycopersicum* collection grown in Valencian field under organic conditions. The specif kind of cultivation is called “baraca” and it is represented by the plants which are supported by canes arranged in a triangular pattern.

During the growing cycles plants were characterized by the qualitative and quantitative descriptors related to the plants, leaves, inflorescence, and fruits, analysing the fruits setting, the ripening earliness and uniformity (Table 4).

Table 4. List of the descriptors adopted for the trial with the International code of the IBPGR.

Organ/Plant	Descriptor ECPGR	Code	Descriptor
Plant	7.1.2.1	**PGH**	Plant grow habit
Plant	7.1.2.3	**PFD**	Plant foliage density
Plant	7.1.2.4	**PSP**	Plant stem Pubescence Density
Plant	7.1.2.5	**PSL**	Plant stem Internode length
Plant		**PV**	Plant vigour
Plant		**PFP**	Plant fruits production (g)
Root		**RRB**	Radicular angle (°)
Root		**RDMR**	Diameter of main root at the union with the plant stem (cm)
Root		**RDF**	Density of fine roots (diameter < 0,05 mm)
Leaf	7.1.2.9	**LLT**	Leaf Type
Leaf	7.1.2.8	**LLA**	Leaf Attitude
Leaf		**LSC**	Leaf Shape of central lobe
Leaf	7.1.2.11	**LAC**	Leaf Anthocyanin colouration of leaf veins
Leaf		**LSI**	Leaf SPAD index (0 - 99,9)
Leaf		**LCl**	Leaf Chlorophyll (µg cm^{-2})
Leaf		**LFl**	Leaf Flavanols
Leaf		**LAn**	Leaf Anthocyanins
Leaf		**LNBI**	Leaf Nitrogen balance index
Leaf	9	**LPL**	Presence and incidence of pests in foliage
Leaf	9	**LDL**	Presence and incidence of disease in foliage
Inflorescence	7.2.1.1	**IT**	Inflorescence type
Inflorescence		**IL**	Leafy inflorescence
Flower	7.2.1.2	**FlCC**	Corolla colour
Flower	7.2.1.7	**FlSP**	Flower Style position
Flower	7.2.1.8	**FlSS**	Style shape
Flower	7.2.1.9	**FlSH**	Style hairiness
Flower	7.2.1.5	**FlPL**	Petal length (mm)
Flower	7.2.1.6	**FlSL**	Sepal length (mm)
Flower	7.2.1.10	**FlSTL**	Stamen length (mm)
Fruit	7.2.2.1	**FEIC**	Exterior Colour of Immature Fruits
Fruit	7.2.2.3	**FGC**	Fruit Green shoulder
Fruit	7.2.2.4	**FP**	Fruit Pubescence
Fruit	7.2.2.11	**FEC**	Fruit External Colour
Fruit	7.2.2.5	**FPS**	Fruit predominant shape
Fruit		**FSS**	Fruit set sequence
Fruit	7.2.2.34	**FBS**	Fruit Blossom End Scar Condition
Fruit	7.2.2.14	**FRC**	Fruit Ribbing at Calyx End
Fruit	7.2.2.9	**FLD**	Fruit Longitudinal Diameter (cm)
Fruit	7.2.2.10	**FTD**	Fruit Transversal Diameter (cm)
Fruit	7.2.2.31	**FNL**	Fruit Number of Locules (n)
Fruit	8.2.9	**FPA**	Fruit Puffiness Appearance
Fruit	8.2.3	**FPRC**	Fruit Presence and Incidence of Radial Cracking
Fruit	8.2.4	**FPCC**	Fruit Presence and Incidence of Concentric Cracking
Fruit	8.2.5	**FF**	Fruit Fasciation
Fruit		**FBE**	Fruit Blossom-end Rot
Fruit	9	**FPFR**	Presence and incidence of pests in fruits
Fruit	9	**FDF**	Presence and incidence of disease in fruits
Fruit	8.1.2	**RE**	Day for detecting the first ripe fruit for the 50% of the plants
Fruit	8.1.3	**RU1**	Day for detecting the first ripe fruit for one plant
Fruit	8.1.3	**RU2**	Day for detecting the first ripe fruit for all the plants
Fruit		**FFS**	Number of fruits set (n)
Fruit	8.1.6	**FT**	Fruit number per truss (n)
Fruit		**FTW**	Fruit per Truss weight (g)
Fruit	7.2.2.8	**FW**	Fruit weight (g)
Fruit		**FUL***	Fruit Colour Parameter L* (CIE L*a*b*)
Fruit		**FUa***	Fruit Colour Parameter a* (CIE L*a*b*)
Fruit		**FUb***	Fruit Colour Parameter b* (CIE L*a*b*)
Fruit		**FD**	Durometer (shore)
Fruit	8.3.1	**FSSC**	Soluble Solid Content (°Brix)
Fruit	8.3.2	**FPh**	Ph
Fruit		**FAC**	Acidity (g)

Leaves were analysed for their chlorophyll index by the SPAD 502 (Minolta, Japan), in addition to their anthocyanin and flavanols content and their nitrogen balance index (NBI) by the DUALEX™ (Force A, France). Fruits were harvested at the commercial stage and were characterized for their morphometric traits and for their soluble solid content and for acidity. All the descriptors are listed in Table 4. Genotypes were characterized following the International Board of Plant Genetic Resources (IBPGR) descriptors for *Solanum lycopersicum*.

Fruit quality analysis was carried out at the Universitat Politècnica of València following the IBPGR descriptors. The analysed traits include the fruit chromatic parameters CIE L*a*b* using the colorimeter. The soluble solid content was analysed by the refractometer Chroma meter CR-400 (Minolta, Japan). With regards to the fruit acidity, it was calculated using the TitraLab AT1000 series (Hach, Italia), registering the pH and the titratable acidity.

We applied two different protocol of DNA extraction, basing on the two different analysis that were performed, which were the molecular markers genotyping and the SPET analysis. For the molecular markers assay, was used the CTAB method adopting a modification of the Doyle and Doyle (1991) DNA extraction protocol. Conversely, for the SPET analysis, the genomic DNA was extracted using the SILEX extraction protocol, developed by Vilanova *et al*. (2020). DNA yield and quantity were quantified by the spectrophotometer NanoDrop™ 1000 ND-1000 (Thermo Scientific, Waltham, MA, USA). Additionally, the absorbance ratio 260/280 and 260/230 ratios were measured to determine protein and polysaccharide contamination, respectively. After the quantification, to check its suitability and integrity, DNA was also charged into agarose gel at 1%.

For SPET analysis, the tomato SNP data were obtained from the SOL Genomics portal and specifically from the "150 Tomato Genome Resequencing Project" (Aflitos *et al*., 2014) and the "AGIS Tomato 360 Resequencing Project" (Lin *et al*., 2014). Illumina platform

The VCF data was filtered to include only simple biallelic SNPs, as described in the work of Barchi *et al*. (2019). The ITAG SL2.50 genome build was used as the reference throughout the analysis, along with the respective gene models. SNP selection was based on the following criteria: (i) positions with alternative cohort-wise allele count greater than 8 (summing two from homozygous loci or one from heterozygous *loci*) were included, (ii) SNPs within introns and 5' UTRs were selected only if they were at least 15 kbp apart from each other or with SNPs in CDS, (iii) SNPs within CDS were selected only if they were at least 5 kbp apart from other selected SNPs, and (iv) SNPs had to be located on anchored chromosomes.

All the morphometric data were elaborated using the software IBM SPSS version (version 27, IBM, USA) performing the Pearson's correlation among the evaluated traits, and the principal component analysis (PCA). Genotyping data was analysed with Tassel 5 software using different filters for reducing the number of SNPs from 44315, selecting the 460 most informative and robust ones. Subsequently the filtered genotyping data, were elaborated using R studio software.

2.3.3. Results and Discussion

EXPERIMENTAL TRIAL A

Pattern and extent of variation among the genotypes was different for various phenotypic characters. The largest variability among all the set of accession, was observed for the inflorescence appearance, in addition also for root area, leaves area and plant height. The low variation was detected in leaf wings, brunches number and leaf hairiness. In fact, the descriptive statistics revealed considerable level of variability for several agro-morphological traits among these different selected accessions. The diversity highlighting indicates that the studied cultivars are marked by a highly agro-morphological heterogeneity.

The inflorescence appearance (IA) showed high variability among the tested broccoli accessions, varying from 61.3 days for BR20, to 273.6 days for BR27. For the genotypes BR27, BR1, BR2, BR24, BR8, listed in decrescent order, it was registered IA higher than 100.0 days (Table 5).

Table 5. Variation of the epigeal traits analysed for broccoli accessions.

Code	IA	PBR	PGH	PSL	PLS	PLN	LHR	LA	LL	LW	LD	LWN	LPL	LPW
BR1	125.3	1.5	8	23.7	3	25.8	0	2172.1	53.2	40.9	3	0	12.9	2.9
BR2	110.8	2.6	8	67.6	5	71.1	0	1907.6	65.4	29.2	3	0	15.1	2.1
BR3	74.2	2.4	8	22.5	0	22.5	0	977.8	43.0	22.8	3	0	18.3	1.6
BR4	81.8	6.5	8	107.6	5	92.3	0	933.2	39.5	23.7	3	0	13.5	1.2
BR5	83.6	1.3	7	27.4	4	28.8	0	1109.9	38.3	29.3	3	0	18.9	1.4
BR6	99.0	1.8	7	25.7	4	29.4	0	2711.8	64.5	42.3	3	0	11.4	2.5
BR7	85.7	1.3	7	31.6	4	34.5	0	2285.5	44.6	51.3	3	0	15.4	2.4
BR8	100.6	3.4	8	77.4	5	68.7	0	2011.2	60.1	33.5	3	0	12.1	1.9
BR9	69.3	3.7	8	15.7	5	17.7	0	1224.8	34.5	35.5	3	0	10.3	1.5
BR10	79.6	3.5	8	40.6	5	44.4	0	2272.5	45.3	50.5	3	0	22.5	2.1
BR11	87.7	6.5	8	77.9	5	72.3	0	3510.5	59.3	59.5	3	0	29.3	1.3
BR12	95.3	3.5	8	102.4	5	88.9	0	967.3	26.5	36.5	3	0	24.1	2.4
BR13	84.2	4.5	8	105.3	5	93.2	0	1040.3	32.5	32.4	3	0	20.9	1.5
BR14	70.8	3.5	8	93.7	5	85.1	0	1968.1	41.3	48.2	3	0	23.6	1.2
BR15	74.3	3.2	8	85.2	5	79.3	0	3539.3	60.5	58.5	3	0	16.9	1.0
BR16	76.7	2.9	8	90.3	5	81.2	0	675.3	22.5	30.4	3	0	10.9	1.7
BR17	74.4	2.7	8	44.5	5	47.7	0	2446.3	51.5	47.5	3	0	17.8	1.6
BR18	79.3	0.0	7	35.2	5	38.4	0	2205.3	31.2	73.5	3	0	23.8	0.8
BR19	76.4	3.7	8	32.6	5	34.8	0	3288.6	37.8	87.6	3	0	22.1	1.8
BR20	61.3	2.7	8	31.9	5	32.7	0	1898.4	36.5	52.3	3	0	22.1	0.9
BR21	89.5	3.5	8	61.7	5	64.8	0	924.5	21.5	43.8	3	0	21.2	1.7
BR22	79.5	3.9	8	103.5	5	91.4	0	2400.2	32.5	75.3	2	0	17.3	1.5
BR23	69.4	6.2	8	10.4	5	30.6	0	2673.1	33.8	81.2	3	0	15.2	1.3
BR24	110.6	6.6	8	75.9	5	75.9	0	1278.4	34.6	37.6	2	0	17.3	1.1
BR25	83.8	3.5	8	36.3	5	36.9	0	2358.5	44.5	52.6	3	0	18.5	1.5
BR26	77.7	0.0	7	29.5	5	32.7	0	3141.5	51.5	61.7	3	0	12.5	1.7
BR27	273.6	3.5	8	38.6	4	41.4	0	2386.5	43.8	55.5	3	0	13.6	1.9
BR28	84.9	3.7	8	25.3	5	28.8	0	2184.1	42.1	52.3	3	0	24.6	0.8
BR29	79.8	3.5	8	44.7	5	46.8	0	1268.3	28.5	44.5	3	0	15.2	1.6
BR30	69.3	0.0	7	45.6	5	45.9	0	507.1	19.5	26.7	3	0	16.3	0.9
BR31	89.3	4.5	8	53.7	1	54.6	0	816.8	36.3	22.5	3	0	12.4	1.3
BR32	95.6	3.6	8	46.8	4	48.9	0	2363.8	44.6	53.6	3	0	10.2	1.4
BR33	89.6	3.7	8	39.5	3	41.1	0	1024.3	16.9	64.7	3	0	15.6	2.6
BR34	89.9	3.7	8	90.3	5	84.8	0	704.1	22.3	32.6	3	0	19.4	2.5
BR35	89.6	3.4	8	80.2	5	80.1	0	2466.3	45.3	54.8	3	0	12.5	1.7
BR36	89.5	3.2	8	71.8	5	71.4	0	3300.2	52.8	62.5	3	0	10.4	2.6
BR37	89.6	3.3	8	50.3	4	51.9	0	2620.4	40.6	65.5	3	0	19.2	2.6

The genotypes BR6, BR32, BR12, BR34, BR33, BR35, BR37, BR21, BR36, BR31, BR11, BR7, BR28, BR13, BR25, BR5 and BR4 which are listed in decrescent order, showed an intermediate IA parameter, and it ranged of 100.0 days and 80.0 days. The genotypes BR29, BR10, BR22, BR18, BR26, BR16, BR19, BR17, BR15, BR3, BR14, BR23, BR9, BR30 and BR20, represented the earliest ones showing IA values lower than 80.0 days. In broccoli, an early IA, represents a crucial parameter because it can allow the reduction of the growing cycle.

With regards to the number of plant branches (PB), it varied from 0.0 branches for the apical dominance types BR30, BR26 and BR18, to 6.6 branches for BR24. The genotypes which showed PB higher than 3.0 were BR4, BR11, BR23, BR13, BR31, BR22, BR19, BR9, BR28, BR33, BR34, BR32, BR10, BR12, BR14, BR21, BR25, BR27, BR29, BR8, BR35, BR37, BR15 and BR36, respectively (Table 5). On the other hand, in addition to the previous mentioned BR30, BR26 and BR18 which showed no branches, the genotypes BR16, BR17, BR20, BR2, BR3, BR6, BR1, BR5 and BR7, showed PB values lower than 3.0 branches, and they were reported in decrescent order, respectively. In relation to the plant growth habit (PGH), in accordance with the IBPGR descriptors for Brassica and Raphanus, only the genotypes BR6, BR5, BR7, BR18, BR26 and BR30 showed the elongate nonbranching stem terminating in enlarged floral or prefloral apex, while all the other broccoli genotypes showed the elongate branching stems terminating in enlarged floral or prefloral apices (Table 5). Concerning the plant stem length (PSL), it fluctuated from 10.4 cm to 107.6 cm, for BR23 and BR4, respectively. The genotypes BR4, BR13, BR22, BR12, BR14, BR34, BR16, BR15, BR35 showed values higher than 80.0 cm for the above-mentioned genotypes listed in decrescent order. Concerning the genotypes BR33, BR27, BR25, BR18, BR19, BR20, BR7, BR26, BR5, BR6, BR28, BR1, BR3, ¬BR9 and BR23, was registered a PSL value lower than 40.0 cm. With regards to the plant leaf shape (PLS), in accordance with the IBPGR descriptors, it was ovate for all the tested broccoli accessions except for BR37, BR32, BR27, BR7, BR5 and BR6 that showed spathulate leaf, while the BR33 and BR1 showed obovate leaf, finally BR31 showed orbicular leaf (Table 5).

For the plant leaf number (PLN), we observed high variability among the 37 examined broccoli accessions, ranging from 17.7 leaves for BR9, to 93.2 leaves for BR13. In addition to BR13, for the genotypes BR4, BR22, BR12, BR14, BR34, BR16, BR35, BR15, BR24, BR11, BR36, and BR2, listed in decrescent order, we observed PLN values higher than 70.0 leaves. Furthermore, for BR8, BR21, BR31, BR37, BR32, BR17, BR29, BR30 and BR10, the PLN varied between 70.0 to 40.0 leaves per plant, while for all the other accessions we ascertained PLN values lower than 40.0 leaves. With regards to the leaf hairiness (LHR), all the accessions showed no hairiness in the leaf surface. Regarding the leaf area (LA), it exhibited high variability among the 37 broccoli accessions, ranging from 507.1 cm2 for BR30, to 3539.3 cm2 for BR15. The accessions

BR15, BR11, BR36, BR19, BR26, BR6, BR23, BR37, BR35, BR17, BR22, BR27, BR32, BR25, BR7, BR10, BR18, BR28, BR1 and BR8, listed in decrescent order, showed LA values higher than 2000.0 cm^2. Only for the genotypes BR3, BR12, BR4, BR21, BR31, BR34, BR16, and BR30, were registered LA values lower than 1000.0 cm^2. In relation to the leaf lamina length (LL), it varied from 16.9 cm to 65.4 cm for BR33 and BR2, respectively. For the genotypes BR2, BR6, BR15, BR8, BR11, BR1, BR36, BR26, BR17, BR35, BR10, BR32, BR7, BR25, BR27, BR3, BR28, BR14 and BR37, it was registered LL parameter higher than 40.0 cm. The leaf width (LW) varied significantly among the 37 evaluated broccoli accessions, and it fluctuated from 22.5 cm for BR31, to 87.6 cm for BR19. The LW parameter showed values higher than 50.0 cm for BR19, BR23, BR22, BR18, BR37, BR33, BR36, BR26, BR11, BR15, BR27, BR35, BR32, BR25, BR28, BR20, BR7 and BR10, listed in decrescent order, respectively. With regards to the qualitative descriptor of the leaf division (LD), it was uniform for 35 broccoli accession that showed lyrate leaf while, only BR22 and

BR24 showed sinuate leaf. Concerning the leaf wings number (LWN), all the 37 evaluated broccoli accessions showed no wings in correspondence of the petiole base. The leaf petiole length (LPL), varied among the tested accession from 10.2 cm to 29.3 cm for BR32 and BR11, respectively. On the other hand, the leaf petiole width (LPW), varied from 0.8 cm for BR18 and BR28, to 2.9 cm for BR1. With regards main secondary root angle (MSA), it ranged from 13.4 ° to 61.0 ° for BR13 and BR6, respectively. MSA values higher than 40.0, in addition to BR6, were registered for BR31, BR32, BR23, BR11, BR28, BR18 and BR24, which were mentioned in decrescent order (Table 6).

Table 6. Variation of the hypogeal traits for the broccoli genotypes.

Code	MSA	BRD	MRD	MRL	LRD	RA	RFW	RDM
BR1	33.6	38.2	51.5	18.3	40.4	171.2	192.3	115.3
BR2	32.4	30.4	55.3	14.3	45.8	102.2	84.5	41.2
BR3	17.1	48.9	90.6	19.8	40.1	98.6	262.6	155.1
BR4	24.2	25.5	52.5	13.6	43.4	131.1	84.3	41.3
BR5	38.6	39.6	47.1	10.1	41.9	79.6	180.5	111.5
BR6	61.0	42.1	40.4	11.2	28.8	66.5	126.7	62.2
BR7	21.5	36.7	43.5	19.9	36.8	140.5	148.3	78.3
BR8	24.0	31.2	70.8	18.7	50.1	162.3	138.7	66.7
BR9	24.0	34.4	71.8	22.3	40.7	174.0	186.3	111.6
BR10	19.1	34.6	40.4	16.4	39.2	72.8	53.6	22.3
BR11	48.1	34.9	50.5	41.9	41.3	111.3	92.4	55.4
BR12	28.7	11.1	50.2	13.8	47.4	94.7	56.3	22.7
BR13	13.4	40.5	40.4	13.2	28.1	80.2	86.9	45.7
BR14	21.7	29.2	50.4	11.8	35.6	90.0	94.3	59.9
BR15	25.9	55.7	40.8	18.7	68.4	235.3	246.2	155.7
BR16	19.8	38.7	30.7	18.3	55.6	124.9	138.3	74.3
BR17	36.8	41.6	50.7	15.8	34.3	113.8	162.7	88.1
BR18	41.3	46.4	60.7	21.4	50.3	143.4	156.2	87.3
BR19	13.5	24.5	40.3	11.2	30.3	70.9	50.3	29.4
BR20	21.6	56.3	39.9	11.3	29.3	92.4	314.8	177.4
BR21	23.4	21.1	40.4	10.2	30.3	56.7	133.6	56.7
BR22	16.9	31.5	50.6	14.3	31.5	98.5	118.2	56.6
BR23	49.4	29.8	40.7	23.6	45.5	83.2	108.7	66.3
BR24	40.1	36.7	40.9	27.3	70.1	152.4	172.9	111.4
BR25	27.0	52.5	50.9	26.5	69.3	299.3	258.5	166.7
BR26	35.9	39.5	30.9	24.1	50.2	284.1	232.4	144.6
BR27	31.9	23.3	40.5	15.8	31.8	121.0	78.9	45.4
BR28	42.7	29.1	62.1	16.2	30.2	66.5	76.2	41.2
BR29	21.4	27.7	40.1	10.6	29.9	65.5	68.3	42.4
BR30	35.7	20.6	50.9	22.3	40.7	58.7	144.9	89.8
BR31	58.6	30.2	49.6	11.3	30.1	40.5	52.7	22.6
BR32	50.9	27.7	90.6	29.3	65.0	81.7	62.2	35.5
BR33	14.0	26.6	80.6	26.3	51.3	115.1	73.5	37.2
BR34	38.0	23.6	52.6	15.3	20.8	109.0	58.7	33.6
BR35	17.5	36.8	40.1	17.4	30.6	207.5	183.6	98.6
BR36	18.9	33.7	80.1	11.6	40.1	67.4	82.5	42.1
BR37	39.4	32.0	79.6	24.3	59.0	89.9	106.7	54.6

The basal root diameter (BRD) showed high variability among the 37 broccoli accessions, fluctuating from 11.1 mm for BR12, to 56.3 mm for BR20. Only for the accessions BR20, BR15, BR25, BR3, BR18, BR6, BR17 and BR13, were registered BRD values higher than 40.0 mm. With regards to the main root diameter (MRD), it fluctuated among the tested genotypes from 30.7 mm for BR16, to 90.6 mm for both BR32 and BR3. The genotypes BR32, BR3, BR33, BR36, BR37, BR9, BR8, BR28, BR18, BR2, BR34, BR4, BR1, BR25, BR30, BR17, BR22, BR11, BR14 and BR12 listed in decrescent order, showed MRD values higher than 50.0 mm, respectively. The main root length (MRL), varied among the 37 broccoli accessions from 10.1 cm to 41.9 cm for BR5 and BR11, respectively. MRL values higher than 20.0 cm were registered only for the genotypes BR11, BR32, BR24, BR25, BR33, BR37, BR26, BR23, BR9, BR30 and BR18, respectively (Table 6). The lateral root diameter (LRD), ranged from 20.8 mm to 70.1 mm for BR34 and BR24, respectively. For

the genotypes BR24, BR25, BR15, BR32, BR37, BR16, BR33, BR18, BR26 and BR8, it was registered LRD parameter higher than 50.0 mm, while, for all the other genotypes, it was lower than 50.0 mm. Concerning the root area (RA), it varied from 40.5 cm2 for BR31, to 299.3 cm2 for BR25. Only the genotypes BR25, BR26, BR15, BR35, BR9, BR1, BR8, BR24, BR18, BR7, BR4, BR16, BR27, BR33, BR17, BR11, BR34 and BR2, exhibited RA values higher than 100.0 cm2. For the root fresh weight (RFW), we observed high variability among the tested broccoli accession, varying from 50.3 g to 314.8 g for BR19 and BR20, respectively (Table 6). The genotypes BR20, BR3, BR25, BR15, BR26, BR1, BR9, BR35, BR5, BR24, BR17, BR18., BR7, BR30, BR8, BR16, BR21, BR6, BR22, BR23 and BR37 showed RFW values higher than 100 g, listed in decrescent order (Table 6). On the other hand, the root dry matter (RDM), varied from 22.3 g for BR10, to 177.4 g for BR20, registering values higher than 100.0 g only for BR20, BR25, BR15, BR3, BR26, BR1, BR9, BR5 and BR24, respectively.

The inflorescence appearance (IA) of the examined 60 cabbage accessions, varied from 67.3 days to 114.5 days for CC18 and CC23, respectively. The earliest accession were the ones which showed IA values lower than 75.0 days, and they were CC36, CC35, CC6, CC34, CC32, CC7, CC11, CC17, and CC18, respectively. Only the accessions CC23, CC44, CC4, CC3, CC2, CC1, CC5, CC41, CC38, CC43, CC21 and CC45, registered IA values higher than 100.0 days. The PB trait showed high uniformity among the different cabbage accessions showing 0.0 branches for all the tested accessions. With regards to the PGH, it was uniform among all the tested genotypes corresponding to the shortened nonbranching stem terminated in leafy head of the IBPGR descriptors. With regards to the PSL, it varied among the tested 60 cabbage accessions from 10.2 cm to 95.5 cm, for CC18 and CC55, respectively. The genotypes CC55, CC19, CC59, CC22, CC35, CC51 and CC3, showed PSL values higher than 50.0 cm. With regards to the genotypes CC16, CC1, CC9, CC46, CC24, CC30, CC36, CC8, CC38, CC50, CC32, CC2, CC6, CC29, CC4, CC41, CC20, CC37, CC40 and CC18, they registered PSL values lower than 20.0 cm. In relation to the qualitative trait plant leaf shape (PLS), it shows high variability among the 60 cabbage accessions analyzed. In accordance to the descriptors for Brassica and Raphanus, the genotypes CC55, CC19, CC35, CC33, CC25, CC14, CC15, CC12, CC36, CC20 and CC37, showed ovate leaf, while for CC59, CC58, CC47, CC57, CC5 and CC30 it was spathulate, for CC56, CC48, CC54, CC39, CC42, CC52, CC13, CC31, CC21, CC53, CC17, CC49, CC28, CC26, CC34, CC1, CC46, CC24, CC50, CC2 and CC29 it was obovate, for CC51, CC10, CC23, CC43, CC16 and CC38 it was elliptic, and finally for CC3, CC45, CC11, CC44, CC7, CC27, CC60, CC9, CC8, CC32, CC6, CC4, CC41, CC40 and CC18 it was orbicular. In relation to the PLN, we observed high variability for the examined cabbage accessions, and its values ranged from 33.6 for both CC41 and CC20, to 111.0 for CC51. PLN values higher than 70.0 leaves were registered for 16 cabbage accessions which were, in decrescent order, CC51, CC55, CC3, CC22, CC56, CC19, CC35, CC33, CC59, CC25, CC14, CC12, CC15, CC45, CC58 and CC48. With regards to the leaf hairiness (LHR), all the examined cabbage accessions showed no hairs in the leaf surface. In relation to the leaf area (LA), it exhibited high variability among the tested accessions, varying from 446.2 cm2 to 5329.9 cm^2 for CC49 and CC4, respectively. The genotypes CC4, CC14, CC6, CC60, CC26, CC1, CC55, CC19, CC52, CC3, CC58, CC7, CC15, CC54, CC41, CC8, CC2, CC9, CC38, CC36, CC16, CC11,

CC5, CC46, CC59, CC32, CC25, CC48, CC30, CC17, CC50, CC33 and CC34 showed LA values higher than 2000.0 cm^2 (Table 7).

Table 7. Variation of the epigeal traits for the cabbage genotypes.

Code	IA	PBR	PGH	PSL	PLS	PLN	LHR	LA	LL	LW	LD	LWN	LPL	LPW
CC1	110.3	0.0	2	19.2	3	45.6	0	3747.6	60.7	62.5	2	0	11.9	4.1
CC2	110.5	0.0	2	15.2	3	45.0	0	3015.7	64.6	46.7	3	0	20.9	2.6
CC3	110.6	0.0	2	50.4	1	107.4	0	3363.1	63.9	52.6	2	0	18.6	1.8
CC4	110.8	0.0	2	14.1	1	40.8	0	5329.9	123.6	43.1	2	0	15.7	2.1
CC5	110.2	0.0	2	25.4	4	57.6	0	2400.4	51.2	46.9	2	0	18.4	2.4
CC6	69.6	0.0	2	14.7	1	41.4	0	4768.8	68.6	70.2	3	0	11.6	3.3
CC7	69.3	0.0	2	23.6	1	51.0	0	3215.5	71.3	45.1	3	0	13.2	3.2
CC8	89.8	0.0	2	16.6	1	40.2	0	3072.8	65.8	46.7	2	0	10.4	3.6
CC9	95.3	0.0	2	18.9	1	45.0	0	3013.7	65.3	46.2	2	0	14.3	2.6
CC10	89.5	0.0	2	31.3	2	69.6	0	1002.3	33.3	30.1	2	0	9.4	2.2
CC11	69.3	0.0	2	31.5	1	67.2	0	2479.6	62.3	39.8	2	0	14.3	2.2
CC12	89.7	0.0	2	33.6	5	76.8	0	1567.2	43.2	36.3	2	0	12.1	2.1
CC13	89.8	0.0	2	25.8	3	57.0	0	1627.2	52.7	30.9	2	0	15.3	2.3
CC14	79.9	0.0	2	39.4	5	83.4	0	4789.6	89.1	53.8	1	0	10.1	3.7
CC15	79.3	0.0	2	38.3	5	76.2	0	3174.3	59.9	53.9	1	0	20.3	1.8
CC16	89.3	0.0	2	19.5	2	45.0	0	2707.5	47.5	57.3	2	0	20.2	1.9
CC17	69.2	0.0	2	24.7	3	51.6	0	2108.2	34.7	62.4	2	0	11.2	1.6
CC18	67.3	0.0	2	10.2	1	35.4	0	1769.1	29.6	61.3	2	0	19.6	1.8
CC19	79.6	0.0	2	79.3	5	93.2	0	3593.9	43.3	83.7	2	0	18.8	2.2
CC20	89.7	0.0	2	11.4	5	33.6	0	888.3	24.9	37.6	1	0	15.8	1.2
CC21	100.3	0.0	2	25.2	3	46.8	0	1974.7	43.4	45.5	2	0	16.9	1.6
CC22	91.3	0.0	2	56.6	0	94.2	0	1415.5	43.9	32.2	2	0	12.4	2.4
CC23	114.5	0.0	2	29.9	2	69.6	0	651.8	29.7	22.8	1	0	12.3	1.3
CC24	83.3	0.0	2	18.3	3	40.2	0	1608.9	44.7	36.9	1	0	10.7	2.2
CC25	80.8	0.0	2	41.4	5	86.1	0	2124.2	59.8	35.5	2	0	18.1	2.6
CC26	89.2	0.0	2	20.7	3	45.6	0	4010.1	75.8	52.9	3	0	10.8	4.2
CC27	80.5	0.0	2	21.9	1	45.0	0	1531.2	45.6	33.6	2	0	13.3	2.2
CC28	79.3	0.0	2	21.5	3	49.8	0	1151.9	40.9	28.2	2	0	19.6	2.7
CC29	82.6	0.0	2	14.3	3	39.6	0	703.3	32.3	21.8	2	0	17.3	1.4
CC30	75.6	0.0	2	17.9	4	45.0	0	2117.9	56.9	37.8	2	0	15.7	1.9
CC31	81.8	0.0	2	25.6	3	57.0	0	1698.6	51.4	33.6	2	0	16.3	2.9
CC32	69.4	0.0	2	15.3	1	45.6	0	2201.5	31.4	71.5	3	0	15.6	2.1
CC33	89.3	0.0	2	41.7	5	87.6	0	2044.5	29.3	70.5	2	0	13.7	2.3
CC34	69.6	0.0	2	20.5	3	45.0	0	2000.6	50.2	41.2	2	0	18.3	1.8
CC35	70.6	0.0	2	55.3	5	91.2	0	1342.5	30.5	44.6	2	0	11.7	2.3
CC36	73.6	0.0	2	17.8	5	40.8	0	2793.5	37.3	75.5	2	0	15.3	2.2
CC37	82.3	0.0	2	10.6	5	45.6	0	1479.7	34.6	43.5	2	0	10.6	2.7
CC38	105.5	0.0	2	15.7	2	51.6	0	3001.5	30.4	100.1	2	0	16.7	1.9
CC39	89.9	0.0	2	28.3	3	64.2	0	733.4	31.8	23.7	2	0	12.4	1.1
CC40	94.7	0.0	2	10.4	1	45.6	0	1706.3	37.5	45.5	2	0	13.8	1.9
CC41	105.6	0.0	2	11.6	1	33.6	0	3074.1	53.3	58.6	2	0	15.3	2.4
CC42	89.3	0.0	2	26.7	3	61.2	0	1803.8	32.5	55.5	1	0	19.3	2.4
CC43	100.4	0.0	2	25.8	2	57.6	0	1660.5	40.5	41.3	3	0	17.5	2.3
CC44	111.6	0.0	2	27.6	1	53.4	0	1745.7	25.3	69.3	2	0	20.4	2.2
CC45	100.3	0.0	2	35.3	1	76.2	0	1068.8	28.5	37.5	3	0	15.1	1.7
CC46	79.8	0.0	2	18.4	3	45.0	0	2322.5	43.6	54.2	3	0	12.3	0.7
CC47	77.3	0.0	2	28.9	4	69.6	0	1677.4	43.9	39.6	2	0	18.4	1.1
CC48	77.5	0.0	2	33.7	3	71.4	0	2120.3	40.8	53.7	2	0	10.6	2.4
CC49	78.6	0.0	2	24.1	3	51.6	0	446.2	16.9	26.4	2	0	11.5	2.2
CC50	78.4	0.0	2	15.3	3	38.4	0	2048.8	39.4	52.3	2	0	18.1	1.7
CC51	79.6	0.0	2	51.2	2	111.0	0	892.5	25.5	35.9	2	0	12.5	0.6
CC52	79.3	0.0	2	26.7	3	57.6	0	3413.1	79.8	42.8	2	0	16.7	2.6
CC53	77.8	0.0	2	25.2	3	58.2	0	1471.8	54.1	27.2	2	0	20.6	2.2
CC54	76.9	0.0	2	31.6	3	69.0	0	3126.5	96.1	32.5	2	0	23.3	2.1
CC55	89.3	0.0	2	95.6	5	110.8	0	3680.8	67.6	54.5	2	0	27.6	3.3
CC56	88.6	0.0	2	45.4	3	93.6	0	1672.3	53.2	31.4	2	0	20.1	2.9
CC57	89.7	0.0	2	25.6	4	55.8	0	1903.8	66.3	28.7	2	0	19.2	3.4
CC58	98.3	0.0	2	36.9	4	74.4	0	3284.9	85.4	38.5	2	0	15.3	2.7
CC59	79.4	0.0	2	60.2	4	87.4	0	2204.5	77.4	28.5	2	0	9.9	2.1
CC60	89.5	0.0	2	20.4	1	57.6	0	4320.2	74.2	58.2	2	0	13.3	1.8

With regards to the leaf lamina length (LL), it also varied significantly among the tested cabbage accessions, ranging from 16.9 cm to 126.3 cm for CC49 and CC4, respectively (Table 7). For the genotypes CC4, CC54, CC14, CC58, CC52, CC59, CC26, CC60, CC7, CC6, CC55, CC57, CC8, CC9, CC2, CC3, CC11 and CC1 were registered LL values higher than 60.0 cm. With regards to the leaf width (LW), it varied among the tested genotypes from 21.8 cm to 100.1 cm for CC29 and CC38, respectively (Table 7). LW trait was higher than 50.0 cm for CC38, CC19, CC36, CC32, CC33, CC6, CC44, CC1, CC17, CC18, CC41, CC60, CC16, CC42, CC55, CC46, CC15, CC14, CC48, CC26, CC3 and CC50, respectively. For the qualitative trait of the leaf division (LD), the genotypes CC32, CC6, CC46, CC26, CC2, CC7, CC43 and CC45 showed lyrate leaf, while CC42, CC15, CC14, CC20, CC24 and CC23 exhibited entire leaf (Table 7). All the other 46 cabbage accessions showed sinuate leaf, in accordance with the IBPGR descriptors for Brassica and Raphanus. In relation to the number of wings (LWN), all the tested cabbage accessions showed no wings at the petiole base. With regards to the leaf petiole length (LPL), it ranged from 9.4 cm for CC10, to 27.6 cm for CC55. For the genotypes CC55, CC54, CC2, CC53, CC44, CC15, CC16 and CC56, listed in the decrescent order, it was registered LPL value higher than 20.0 cm. On the other hand, the leaf petiole width (LPW), varied from 0.6 cm to 4.2 cm for CC51 and CC26, respectively. With regards to the MSA, it fluctuated among the evaluated accessions from 13.8 ° to 66.6 ° for CC34 and CC16, respectively. MSA values higher than 50.0 ° were registered for CC16, CC6, CC22, CC52, CC26, CC30, CC56, CC47, CC50, CC5 and CC7 and they were reported in decrescent order. The BRD values varied among the examined genotypes from 12.5 mm to 46.6 mm for CC56 and CC2, respectively. BRD value higher than 30.0 mm was registered for the genotypes CC2, CC13, CC58, CC36, CC6, CC22, CC23, CC19, CC11, CC10, CC32, CC16, CC1, CC18, CC21, CC17, CC4, CC5, CC52, CC44 and CC3, while all the other ones registered lower values (Table 7).

In relation of the main root diameter (MRD), we ascertained high variability among all the examined cabbage accessions, and it ranged from 22.8 mm to 92.4 mm, for CC56 and CC2, respectively. The genotypes CC2, CC11, CC58, CC10, CC27, CC39, CC19, CC4, CC52, CC33, CC3, CC36 and CC38 showed MRD values higher than 60.0 mm and they were listed in decrescent order, respectively. The main root length (MRL), varied among the tested cabbage accessions, from 7.4 cm to 28.8 cm for CC37 and CC6, respectively (Table 8). For the genotypes CC6, CC10, CC27, CC17, CC35, CC16, CC50, CC18, CC25 and CC11 were registered values higher than 20.0 cm. Concerning the lateral root diameter (LRD), the examined genotypes showed values ranging from 18.6 mm for CC16, to 70.5 mm for CC6. LRD values higher than 50.0 mm were registered for CC6, CC2, CC33 and CC45, respectively. In relation to the root area (RA), genotypes showed values between 21.7 cm2 and 191.8 cm2 for CC37 and CC2, respectively. The genotypes which showed RA values higher than 100.0 cm2 were CC2, CC4, CC10, CC11, CC8, CC27, CC6, CC40, CC50, CC3, CC20, CC36, CC42, CC52, CC21, CC31, CC14, CC16, CC18 and CC53, and they were ordered in decrescent order (Table 8). The root fresh weight (RFW), fluctuated from 21.7 g to 374.7 g for CC45 and CC2, respectively. The genotypes CC2, CC4, CC37, CC10, CC36, CC11, CC13, CC58, CC5, CC22, CC16, CC50, CC60, CC56, CC32, CC31, CC27¬ and CC38 registered RFW values higher than 100.0 g. With regards RDM, it varied from 14.3 g for CC41, to 212.3 for CC2 (Table 8). For the IA we ascertained high variability among the 26 tested

cauliflower accessions, ranging from 69.1 days for CV16, to 120.8 for CV2. In addition to CV2, only CV26 showed the IA higher than 100.0 days, while for CV25, CV1, CV6, CV5, CV8, CV24, CV9, CV14, CV15, CV12 and CV22, the IA was between 91.5 days and 80.4 days, respectively.

Table 8. Variation of the hypogeal traits for the cabbage genotype.

Code	MSA	BRD	MRD	MRL	LRD	RA	RFW	RDM
CC1	40.1	33.2	40.9	12.1	39.9	60.6	66.1	32.5
CC2	15.4	46.6	92.4	15.8	61.2	191.8	374.7	212.3
CC3	31.9	30.1	62.4	15.7	40.1	113.4	98.4	44.7
CC4	36.3	31.9	69.2	16.2	41.1	151.8	202.3	122.6
CC5	50.1	31.7	50.9	15.8	36.7	95.9	134.5	74.3
CC6	62.5	40.5	49.9	28.8	70.5	124.3	82.3	41.4
CC7	50.1	14.9	33.6	9.3	30.2	22.5	54.2	23.7
CC8	15.4	25.6	42.1	14.4	38.2	133.8	98.4	52.8
CC9	20.6	24.7	39.7	12.5	31.6	33.6	38.6	21.3
CC10	29.1	36.1	75.8	27.3	44.1	144.5	172.8	101.5
CC11	21.5	36.7	88.2	20.1	42.3	135.1	146.3	99.2
CC12	41.0	23.3	40.7	12.9	20.2	73.4	76.7	56.5
CC13	27.6	44.1	45.1	17.9	22.2	99.6	144.4	89.2
CC14	20.4	18.5	35.9	15.3	30.1	103.3	40.6	29.4
CC15	38.6	15.4	40.1	14.2	35.1	49.5	52.1	22.3
CC16	66.6	34.4	30.1	21.2	18.6	100.9	130.4	74.9
CC17	24.2	32.2	29.4	21.6	30.2	98.9	72.6	38.4
CC18	20.1	32.9	32.6	20.5	25.6	100.4	78.5	45.9
CC19	37.6	37.9	70.1	19.8	30.3	77.6	94.9	58.2
CC20	26.2	26.8	55.1	18.4	31.3	110.7	68.7	44.3
CC21	18.5	32.5	30.3	15.8	28.2	103.6	81.4	47.3
CC22	61.6	40.3	50.6	12.5	30.4	62.9	130.9	77.5
CC23	31.9	40.1	53.3	16.4	32.6	78.9	78.3	41.8
CC24	42.1	25.9	39.9	14.9	28.2	69.2	80.6	47.8
CC25	38.3	25.6	45.5	20.4	32.4	87.3	82.5	45.2
CC26	57.8	23.5	38.2	8.6	25.1	22.8	42.2	32.4
CC27	32.1	30.0	70.1	21.6	29.0	131.7	120.6	74.9
CC28	31.1	22.8	55.2	11.8	32.3	64.5	54.3	25.2
CC29	42.0	17.3	40.6	9.1	30.0	40.8	26.5	14.4
CC30	56.9	22.5	38.1	17.0	35.6	42.9	60.7	36.3
CC31	20.6	25.1	35.9	13.3	27.2	103.4	124.2	74.1
CC32	15.4	35.4	40.2	11.4	30.1	95.6	124.4	75.7
CC33	41.9	27.6	65.3	12.3	55.5	70.1	70.6	44.2
CC34	13.8	25.5	39.4	18.5	49.5	74.6	76.3	48.6
CC35	29.4	24.9	30.1	21.4	39.1	95.8	87.2	65.8
CC36	37.5	41.5	62.2	12.8	32.3	107.3	154.1	100.3
CC37	49.4	13.2	30.1	7.4	22.9	21.7	188.6	121.1
CC38	37.5	27.6	61.2	18.1	35.1	87.1	118.9	74.6
CC39	49.3	25.5	70.1	16.9	39.1	68.8	94.4	55.2
CC40	33.1	16.4	55.8	15.1	40.3	116.6	48.4	25.1
CC41	19.2	23.3	48.2	11.1	42.1	31.6	22.3	14.3
CC42	44.0	22.1	47.3	17.3	40.2	105.5	47.2	26.5
CC43	23.8	24.7	34.9	14.1	35.3	77.3	63.8	33.1
CC44	27.0	30.2	41.2	16.2	30.3	50.3	38.6	22.6
CC45	29.9	23.7	59.7	12.3	50.1	59.5	21.7	16.7
CC46	32.3	16.6	29.9	8.2	20.1	40.6	30.8	22.6
CC47	54.6	22.3	41.3	14.3	33.3	57.2	42.4	30.6
CC48	27.0	20.6	50.1	12.3	30.6	82.8	46.5	31.5
CC49	48.0	22.1	49.4	10.3	32.3	40.5	38.7	23.7
CC50	50.6	23.3	51.2	20.9	38.6	114.8	128.7	88.6
CC51	14.3	20.9	36.1	11.6	36.3	77.3	60.9	45.2
CC52	58.2	30.9	66.7	17.4	29.6	104.3	90.7	68.3
CC53	46.2	29.9	23.9	15.6	30.1	100.2	96.2	77.3
CC54	34.7	20.2	40.1	13.9	20.9	54.6	68.6	44.1
CC55	39.2	22.6	37.1	18.6	30.3	72.3	82.3	54.6
CC56	55.9	12.5	22.8	10.6	22.7	23.7	126.4	78.4
CC57	26.5	18.4	51.6	12.9	30.2	66.0	58.6	40.2
CC58	21.1	43.4	78.8	10.5	29.9	88.1	138.7	88.2
CC59	30.1	14.4	40.2	10.8	26.8	45.8	28.6	20.3
CC60	28.6	15.9	38.8	9.6	20.4	28.4	128.7	89.1

For the genotypes CV3, CV7, CV10, CV4, CV23, CV19, CV11, CV21, CV17, CV13, CV18, CV20 and CV16, listed in decrescent order, we observed the IA lower than 80.0 days. In relation to the PB, each cauliflower accession examined showed no branches linked to the main stem (Table 9). With regards to the PGH, all the 26 cauliflowers genotype showed, in accordance with the IBPGR descriptors for Brassica and Raphanus the elongate nonbranching stem terminating in enlarged floral or prefloral apex. In relation to the PSL, it varied from 12.6 cm for CV13, to 79.3 cm for CV26. In addition to CV26, only CV8 and CV22 exhibited a PSL higher than 50.0 cm. The genotypes CV11, CV16, CV18, CV2, CV20, CV15, CV7, CV24, CV5, CV3 and CV13, showed the PSL values lower than 20.0 cm, respectively. For the PLS we ascertained high variability among the different accessions, registering for CV5, CV22, CV10, CV26, CV14, CV1, CV16, CV13, CV19, CV6, CV15, CV7, CV25 and CV8 an ovate leaf, while for CV24, CV23, CV3, CV18, CV21, CV17, CV4, CV20, CV12 and CV11 we observed an elliptic leaf, and finally only for CV2 and CV9 we registered an orbicular leaf. In relation to the PLN, it fluctuated from 22.4 leaves for CV24, to 86.4 leaves for CV26 (Table 9). Regarding the LHR trait, it was uniform for all the tested cauliflower genotypes, showing no hairiness in the surface of all the leaves tested. Contrarily, for the LA, we ascertained high variability among the tested accessions, varying from 500.4 cm2 to 3314.8 cm2 for CV24 and CV26, respectively. For CV26, CV16, CV6, CV13, CV5, CV3 and CV18, we observed LA values higher than 2000 cm2, while for CV8, CV12, CV20, CV19, CV22, CV21, CV25, CV2, CV14, CV1, CV9, CV4, CV23 and CV11, the LA value ranged between 2000.0 cm2 and 1000.0 cm2, and only for CV7, CV10, CV17, CV15 and CV24, we observed LA values lower than 1000.0 cm2, respectively (Table 9). Furthermore, also the LL trait, showed high variability among the tested accessions, varying from 23.5 cm for CV7, to 67.6 cm for CV16.

Table 9. Variation of the epigeal traits for the cauliflower's genotypes tested.

Code	IA	PBR	PGH	PSL	PLS	PLN	LHR	LA	LL	LW	LD	LWN	LPL	LPW
CV1	89.6	0.0	7	30.7	5	42.0	0	1413.8	32.5	43.5	1	0	12.6	1.5
CV2	120.8	0.0	7	17.5	1	30.0	0	1454.4	52.9	50.5	1	0	0.0	1.7
CV3	79.7	0.0	7	13.4	2	34.4	0	2066.3	49.6	72.5	1	0	0.0	2.3
CV4	79.3	0.0	7	30.2	2	41.2	0	1328.3	51.0	41.5	1	0	0.0	1.9
CV5	89.5	0.0	7	13.4	5	30.4	0	2501.1	41.6	61.5	1	0	19.2	1.9
CV6	89.6	0.0	7	20.6	5	28.8	0	2835.6	35.9	81.2	1	0	12.6	1.8
CV7	79.6	0.0	7	15.3	5	24.8	0	940.7	23.5	40.3	1	0	12.8	2.8
CV8	89.3	0.0	7	56.7	5	77.2	0	1950.6	30.7	66.2	1	0	13.1	2.1
CV9	84.2	0.0	7	43.6	1	60.8	0	1379.5	48.9	44.5	1	0	0.0	0.8
CV10	79.6	0.0	7	28.2	5	41.2	0	892.5	25.5	35.3	1	1	11.8	0.7
CV11	76.8	0.0	7	19.6	2	30.0	0	1072.5	41.1	37.5	3	1	12.5	1.6
CV12	82.5	0.0	7	32.4	2	45.2	0	1939.3	61.1	38.9	1	0	0.0	2.1
CV13	74.7	0.0	7	12.6	5	30.4	0	2535.8	63.7	39.8	1	0	11.9	2.3
CV14	83.6	0.0	7	32.4	5	46.4	0	1426.3	47.9	29.8	1	1	11.8	1.5
CV15	83.3	0.0	7	15.9	5	30.4	0	581.8	34.5	21.1	1	0	15.5	1.7
CV16	69.1	0.0	7	19.3	5	31.6	0	2963.6	67.6	44.2	1	0	19.4	2.4
CV17	75.5	0.0	7	27.5	2	37.2	0	755.8	52.3	24.3	3	1	10.8	1.3
CV18	72.2	0.0	7	19.1	2	34.4	0	2018.2	59.3	43.3	3	1	12.7	2.3
CV19	77.4	0.0	7	20.7	5	29.4	0	1884.9	52.6	35.9	1	0	13.4	2.3
CV20	70.6	0.0	7	16.8	2	25.2	0	1886.8	66.8	43.9	1	0	0.0	1.7
CV21	75.8	0.0	7	24.3	2	30.0	0	1550.8	63.7	40.2	1	0	0.0	1.8
CV22	80.4	0.0	7	52.8	5	73.2	0	1668.5	49.4	35.3	1	0	18.5	1.9
CV23	79.3	0.0	7	22.9	2	34.4	0	1302.1	60.2	33.9	1	0	0.0	1.9
CV24	85.7	0.0	7	14.5	2	22.4	0	500.4	58.6	20.4	1	0	0.0	2.3
CV25	91.5	0.0	7	36.7	5	49.2	0	1535.5	51.5	37.1	3	1	15.2	2.1
CV26	100.4	0.0	7	79.3	5	86.4	0	3314.8	57.8	57.4	1	1	20.5	1.9

For the 14 cauliflower genotypes which were CV16, CV20, CV21, CV13, CV12, CV23, CV18, CV24, CV26, CV2, CV19, CV17, CV25 and CV4, we observed LL values higher than 50.0 cm, and they were mentioned in decrescent order. On the other hand, for CV6, CV15, CV1, CV8, CV10 and CV7, were registered values lower than 40.0 cm. With regards to the LW, it varied from 20.4 cm to 81.2 cm for CV24 and CV6, respectively. In addition to CV26, only for CV3, CV8 and CV5 were obtained LW values higher than 60.0 cm, respectively. For CV26, CV2, CV9, CV16, CV20, CV1, CV18, CV4, CV7 and CV21 the LW registered was between 50.0 cm and 40.0 cm while for all the other genotypes we observed values lower than 40.0 cm. In relation to the leaf division, all the 26 cauliflowers accessions exhibited uniformity registering, in accordance with the IBPGR descriptors, an entire leaf. Concerning the LWN traits, only for CV26, CV18, CV11, CV25, CV10, CV14 and CV17 genotypes, we observed leaf at the petiole bases, while all the other ones show no wings. In relation to the LPL, we ascertained high variability in the panel analyzed, and its values varied from 0.0 cm for CV24, CV23, CV3, CV21, CV4, CV20, CV12, CV2 and CV9, to 20.5 cm for CV26. For the genotypes CV16, CV5, CV22, CV15 and CV25 were registered values higher than 15.0 cm, in addition to the previously mentioned CV26. Concerning the LPW, it fluctuated among the evaluated genotypes from 0.7 cm for CV10, to 2.8 cm for CV7. For the CV7, CV16, CV19, CV13, CV18, CV3, CV24, CV25, CV8 and CV12, listed in decrescent order, we ascertained LPW values higher than 2.0 cm. With regards to the MSA, it varied in the evaluated cauliflower accessions from 11.4 ° to 70.4 ° for CV11 and CV24, respectively (Table 10).

Table 10. Variation of the hypogeal traits for the cauliflower's genotypes tested.

Code	MSA	BRD	MRD	MRL	LRD	RA	RFW	RDM
CV1	35.2	24.8	70.2	15.4	32.2	158.4	164.9	69.3
CV2	40.0	31.7	50.6	17.3	30.9	119.9	152.3	75.4
CV3	37.8	33.3	72.8	13.5	41.1	85.1	115.7	48.5
CV4	17.4	37.2	83.4	19.6	65.4	207.6	176.2	77.9
CV5	65.3	21.6	80.6	20.4	60.9	53.5	72.6	45.5
CV6	26.5	45.4	70.6	17.6	50.1	274.3	489.7	355.4
CV7	21.6	29.2	60.7	15.4	49.6	84.7	72.8	42.5
CV8	16.9	29.3	69.6	12.3	50.2	113.4	129.7	45.8
CV9	33.7	27.5	63.9	17.3	42.6	116.4	200.5	100.3
CV10	44.0	25.7	50.6	19.3	30.3	77.8	78.4	38.6
CV11	11.4	32.2	75.2	21.9	58.3	199.8	190.5	101.0
CV12	14.8	35.1	50.2	14.3	20.2	187.9	210.6	111.3
CV13	28.5	36.1	66.4	15.8	40.4	156.3	160.4	33.5
CV14	35.7	29.1	79.3	13.1	40.3	77.2	84.3	32.7
CV15	25.4	22.5	55.1	10.9	43.6	95.6	46.1	30.5
CV16	34.4	32.5	50.2	15.5	42.8	75.8	104.3	49.4
CV17	18.3	23.2	40.1	14.9	20.3	81.8	95.3	44.3
CV18	37.1	27.6	80.5	13.1	40.4	63.2	90.8	55.9
CV19	27.3	38.2	75.1	17.6	35.9	83.5	70.6	44.3
CV20	17.0	41.8	70.2	11.8	40.6	87.9	109.3	69.4
CV21	31.9	37.6	56.6	21.2	60.3	208.6	136.6	88.2
CV22	46.5	39.2	70.8	17.6	40.9	116.7	128.9	77.3
CV23	38.0	38.9	59.4	13.4	50.2	110.6	166.4	98.2
CV24	70.4	20.4	50.4	19.5	28.3	41.6	34.2	22.3
CV25	17.3	36.2	60.2	19.3	50.3	128.3	129.3	55.4
CV26	40.4	54.4	55.7	18.8	32.1	95.8	298.9	177.3

MSA values higher than 40.0 were registered only for the genotypes CV24, CV5, CV22, CV10, CV26 and CV2. The BRD varied in the examined cauliflower genotypes from 20.4 mm for CV24, to 54.4 mm for CV26. BRD value higher than 30.0 mm was registered for the genotypes CV26, CV6, CV20 CV22, CV23,

CV19, CV21, CV4, CV25, CV13, CV12, CV3, CV16, CV11 and CV2, listed in decrescent order, respectively. On the other hand, for CV8, CV7, CV14, CV18, CV9, CV10, CV1, CV17, CV15, CV5, and CV24, we observed BRD values lower than 30.0 mm, respectively (Table 10). For the MRD, we ascertained high variability in the tested accessions, varying from 40.1 mm to 83.4 mm, for CV17 and CV4, respectively. In addition to CV4, the genotypes CV5, CV18, CV14, CV11, CV19, CV3, CV22, CV6, CV20 and CV1, mentioned in decrescent order, registered MRD values higher than 70.0 mm, while for CV8, CV13, CV9, CV7, CV25, CV23, CV21, CV26, CV15, CV10, CV2, CV24, CV12, and CV16, we observed values between 70.0 mm and 50.0 mm, respectively. The MRL, it varied from 10.9 cm for CV15, to 21.9 mm for CV11.Furthermore, the LRD varied from 20.2 mm for CV12, to 65.4 mm for CV4. In addition to CV4, only for CV5, CV21, CV11, CV25, CV8, CV23 and CV6 we observed LRD values higher than 50.0 mm while for CV7, CV15, CV16, CV9, CV3, CV22, CV20, CV13, CV18 and CV14 it was between 50.0 mm and 40.0 mm and for all the other accessions it was lower than 40.0 mm. The RA, from 41.6 cm^2 to 274.3 cm^2, for CV24 and CV6, respectively. In addition to CV6, the genotypes CV21 and CV4 showed the RA higher than 200.0 cm^2 while, for CV11, CV12, CV1, CV13, CV25, CV2, CV22, CV9, CV8 and CV23 we observed RA values between 200.0 cm^2 and 100.0 cm^2, and for all the other accessions we registered values lower than 100.0 cm^2. Concerning the RFW, we registered high variation among the different accessions, and it varied from 34.2 g to 489.7 g, for CV24 and CV6, respectively. The CV26, CV12 and CV9 genotypes, showed the RFW between 300.0 g and 200.0 g, while for CV11, CV4, CV23, CV1, CV13, CV2, CV21, CV8, CV25 and CV22, respectively, it was between 200.0 g and 100.0 g, while for the other ones it was lower than 100.0 g. On the other hand, the RDM, it ranged between 22.3 g for CV24, to 355.4 g for CV6.

The inflorescence appearance (IA) of the examined 18 Chinese kale accessions, varied from 69.8 days for CK3, to 115.3 days for both CK4 and CK8. The genotypes CK4, CK8, CK5, CK6 and CK17 showed IA values higher than 100.0 days while, CK7, CK2, CK18, CK1, CK16, CK12, CK11, CK15, CK13, CK14, CK9, CK10 and CK3 showed IA values lower than 100.0 days (Table 11).

Table 11. Variation of the epigeal traits in relation to the different Chinese kale accessions tested.

Code	IA	PBR	PGH	PSL	PLS	PLN	LHR	LA	LL	LW	LD	LWN	LPL	LPW
CK1	95.3	1.2	8	99.1	5	33.5	0	2156.3	38.5	56.7	2	0	15.3	1.2
CK2	98.2	3.0	8	80.3	5	27.4	0	1620.5	36.8	45.2	3	0	13.4	1.6
CK3	69.8	3.0	8	59.4	5	20.6	0	602.6	21.5	28.3	3	0	15.7	2.7
CK4	115.3	3.0	8	160.7	5	54.9	0	1916.3	36.5	52.5	3	0	13.5	1.8
CK5	110.7	3.0	8	40.6	7	14.6	0	1894.1	46.4	40.8	3	0	20.8	2.6
CK6	110.6	3.0	8	49.8	5	17.9	0	1511.6	51.7	29.3	3	0	21.9	1.8
CK7	99.5	3.0	8	45.6	2	15.6	0	2258.6	65.6	34.4	3	0	29.6	2.4
CK8	115.3	0.0	8	130.4	5	44.3	0	751.6	31.7	23.7	3	0	21.4	1.3
CK9	74.4	0.0	8	87.4	1	29.8	0	1469.4	39.3	37.4	3	0	20.1	1.6
CK10	70.2	3.0	8	61.2	5	21.7	0	3024.8	48.6	63.4	3	0	21.2	1.6
CK11	88.3	3.0	8	76.3	5	26.3	0	4060.5	56.9	72.5	3	0	23.2	2.5
CK12	89.2	3.0	8	45.7	5	15.5	0	2160.7	30.7	72.4	3	0	22.1	2.1
CK13	79.5	0.0	8	16.4	5	7.6	0	2520.3	40.3	62.6	3	0	18.3	0.9
CK14	75.4	3.0	8	31.4	3	11.3	0	1056.7	48.3	22.6	3	0	10.3	2.4
CK15	80.6	3.0	8	30.6	5	10.7	0	1370.4	54.4	25.2	3	0	15.4	2.6
CK16	89.6	3.0	8	82.3	2	28.6	0	1858.1	57.1	32.6	3	0	19.8	3.3
CK17	110.3	3.0	8	103.6	5	35.3	0	1364.1	48.2	28.3	3	0	12.5	2.4
CK18	95.9	3.0	8	118.2	5	38.7	0	1167.7	37.1	31.4	4	0	11.3	1.6

With regards to plant branches (PB), it shows low variability among the tested genotypes. The genotypes CK4, CK5, CK6, CK17, CK7, CK2, CK18, CK16, CK12, CK11, CK15, CK14, CK10 and CK3 showed PB value of 3.0 branches while CK1 showed 1.0 branch and CK8, CK13 and CK9 showed 0.0 branches. In relation to the plant growth habit (PGH), it was uniform for all the tested Chinese kale accessions, and, in accordance with the IBPGR descriptors, they showed an elongate branching stem terminating in enlarged floral or prefloral apices. For the plant stem length (PSL), we observed high variability among the different Chinese kale accession, registering values between 16.4 cm and 160.7 cm for CK13 and CK4, respectively (Table 11).

The genotypes CK4, CK8, CK18, CK17, CK1, CK9, CK16 and CK2, registered a PSL values higher than 80.cm. The qualitative trait of the plant leaf shape (PLS) also showed high variability among the different accessions, and it was oblong for the accession CK5, it was ovate for CK4, CK8, CK18, CK17, CK1, CK2, CK11, CK10, CK3, CK6, CK12, CK15 and CK13, it was obovate for CK14, elliptic for CK16 and CK7, and finally orbicular for CK9. PLN spanned from 7.6 leaves to 54.9 leaves for CK13 and CK4, respectively. The genotypes which exhibited PLN values higher than 30.0 were CK4, CK8, CK18, CK17 and CK1. For the leaf hairiness (LHR) trait, all the genotypes showed no hairiness in the leaves surface. The leaf area (LA), fluctuated from 602.6 cm^2 to 4060.5 cm^2 for CK3 and CK11, respectively. Concerning the LL value, it spanned from 21.5 cm for CK3, to 65.6 cm for CK7. LL value higher than 45.0 cm was registered for the genotypes CK7, CK16, CK11, CK15, CK6, CK10, CK14, CK17 and CK5, respectively. The leaf width (LW), ranged from 22.6 cm to 72.4 cm for CK14 and CK12, respectively. For CK12, CK11, CK10, CK13, CK1 and CK4, we observed LW values higher than 45.0 cm. In relation to the qualitative trait of the leaf division (LD), in accordance with the IBPGR descriptors for Brassica and Raphanus, it was lacerate for the accession CK18, while it was sinuate for CK1. On the other hand, all the others Chinese accession registered a lyrate leaf. The leaf wing trait (LWN) was uniform among all the examined accessions, which showed no wings at the base of their leaf's petiole. With regards to the LPL, it varied from 10.3 cm to 29.6 for CK14 and CK7, respectively. The genotypes that registered LPL values higher than 20.0 cm were CK7, CK11, CK12, CK6, CK8, CK10, CK5 and CK9. Conversely, the LPW varied among the analysed Chinese kale accessions from 0.9 for CK13, to 3.3 cm for CK16. The accessions CK16, CK3, CK5, CK15, CK11, CK7, CK17, CK14 and CK12, exhibited LPW higher than 2.0 cm. With regards to the MSA it varied from 16.4 ° for CK16 to 61.1 ° for CK2, showing values higher than 30.0 ° for CK2, CK6, CK4, CK17, CK14, CK1, CK5, CK13 and CK7 (Table 12). Concerning the BRD, we observed variability among the accessions, varying from 14.1 mm to 55.1 mm for CK9 and CK11, respectively (Table 12). Only for the genotypes CK11, CK12 and CK8, were registered BRD values higher than 50.0 mm. Also, for the main root diameter (MRD) we ascertained high variability among the different genotypes analysed, and it varied from 30.5 mm for CK9 and CK16, to 85.8 mm for CK17. The accessions CK17, CK1, CK8, CK12, CK2 and CK6, showed MRD values higher than 70.0 mm. With regards to the lateral root diameter (LRD), it varied from 22.9 mm to 79.9 mm for CK16 and CK1, respectively. For the genotypes CK1, CK15, CK18, CK12, CK11, CK2 and CK8, were registered LRD values higher than 60.0 mm. Concerning the root area (RA), it varied from 40.8 cm2 for CK9, to 261.8 cm2 for CK1, respectively.

The genotypes CK1, CK4, CK3, CK15, CK12, CK17, CK11 and CK8, showed RA values higher than 150 cm^2, and they were listed in decrescent order. The root fresh weight (RFW), ranged from 43.4 g to 399.8 g for CK13 and CK1, respectively. Only CK1, CK4 and CK12 exhibited RFW values higher than 300.0 g. On the other hand, the root dry matter (RDM) trait, it varied from 22.3 g to 254.5 g for CK14 and CK1, respectively. Only CK1 and CK4, registered RDM values higher than 200 g.

Table 12. Variation of the hypogeal traits in relation to the different Chinese kale accessions tested.

Code	MSA	BRD	MRD	MRL	LRD	RA	RFW	RDM
CK1	38.6	23.5	85.2	62.4	79.9	261.8	399.8	254.6
CK2	61.1	28.3	70.2	23.1	60.4	143.9	190.7	111.2
CK3	24.1	37.3	40.3	22.2	30.6	200.5	168.4	101.5
CK4	44.5	41.2	50.1	26.4	32.6	253.6	348.6	252.8
CK5	37.5	33.5	60.2	21.9	33.9	141.6	164.3	100.4
CK6	53.5	33.6	70.1	22.9	40.3	120.7	140.8	88.3
CK7	30.2	42.4	68.8	10.6	46.3	100.5	136.7	75.1
CK8	20.1	51.4	79.7	22.3	60.3	169.1	179.2	101.6
CK9	23.0	14.1	30.5	10.8	28.4	40.8	123.3	77.2
CK10	25.6	30.5	58.2	11.6	40.1	89.4	93.3	55.6
CK11	16.9	55.1	68.3	25.6	60.6	180.9	226.8	144.1
CK12	24.5	53.1	77.8	22.3	61.3	196.4	310.6	166.4
CK13	35.5	26.3	63.1	16.5	36.3	65.9	43.4	25.7
CK14	39.1	22.4	50.2	17.6	30.1	62.7	46.7	22.3
CK15	25.3	33.1	67.1	19.3	61.9	198.9	182.3	144.5
CK16	16.4	33.9	30.5	11.2	22.9	52.2	128.5	81.5
CK17	40.3	39.5	85.8	16.5	59.3	193.3	214.2	156.3
CK18	29.2	33.1	37.9	12.2	61.6	137.2	140.4	78.5

In relation to the 6 different CWRs accessions, we observed high value of IA, which ranged from 122.3 days for BS1 to 149.6 days for BS4. The PB, it varied form 3.2 branches to 4.6 branches for BS4 and BS2, respectively. For the PGH, we ascertained uniformity among the six CRWs tested, and it was, in accordance with the IBPGR descriptors, elongate branched stems terminating in enlarged floral apex or prefloral apices. The PSL, varied from 17.5 cm to 70.9 cm for BS4 and BS3, respectively. The genotypes BS6, BS5 and BS1, showed PSL higher than 35.0 cm, in contrast to BS2 and BS4 which registered lower values. For the PLS, the genotypes BS6 and BS4 showed ovate leaf while for BS3, BS5, BS1 and BS2, we observed the elliptic leaf (Table 13). The PLN, ranged among the different CWRs genotypes from 15.2 to 47.4 leaves for BS4 and BS3, respectively. All the CWRs examined, except for the B. rupestris, BS2, showed the hairiness in the leaf surface and the two *B. villosa* genotypes BS6 and BS5 registered the highest value in accordance with the IBPGR descriptors of leaf hairiness.

Table 13. Variation of the epigeal traits in relation to the different crop wild relatives (CWRs) tested.

Code	IA	PBR	PGH	PSL	PLS	PLN	LHR	LA	LL	LW	LD	LWN	LPL	LPW
BS1	122.3	3.5	8	36.7	2	12.6	1	3385.2	54.6	62.3	2	0	17.4	3.4
BS2	133.5	4.6	8	22.6	2	8.7	1	2110.9	46.9	45.3	2	0	18.7	3.1
BS3	144.4	3.4	8	70.9	2	23.7	5	1503.4	42.2	35.6	3	1	14.2	2.4
BS4	149.6	3.2	8	17.5	5	7.6	4	1940.5	47.3	41.1	3	1	19.2	2.6
BS5	127.8	3.6	8	47.3	2	16.8	7	1767.5	43.8	40.4	2	0	13.6	2.2
BS6	128.9	4.5	8	59.3	5	20.9	7	733.4	31.8	23.7	2	0	12.8	1.3

Concerning the LA, it ranged from 733.4 cm2 to 3385.2 cm2, for BS6 and BS1, respectively. In addition to BS1, only BS2 registered LA values higher than 2000.0 cm2. With regards to the LL, it varied

between 31.8 cm for BS6 to 54.6 cm for BS1. On the other hand, the LW ranged among the six examined CWRs accessions from 23.7 cm to 62.3 cm for BS6 and BS1, respectively. For the LD trait, BS4 and BS3 showed the lyrate leaf while for BS1, BS2, BS5 and BS6 it was sinuate (Table 14). Only the accessions BS3 and BS4 showed LWN at the petiole base, while for all the other ones was registered no wings in the petiole. Concerning the LPL, it varied from 12.8 cm to 19.2 cm for BS6 and BS4, respectively. Concerning the LPW, it varied from 1.3 cm to 3.4 cm from BS6 to BS1, respectively (Table 4). All the CWRs genotypes showed LPW higher than 2.0 cm, except for BS6. The MSA it fluctuated from 13.1 ° to 54.0 ° for BS3 and BS4. In addition to BS4, only BS5 and BS1 registered values higher than 40.0 °. Regarding the BRD, it ranged from 19.7 mm for BS2, to 46.7 mm for BS3.The MRD varied from 40.7 mm for BS2, to 65.3 for BS1.In addition to BS1, only BS6 exhibited MRD value higher than 60.0 mm. In relation to the MRL, we observed variation among the genotypes from 15.4 cm for BS6 to 24.3 cm for BS1. The LRD fluctuated from 30.0 mm to 43.1 mm for BS5 and BS1, respectively. For the RA, we observed a variation from 48.9 cm2 for BS3, to 121.5 cm2 for BS6. The RFW varied from 56.4 g to 142.5 g for BS3 and BS6, respectively. On the other hand, the RDM varied from 22.5 g to 78.3 for BS3 and BS1, respectively (Table 4).

Table 14. Variation of the hypogeal traits in relation to the different CWRs accessions tested.

Code	MSA	BRD	MRD	MRL	LRD	RA	RFW	RDM
BS1	40.4	34.8	65.3	24.3	43.1	90.5	138.1	78.3
BS2	18.7	19.7	40.7	20.2	30.2	62.3	69.3	29.7
BS3	13.1	46.7	59.5	16.4	40.0	48.9	56.4	22.5
BS4	54.0	23.3	50.1	19.6	42.8	73.0	72.3	33.6
BS5	41.0	23.2	42.7	18.3	30.0	94.3	124.8	77.8
BS6	30.6	33.4	61.2	15.4	30.7	121.5	142.5	75.6

The IA varied from the tested *B. oleracea* var. *acephala* accessions from 61.3 days to 118.6 days for BH5 and for BH15, respectively. Genotypes which registered IA values higher than 85.0 were, in decrescent order, BH15, BH12, BH18, BH3, BH6, BH17, BH14, BH10, BH13, BH16, respectively (Table 15).

Table 15. Variation of the epigeal traits in the analysed kale accessions.

Code	IA	PBR	PGH	PSL	PLS	PLN	LHR	LA	LL	LW	LD	LWN	LPL	LPW
BH1	84.2	6.5	6	38.3	5	44.6	0	2529.7	22.3	34.5	3	0	28.9	2.4
BH2	75.5	6.5	6	32.4	5	65.4	0	1245.1	44.9	23.7	2	0	15.6	3.6
BH3	100.3	3.4	6	71.3	5	60.8	0	1003.8	51.4	31.2	3	0	14.8	2.7
BH4	84.4	1.6	6	95.4	5	16.6	0	918.7	52.3	68.5	3	0	14.8	1.7
BH5	61.3	6.3	6	74.6	5	50.6	0	1745.3	57.3	34.6	2	0	25.4	1.2
BH6	89.8	5.2	6	83.2	5	23.0	0	832.5	43.1	100.2	3	0	11.3	1.7
BH7	79.4	6.5	6	50.3	5	83.4	0	3010.9	27.5	36.5	3	0	22.3	1.7
BH8	79.6	6.7	6	18.2	5	33.6	0	2691.8	26.4	35.5	3	0	22.1	1.7
BH9	83.4	1.9	6	65.8	5	49.8	0	1980.5	56.4	31.2	3	0	18.7	2.9
BH10	88.3	7.2	6	65.7	5	25.2	0	4308.6	22.3	36.2	3	0	18.6	2.3
BH11	83.7	4.5	6	71.6	5	55.2	0	793.5	22.5	37.5	3	0	13.8	1.5
BH12	110.3	3.4	6	149.6	5	45.2	0	1241.3	40.5	30.7	3	0	23.7	1.8
BH13	85.4	3.3	6	65.4	5	65.6	0	792.3	43.9	21.5	3	0	8.2	1.2
BH14	88.7	6.6	6	85.3	5	35.0	0	1593.3	71.6	65.7	3	0	19.4	0.9
BH15	118.6	3.5	6	94.7	5	57.2	0	923.6	48.5	55.5	3	0	14.8	0.7
BH16	85.4	1.2	6	48.2	5	49.2	0	4701.3	63.8	47.2	3	0	13.8	0.7
BH17	89.3	3.6	6	36.9	5	45.8	0	1065.5	58.5	43.3	2	0	14.9	1.6
BH18	110.3	4.5	6	91.3	5	26.8	0	3536.2	31.2	41.5	3	0	15.5	2.2

On the other hand, BH4, BH1, BH11, BH9, BH8, BH7, BH2 and BH5, showed values lower than 85.0, in decrescent order. With regards to the PB, it ranged from 1.2 to 7.2 branches, for BH16 and BH10,

respectively. Genotypes which showed PB values higher than 5.0 branches were BH10, BH8, BH14, BH1, BH7, BH2, BH5 and BH6, and they were reported in decrescent order. In contrast, the genotypes BH18, BH11, BH17, BH15, BH12, BH3, BH13, BH9, BH4, BH16 exhibited values lower than 5.0 branches. The qualitative PGH showed uniformity for all the examined kale accessions, and it was 6.0 which correspond to the elongated branching stem supporting leaves and/or head in the IBPGR descriptors for Brassica and Raphanus. With regards to the PSL, it fluctuated from 18.2 cm for BH8, to 149.6 cm for BH12. The accessions that registered PSL values higher than 85.0 cm were BH12, BH4, BH15, BH18 and BH14. Contrarily, BH6, BH5, BH11, BH3, BH9, BH10, BH13, BH7, BH16, BH1, BH17, BH2 and BH8, listed in decrescent order, registered PSL values lower than 85.0 cm. The PLS showed uniformity for all the kale genotypes showing ovate leaves for all the accessions. In relation to the PLN, it ranged from 16.6 to 83.4 leaves per plant for BH4 and BH7, respectively. In decrescent order, BH7, BH13, BH2, BH3, BH15, BH11, BH5, BH9, BH16, BH17, BH12 and BH1, exhibited values higher than 40.0 leaves, while all the other genotypes showed lower values. Concerning the LHR, all the evaluated kales, showed absence of hairiness in their leaves (Table 15).

The LA values registered showed high variability among the tested genotypes, varying from 792.3 cm^2 to 4701.3 cm^2 for BH13 and BH16, respectively. The genotypes BH16, BH10, BH18, BH7, BH8 and BH1 showed values higher than 2000.0 cm^2, while BH9, BH5, BH14, BH2, BH12, BH17 and BH3, exhibited values within 2000.0 cm^2 and 1000.0 cm^2. Finally, for BH15, BH4, BH6, BH11 and BH13, it was registered values lower than 1000.0 cm2. Regarding the LL trait, it varied from 22.3 cm for the genotypes BH1 and BH 10, to 71.6 cm for BH14. For the genotypes BH14, BH16, BH17, BH5, BH9, BH4 and BH3 which are listed in decrescent order, were registered LL values higher than 50.0 cm, while BH15, BH2, BH13, BH6, BH12, BH18, BH7, BH8, BH11, BH10 and BH1 showed LL values lower than 50.0 cm, in decrescent order. Concerning the LW, it shows a high variability among the tested kale accessions, varying from 21.5 cm to 100.2 cm for BH13 and BH6, respectively. The genotypes BH6, BH4, BH14 and BH15, showed LW values higher than 50.0 cm, while all the other ones showed LW values lower than 50.0 cm. Regarding the leaf division (incision) parameter (LD), all the tested kale accession showed lyrate leaf except for BH17, BH5 and BH2, which showed sinuate leaf. The leaf wings number (LWN) showed no variability among the examined kale accessions, exhibiting no wings for each accession. The LPL varied among the tested accessions from 8.2 cm to 28.9 cm for BH13 and BH1, respectively. Only for the genotypes BH1, BH5, BH12, BH7 and BH8, were registered values higher than 20.0 cm. With regards to the LPW, it ranged from 0.7 cm for BH15 and BH16, to 3.6 cm for BH2. LPW values higher than 2.0 cm were registered just for BH2, BH9, BH3, BH1, BH10 and BH18, respectively while BH12, BH7, BH4, BH8, BH6, BH17, BH11, BH13, BH5, BH14, BH15 and BH16 showed LPW values lower than 2.0 cm.

Concerning the MSA, it ranged from 11.4 ° to 81.1 ° for BH5 and BH12, respectively. MSA values higher than 40.0 ° were registered only for BH11, BH2 and BH17, in addition to BH12. Concerning the root left angle (RLA) it varied among the tested accessions from 11.2 ° to 53.1 ° for BH5 and BH11, respectively. Contrarily, the root right angle (RRA), ranged from 11.6 ° for BH5, to 125.4 for BH12. With regards to the basal root diameter (BRD), it varied from 18.2 mm for BH12 to 57.4 mm for BH4. The genotypes which

showed BRD values higher than 40.0 mm were BH4 BH6, BH3, BH8, BH15, BH17, BH9, BH10 and BH2, in decrescent order, while BH5, BH16, BH14, BH13, BH7, BH18, BH1, BH11 and BH12, registered values lower than 40.0 mm. The main root diameter (MRD) ranged from 22.3 mm to 93.2 mm for BH16 and BH6, respectively. For the genotypes BH6, BH3, BH14, BH13, BH9 and BH17, were registered MRD values higher than 90.0 mm, while BH1, BH2, BH10, BH12, BH5, BH4, BH18, BH8, BH15 showed MRD values within 90.0 mm and 50.0 mm and finally, only BH7, BH11 and BH16, showed values lowed than 50.0 mm. The MRL varied among the tested genotypes from 12.3 cm to 29.3 for BH7 and BH17, respectively. The genotypes BH17, BH2, BH4, BH12, BH6, BH3, BH11, BH1, BH15, BH8, BH9 and BH13 exhibited MRL values higher than 20.0 cm, while BH14, BH16, BH5, BH10, BH18 and BH7, reported MRL values lower than 20.0 cm, and they were listed in decrescent order. Concerning the lateral root diameter (LRD), it ranged from 20.2 mm to 62.2 mm for BH16 and BH17, respectively. The genotypes BH17, BH14, BH2, BH6, BH1, BH3 and BH12, registered LRD values higher than 50.0 mm following the decrescent order (Table 16). Only for the genotypes BH18, BH11 and BH16 were registered LRD values lower than 30.0 mm. The root area parameter (RA), exhibited high variability among the tested kale accessions, varying from 56.5 cm2 for BH18 to 421.6 cm2 for BH15. The genotypes BH15, BH14, BH4, BH6 and BH2 showed RA values higher than 200.0 cm2, while BH9, BH11, BH10, BH7, BH12, BH1 and BH16 exhibited RA values within 200.0 cm2 and 100.0 cm2, and finally for BH8, BH3, BH13, BH17, BH5 and BH18 were registered values lower than 100.0 cm2. Concerning the root fresh weight (RFW), varied from the tested kale accessions from 74.4 g to 408.2 g, for BH18 and BH14, respectively. In the other hand, the root dry matter (RDM) ranged from 41.4 g to 256.3 g for BH18 and BH14, respectively (Table 16).

Table 16. Variation of the hypogeal traits for the kale accessions tested.

Code	MSA	BRD	MRD	MRL	LRD	RA	RFW	RDM
BH1	14.0	30.8	82.8	22.4	50.6	136.4	222.3	132.6
BH2	46.0	40.5	80.2	29.2	60.2	243.4	310.6	189.5
BH3	36.7	48.8	92.2	25.3	50.4	96.2	103.6	56.7
BH4	24.8	57.4	60.3	29.2	49.9	313.8	302.2	175.9
BH5	11.4	39.3	63.3	13.5	45.7	70.6	86.1	55.6
BH6	28.5	53.7	93.2	26.2	55.2	280.8	348.1	221.1
BH7	19.4	33.6	49.2	12.3	30.6	146.3	108.1	65.3
BH8	39.2	44.3	50.2	21.4	39.7	98.6	97.3	50.6
BH9	34.1	41.5	90.5	21.2	40.1	158.2	158.3	100.7
BH10	32.5	40.7	73.1	13.0	32.6	152.8	98.3	45.2
BH11	54.2	25.4	30.3	22.6	29.2	156.4	140.1	85.7
BH12	81.1	18.2	70.4	28.7	50.1	142.3	186.5	111.6
BH13	18.9	35.5	90.6	20.6	37.3	95.8	138.2	85.4
BH14	23.0	35.9	91.2	18.7	60.8	332.6	408.2	256.3
BH15	17.9	43.1	50.1	22.3	35.5	421.6	363.2	211.3
BH16	16.9	38.7	22.3	16.6	20.2	108.5	151.2	88.6
BH17	42.9	42.6	90.3	29.3	62.2	80.9	233.4	144.2
BH18	37.2	32.9	55.1	12.5	30.2	56.5	74.4	41.4

The IA of the examined 17 kohlrabi accessions, it varied from 69.3 days for CR16 and CR14, to 112.9 days for CR4. IA values higher than 80.0 days were registered for the genotypes CR4, CR12, CR2, CR7, CR15, CR6, CR11, CR1, CR10 and CR3, listed in decrescent order.

With regards to the PB, it was uniform for all the genotypes registering 0.0 branches for all the accessions examined. In relation to the PGH, all the tested 17 kohlrabi accessions showed, in accordance with

the IBPGR descriptors, the elongate or enlarged nonbranching stem supporting leaves. Concerning the PSL, it varied among the examined accessions from 11.5 cm for CR10, to 51.7 cm for CR9. Only CR9, CR2 and CR16, showed PSL values higher than 40.0 cm. With regards to qualitative trait of the PLS, CR9, CR16 and CR13 showed ovate leaf, while CR4 and CR3 exhibited a spathulate leaf, for CR17, CR14, CR15, CR8, CR1, CR5, CR11, CR12, CR7, CR6 and CR10 was registered a lyrate leaf, and only for CR2, it was registered elliptic leaf. The PLN ranged from 17.0 leaves for CR7, to 41.0 leaves for CR9. In relation to the LHR, all the tested kohlrabi accessions showed absence of hairiness in the surface of their leaves. Concerning the LA, we ascertained high variability among the 17 tested accessions, varying from 672.4 cm2 to 4789.6 cm2 for CR16 and CR9, respectively (Table 17). The genotypes which showed LA values higher than 3000.0 were CR4, CR6, CR2, CR3 and CR8 and they were listed in decrescent order. In relation to the LL, it varied from 24.3 cm for CR16, to 126.8 cm for CR3. The genotypes which registered LL values higher than 60.0 cm were CR3, CR2, CR6, CR4, CR8, CR9 and CR5 (Table 17). Regarding the LW trait, it ranged from 26.9 cm to 75.5 cm for CR9 and CR1, respectively. Only CR1, CR10, CR17 and CR15, showed LW value higher than 60.0 cm (Table 17).

Table 17. Variation of the epigeal traits of kohlrabi's genotypes tested.

Code	IA	PBR	PGH	PSL	PLS	PLN	LHR	LA	LL	LW	LD	LWN	LPL	LPW
CR1	88.6	0.0	4	19.7	3	22.4	0	2340.5	31.9	75.5	3	0	12.5	2.5
CR2	90.3	0.0	4	46.8	2	39.8	0	3933.3	103.8	38.2	3	0	21.3	2.3
CR3	84.7	0.0	4	18.3	4	21.2	0	3599.7	126.8	28.4	3	0	19.4	2.9
CR4	111.2	0.0	4	26.9	4	25.0	0	4789.6	89.1	53.8	3	0	20.1	3.7
CR5	79.8	0.0	4	19.7	3	21.3	0	2935.7	70.8	41.5	2	0	13.7	2.2
CR6	89.3	0.0	4	15.1	3	18.6	0	4130.6	94.5	43.7	3	0	18.6	2.1
CR7	89.7	0.0	4	15.6	3	17.0	0	1422.8	43.4	32.8	3	0	18.1	1.4
CR8	79.4	0.0	4	20.6	3	21.2	0	3515.4	85.7	41.6	3	0	17.6	1.6
CR9	77.6	0.0	4	51.7	5	41.0	0	2222.1	82.3	26.9	3	0	11.1	2.6
CR10	88.3	0.0	4	11.5	3	19.2	0	2263.4	31.4	73.8	3	0	16.2	2.9
CR11	89.3	0.0	4	19.4	3	21.6	0	1054.6	31.9	34.2	2	0	26.6	2.3
CR12	90.6	0.0	4	16.3	3	21.6	0	993.8	26.5	37.5	3	0	14.9	2.6
CR13	78.7	0.0	4	25.6	5	23.1	0	1732.5	33.5	52.5	3	0	10.5	1.7
CR14	69.3	0.0	4	21.9	3	23.0	0	1710.3	30.4	57.3	3	0	11.3	1.2
CR15	89.4	0.0	4	21.7	3	21.4	0	2600.8	40.9	65.2	3	0	13.9	1.3
CR16	69.3	0.0	4	41.5	5	35.2	0	672.4	24.3	28.3	3	0	17.4	1.6
CR17	79.4	0.0	4	22.3	3	24.8	0	2445.5	33.5	73.6	3	0	23.2	1.8

On the other hand, the genotypes CR2, CR12, CR11, CR7, CR3, CR16 and CR9, exhibited LW values lower than 40.0 cm. In relation to the qualitative trait LD, all the examined genotypes showed lyrate leaf, except for CR5 and CR11, which showed sinuate leaf. The LWN parameter was uniform for all the evaluated genotypes, showing 0.0 wings at the petiole base. Concerning the LPL, it varied from 10.5 cm to 26.6 cm for CR13 and CR11, respectively. The genotypes which showed LPL higher than 20.0 cm were CR11, CR17, CR2 and CR4 while all the others registered LPW values between 10.0 cm and 20.0 cm. In relation to the LPW, it varied from 1.2 cm for CR14 to 3.7 for CR4. For CR4, CR3, CR10, CR12, CR9, CR1, CR2, CR11, CR5 and CR6 it was observed a LPW higher than 2.0 cm, respectively, while CR17, CR13, CR8, CR16, CR7, CR15 and CR14 exhibited the LPW value lower than 2.0 cm, respectively. For the MSA we observed a variation between 19.5 ° to 57.9 ° for CR12 and CR4, respectively. MSA values higher than 40.0 ° were registered for CR15, CR3, CR2, CR6, CR1 and CR14, in addition to CR4. For the BRD, we observed a variation between

9.4 mm and 58.6 mm for CR8 and CR17, respectively. For CR17, CR16, CR5, CR1, CR2 and CR14, it was registered the BRD higher than 30.0 mm, while for CR12, CR15, CR9, CR7 and CR6, we ascertained BRD values between 20.0 mm and 30.00, and for CR11, CR13, CR4, CR3, CR10 and CR8, were observed values lower than 20.0 mm, respectively. Concerning the MRD, it varied from 28.9 mm to 85.4 mm for CR5 and CR17, respectively. Only for CR17, CR16, CR9, CR13 and CR2, were registered MRD values higher than 50.0 mm. In relation to the MRL, it was observed a variation between 7.6 cm and 27.8 cm for CR13 and CR17, respectively. The higher values were observed for CR17, CR16 and CR1 which showed the MRL longer than 25.0 cm, while for CR15, CR7, CR10, CR9, CR5, CR12, CR8, CR4, CR14, CR3, CR6, we observed the MRL values between 25.0 cm and 10.0 cm. Only for CR11, CR2 and CR13, we observed MRL values lower than 10.0 cm, respectively. The LRD, it varied from 16.7 mm for CR4, to 69.2 mm for CR17. Only for CR17, CR1 and CR16, were observed LRD values higher than 45.0 mm. With regards to the RA, we ascertained high variability among the tested genotypes, varying from 16.5 cm2 for CR3, to 381.7 cm^2 for CR16. Only CR16, CR17 and CR1, registered RA values higher than 300.0 cm^2, while for all the other genotypes we observed RA values lower than 100.0 cm^2, in particular, CR13, CR14, CR4 and CR3, registered RA values lower than 40.0 cm^2, respectively. In relation to the RFW, it ranged from 52.4 g to 640.7 g, for CR8 and CR16, respectively (Table 18). The genotypes CR16, CR17, CR1, CR4, CR14, CR13, CR6, CR3, CR10 and CR5, showed RFW values higher than 100.0 g and they were listed in decrescent order. Contrarily, for the RDM we observed a variation between 55.3 g to 322.3 g, for CR11 and CR16, respectively (Table 18).

Table 18. Variation of the hypogeal traits fort the analysed kohlrabi genotypes.

Code	MSA	BRD	MRD	MRL	LRD	RA	RFW	RDM
CR1	41.9	37.9	40.2	27.5	50.3	307.1	256.3	166.4
CR2	46.1	32.3	50.1	9.2	30.4	54.3	94.8	65.3
CR3	50.9	13.4	44.8	11.2	29.1	16.5	122.6	74.4
CR4	57.9	16.7	30.2	12.2	16.7	29.8	136.9	72.5
CR5	35.0	45.4	28.2	13.2	28.9	99.4	112.5	71.3
CR6	43.1	20.9	41.5	10.1	30.1	55.7	122.6	69.5
CR7	25.4	26.6	39.1	15.2	22.2	94.6	78.7	55.8
CR8	26.0	9.4	40.5	12.4	30.3	75.4	52.4	66.5
CR9	32.0	27.2	51.2	13.4	30.9	68.2	58.6	65.6
CR10	35.1	10.9	49.3	13.8	25.1	58.3	118.2	63.5
CR11	22.7	19.4	40.5	9.4	31.3	64.9	75.4	55.3
CR12	19.5	28.2	40.2	12.6	30.1	82.3	78.9	60.5
CR13	32.4	18.7	50.1	7.6	30.3	39.7	126.1	59.4
CR14	41.1	31.6	29.3	12.0	19.2	31.2	136.1	57.5
CR15	54.6	27.3	40.2	16.3	30.1	94.3	82.2	56.2
CR16	23.5	49.5	78.6	27.6	45.3	381.7	640.7	322.3
CR17	39.9	58.6	85.4	27.8	69.2	370.6	446.8	199.6

EXPERIMENTAL TRIAL B

With regards to the qualitative descriptor of the plant growth habit (PGH), we observed variation among the genotypes tested distinguishing the determinate, semi-determinate and indeterminate types. The genotypes 1, 2, 4, 6, 11, 14, 15, 16, 17, 19, 20, 21, 22, 23, 24, 26, 27, 28, 29, 31, 32, 33, 34 exhibited an indeterminate growth habit, while only for 9, 10, 12 and 25 we registered a determinate one. Furthermore, for 3, 5, 7, 8, 13, 18 and 30 we registered a semi-determinate PGH. For the qualitative descriptors of the plant

foliage density (PFD) and for the stem pubescence density (PSP), we observed intermediate values for all the tested genotypes. For the qualitative trait of the plant internode length (PSL), we detected intermediate value for all the genotypes, except for the genotype 18, which showed the highest value. With regards to the plant vigour (PV), detected by visual evaluation due to it is a qualitative descriptor, we observed high variability among the 34 tested genotypes. The genotypes which showed the lowest PV were 7, 9, 10, 11, 12, 25 and 34, while the ones that showed the highest PV were 1, 14, 17, 20, 22, 24, 28, 31 and 33. All the other genotypes showed an intermediate vigour, in accordance with the IBPGR descriptor examined. Concerning the amplitude of the root angle (RRB), it showed variability among the tested genotypes, varying from 93.33 ° to 166.67 ° for the genotypes 6 and 1, respectively. RRB values higher than 140.0 ° were registered only for 5, 13, 20, 23, 32 and 34. On the other hand, also for the diameter of the main root at the union with the plant stem (RDMR), we observed high variability among the 34 accessions, fluctuating from 1.20 cm for the genotype 10, to 2.47 cm for both the genotypes 20 and 34. With regards to the score for evaluating the density of fine roots (RDF), we registered the lowest values for the genotypes 9, 10, 11 and 25, while the highest ones were registered by 14 and 33. All the other genotypes showed intermediate values. The leaf type, in accordance with the IBPGR tomato descriptors, was uniform for all the tested genotypes, showing the standard tomato leaf for all the genotypes. With regards to the leaf lamina attitude (LLA), it was horizontal for all the tested genotypes except for 1, 17, 28, 31, 32 and 34. Also the shape of the leaf central lobe (LSC) it was uniform, registering a toothed lobe for each genotype tested. The anthocyanin colouration of the leaf veins (LAC), showed high uniformity, registering normal colouration in each examined leaf. Leaf SPAD index (LSI), it indicates the plant nutritional status evaluation, and its value varied from 31.96 to 57.78 for the genotypes 7 and 23, respectively (Table 19). LSI values higher than 50.0 were registered for the genotypes 1, 15, 22, 23, 26, 28, 29, 30 and 32 (Table 19). Concerning the presence of pest (LPL) and disease (LDL) in leaves we have fortunately no detected serious problems in the leaves of the examined plants, grown in Valencian summer conditions. With regards to the leaf flavanols index (LFl), it ranged from 0.27 to 0.90 for the genotypes 10 and 26, respectively. LFl values higher than 40.0 were registered, in addition to the genotype 26, for the accessions 1, 2, 8, 11, 14, 19, 22, 23, 24, 25, 30 and 34. On the other hand, the leaf chlorophyll (LCl), fluctuated from 1.07 μg cm^{-2}, to 2.01 μg cm^{-2}, for the accessions 7 and 30, respectively. Values of chlorophyll higher than 1.80 μg cm^{-2} were registered for the genotypes 5, 23, 26, 28, 29, 30, 32 and 33. The leaf anthocyanin (LAn), varied from 0.10 for the genotype 23, to 0.24 for the genotype 7. We have observed values higher than 0.15 for the genotypes 3, 4, 7, 9, 10, 11, 12, 15, 25, 27 and 31 (Table 19). The leaf nitrogen balance index (LNBI) is one of the most important indicators of the plant growth and development, and it varied from 0.72 to 1.07 for the accessions 27 and 13, respectively. Only for the genotypes 13, 14, 26, 29, 30 and 32, we detected LNBI values higher than 1.0, indicating that the above-mentioned ones took up more nitrogen that they need, and this nitrogen may be stored in the plant tissues (Table 19).

Table 19. Variation of the leaf quantitative traits in relation to the genotypes

Genotype	LSI	LCl	LFl	LAn	LNBI
1	54.80	1.61	0.49	0.14	0.96
2	47.87	1.59	0.45	0.14	0.88
3	41.07	1.63	0.36	0.17	0.96
4	48.06	1.67	0.38	0.16	0.86
5	48.59	1.89	0.39	0.12	0.73
6	45.90	1.69	0.40	0.15	0.87
7	31.96	1.07	0.38	0.24	0.80
8	45.59	1.71	0.46	0.14	0.91
9	43.54	1.58	0.28	0.19	1.00
10	38.18	1.20	0.27	0.21	0.90
11	43.22	1.59	0.43	0.20	0.90
12	38.32	1.38	0.40	0.19	0.85
13	48.04	1.77	0.32	0.13	1.07
14	47.69	1.78	0.41	0.13	1.06
15	52.39	1.51	0.36	0.16	0.95
16	49.08	1.78	0.40	0.15	0.93
17	48.56	1.73	0.32	0.13	0.92
18	43.04	1.73	0.31	0.15	0.93
19	45.30	1.78	0.46	0.15	0.74
20	45.00	1.63	0.39	0.14	0.93
21	49.16	1.56	0.34	0.15	0.88
22	53.26	1.75	0.41	0.14	0.98
23	57.78	1.92	0.46	0.10	0.96
24	47.03	1.79	0.55	0.13	0.80
25	43.64	1.43	0.42	0.16	0.79
26	50.42	1.81	0.90	0.13	1.04
27	46.46	1.59	0.36	0.19	0.72
28	54.95	1.95	0.32	0.13	0.94
29	54.50	1.88	0.34	0.12	1.04
30	54.43	2.01	0.46	0.11	1.02
31	41.47	1.60	0.38	0.16	0.92
32	51.79	1.84	0.38	0.11	1.01
33	48.22	1.81	0.33	0.13	0.94
34	41.30	1.60	0.45	0.15	0.87

With regards to the inflorescence characterization, the inflorescence type (IT) was different among the tested genotypes, and it was generally uniparous except for the accessions 11, 20, 23, 24 and 24 which showed a fishbone inflorescence, moreover, the genotype 4 showed a forked inflorescence, therefore the accessions 6 and 32 exhibited an irregular inflorescence. As concern the corolla colour (FlCC), it was uniform for all the genotypes which showed a yellow one. The leafy inflorescence (IL) was not detected in all the genotypes, apart for 18, 31 and 34 which showed a leafy inflorescence. The style position (FlSP) is an important trait that can determinate a significative increase in terms of allogamy. It was generally inserted for all the genotypes, apart from 1, 6, 11, 25, 28 and 28 which was at the same level of the stamen. As concern the style shape (FlSS), it was simple for all the genotypes, except for the genotypes 3 and 28, for which we detected a fasciated style. In relation to the presence or absence of hairiness in the style, for the genotypes 3, 8, 9, 10, 15, 21, 22, 26, 29 and 33 we observed absence of hairiness in the style, in contrast to all the other ones for which we registered hairiness in the style (Figure 2).

Figure 2. Tomato style detected in open field in Valencia. For the evaluation of the qualitative style descriptors related to the shape and the hairiness, style was peel off from the petals, sepals and stamen and subsequently analysed.

Concerning the petal length of the flower (FlPL), it varied among the genotypes from 11.78 mm to 16.67 mm for the accessions 14 and 7, respectively (Table 20).

Table 20. Variation of the flower petal, sepal and stamen length (FlPL, FlSL and FlSTL, respectively) among the different genotypes tested.

Genotype	FlPL	FlSL	FlSTL
1	14.51	13.22	10.22
2	13.44	11.33	9.89
3	14.40	14.65	9.80
4	11.83	8.61	8.22
5	15.33	16.17	10.07
6	12.78	11.54	9.12
7	16.67	18.25	10.67
8	14.43	12.30	10.12
9	12.26	13.28	9.90
10	13.11	11.00	10.21
11	16.11	18.28	10.17
12	12.88	11.56	9.97
13	13.89	15.33	10.14
14	11.78	10.10	9.18
15	13.08	9.08	9.97
16	12.44	12.00	10.00
17	13.44	11.33	10.06
18	14.67	12.78	10.40
19	13.56	12.00	9.67
20	13.86	13.44	9.44
21	12.72	11.33	9.69
22	13.78	13.00	10.12
23	14.67	13.05	11.34
24	14.57	14.67	10.13
25	14.04	14.86	10.42
26	12.18	10.78	9.33
27	14.00	13.22	10.33
28	15.18	13.92	10.25
29	12.89	10.33	9.73
30	14.44	13.33	10.44
31	13.56	12.33	9.11
32	13.27	12.56	10.17
33	12.17	10.50	9.83
34	16.28	18.11	10.73

FlPL values higher than 14.00 mm, were registered for the genotypes 1, 3, 5, 8, 11, 18, 23, 24, 25, 28, 30, 34, in addition to the accession 7. Concerning the sepal length of the flower (FlSL), its value ranged from 8.61 mm to 18.28 mm for the genotypes 4 and 11, respectively. In addition to the accession 11, we observed FlSL values higher than 14.00 mm only for 3, 5, 7, 13, 24, 25 and 34. On the other hand, the stamen length of the flowers (FlSTL) ranged from 8.22 mm to 11.34 mm for the genotypes 4 and 23, respectively. Also, for 1, 5, 7, 8, 10, 11, 13, 17, 18, 22, 24, 25, 27, 28, 30, 32 and 34 we detected FlSLT values higher than 10.00 mm (Table 20). Concerning the ripening earliness (RE) of the fruits which correspond to the days for detecting the first ripe fruit for the 50% of the plants, it varied from 76.67 days for the genotypes 6 and 33, to 129.33 days for the genotype 31 (Table 20).

Regarding the qualitative characterization of tomato fruits, in the present PhD thesis I examined the morphometric characteristics of the fruits collected at the commercial ripening stage, using both qualitative and quantitative descriptors. Additionally, the biochemical components that determine fruit quality, such as soluble solids content and acidity (expressed in pH and grams of citric acid), were also analyzed.

Concerning the fruit weight (FW), we ascertained high variability among the tested accessions, and its values varied from 13.56 g to 276.92 g for the genotypes 4 and 11, respectively (Table 21).

Table 21. Variation of the fruits production per plant (PFP) components in the examined 34 accessions. The analysed traits were the fruit weight (FW), the number of fruits per truss (FT), and the average weight of truss (FTW).

Genotype	FW	FT	FTW	PFP
1	148.16	6.25	926.0	5555.9
2	25.53	8.36	213.5	1280.8
3	110.18	7.97	878.4	5270.3
4	13.56	22.39	303.7	1822.0
5	195.11	7.33	1430.8	8584.7
6	16.80	29.78	500.2	3001.4
7	115.30	6.06	698.2	4189.1
8	72.80	23.61	1719.0	10313.7
9	38.78	10.50	407.2	2443.4
10	41.95	10.78	452.1	2712.7
11	276.92	2.44	676.9	4061.5
12	23.14	11.44	264.8	1588.8
13	134.36	8.14	1093.5	6561.0
14	27.36	7.97	218.1	1308.6
15	48.29	7.39	356.8	2141.0
16	58.40	6.33	369.9	2219.2
17	45.41	7.17	325.4	1952.4
18	108.06	4.53	489.3	2935.8
19	51.15	9.67	494.4	2966.6
20	71.59	6.75	483.2	2899.5
21	47.68	7.89	376.1	2256.6
22	42.41	8.11	344.0	2064.0
23	61.56	7.55	464.5	2787.1
24	43.55	10.63	462.9	2777.4
25	24.09	13.31	320.5	1922.9
26	52.99	7.53	398.9	2393.3
27	88.76	7.61	675.5	4053.2
28	84.95	9.96	846.0	5075.8
29	32.80	10.42	341.6	2049.8
30	57.32	8.11	464.9	2789.7
31	41.42	8.33	345.2	2071.2
32	44.04	16.86	742.5	4455.2
33	27.48	7.06	193.9	1163.4
34	100.75	4.83	487.0	2921.8

In addition to the genotype 11, we registered FW values higher than 100.0 g only for 1, 3, 5, 7, 13, 18, and 34. In contrast, for 2, 4, 6, 9, 12, 14, 25, 29 and 33 we registered values of FW lower than 40.0 g. The fruit number per truss (FT), showed high variability among the different accessions, varying from 2.44 fruits for the genotype 11, to 29.78 fruits for the accession 6. The genotypes which showed values of FT higher than 15 fruits, were only 4, 6, 8, and 32. Values lower than 10 fruits were registered for 1, 2, 3, 5, 7, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 26, 27, 28, 30, 31, 33 and 34. Concerning the weight of a single truss (FTW) which it was registered for the first six trusses, it also exhibited elevated variability, ranging from 193.9 g, to 1719.0 g for the genotypes 33 and 8, respectively. In addition to the genotype 8, we registered FTW values higher than 700.0 g only for 1, 3, 5, 13, 28 and 32. On the other hand, values lower than 400.0 g were registered for 2, 4, 12, 14, 15, 16, 17, 21, 22, 25, 26, 29 and 31, 33. Also the fruit production per plant (PFP) varied significantly among all the set of accessions tested, fluctuating form 1163.4 g to 10313.7 g for the genotypes and 33 and 8, respectively. PFP showed values higher than 5000.0 g for the genotypes 1, 3, 5, 13, and 28. Contrarily, for 2, 4, 12, 14, 17, 25 and 33, PFP values lower than 2000.0 g per plant (Table 21).

In relation to the external colour of the immature fruits (FEIC), it was, in accordance with the international descriptors adopted, light green for all the genotypes, apart from the genotypes 3, 5, 7, 11, 12, 13, 15, 18, 23, 24, 25, 27, 28, 29, 30, 32, 33 and 34, for which we registered the greenish white colour. Furthermore, for the fruit green shoulder (FGC) we registered the dark green colour only for the genotype 2, while it was medium green for 4, 6, 9, 10, 12, 14, 15, 16, 17, 19, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 31, 32, 33 and 34 and for 1, 3, 5, 7, 8, 11, 13, 18, 27 it was light green. For the qualitative descriptor related to the fruit pubescence (FP), we registered an intermediate pubescence density for all the tested accessions except for the genotype 1 and 12 for which we registered a dense and sparse pubescence density, respectively. Concerning the exterior fruit colour (FEC), it was red for all the genotypes except for 1 and 16, for which we registered a pink colour, moreover for 17, 18, 20, 30 and 31 we registered the orange colour and finally, only for the genotypes 5 and 26 we detected the yellow colour. With regards the qualitative descriptor of the fruit predominant shape (FPS), we observed the obovate shape only for the accessions 32, in addition for 8, 9, 10, 15, 19 and 25 we detected the hearth shaped one, while for 3, 7, 12, 22, 27 and 34 it was elliptic, furthermore for 5, 6, 13, 20, 23, 24, 26, and 30 it was cylindrical, moreover for 2 and 29 it was rectangular, for 4, 14, 16, 17, 21, 28, 31 and 33 it was circular and finally for 1, 11 and 18 slightly flattened. Concerning the fruit blossom end and scar condition (FBS), in accordance with the IBPGR descriptor analysed, it was closed for all the tested genotypes except for the genotypes1, 18 and 34 for which we detected an open scar at the end of the fruit. On the other hand, in relation to the fruit ribbening at calix end (FRC), we detected for all the genotypes a very weak ribbening, except for the accessions 1, 5, 11, 18, 26 and 34, for which we detected a weak ribbening at calix end.

With regards to the fruit locus number (FLN), we ascertained variability among the evaluated genotypes, varying from 2.00 locules for the accessions 6, 9, 19, 23, 25, 26, 32, 33 and 34, to 6.78 locules for the genotype 11. In addition to the genotypes 11, we detected more than 3.00 locules for the genotypes 1, 3, 5, 13 and 18. Concerning the fruit longitudinal diameter (FLD), it ranged from 2.77 cm to 8.99 cm for the accessions 4 and 7, respectively. We detected FLD values higher than 5.00 cm for the accessions 1, 3, 5, 7, 8,

11, 13, 15, 27, 28, 30 and 34. On the other hand, for the fruit transversal diameter (FTD), we observed a variation between 2.83 cm to 8.44 for the genotypes 4 and 11, respectively. Furthermore, for 1, 3, 5, 8, 11, 13, 18, 20, 27, 28 and 34 we observed FTD values higher than 5.00 cm. For the fruit hardness (FD), we observed an elevate variability among the accessions, and its values varied from 39.38 shore to 72.56 shore for the genotypes 3 and 1, respectively. In addition to the genotype 1, FD values higher than 60.00 shore were detected for the genotypes 7, 8, 15, 20, 23, 26, 27, 28, 30 and 31. Besides, FD values lower than 50.00 shore, were registered for the accessions 2, 3, 4, 5, 6, 9, 10, 11, 13, 14, 18, 29 and 32. In relation to the fruit puffiness (FPA), we registered its absence for all the genotypes apart from the genotypes 5, 20, and 26, for which we registered a slight puffiness, and for the genotype 34 for which we registered the intermediate puffiness value. In relation to the fruit radial cracking (FPRC), it was absent for the genotypes 1, 4, 6, 7, 8, 9, 12, 18, 20, 21, 27, 28, 29, 30, 32 and 34, while, in accordance with the international descriptors, we ascertained the presence of radial cracking in the 5% of the ripened fruits for the genotypes 2, 5, 10, 14, 16, 17, 19, 23, 24, 25, 26 and 33. FPRC spanned between the 5% and the 20% for the accessions 3, 11, 13, 15, 22 and 31 (Table 22).

Table 22. Variation of the merceological fruit quality traits, which were the number of fruit locules (FNL), the lateral and transversal diameter (FLD and FTD, respectively), the firmness (FD), the soluble solid content (SSC) and the acidity expressed in pH and in titratable acidity (FpH and FAC, respectively).

Genotype	FNL	FLD	FTD	FD	FSSC	FpH	FAC
1	3.11	5.08	6.73	72.56	3.72	4.38	0.70
2	2.44	3.68	3.56	43.49	5.00	4.27	0.66
3	5.00	6.40	7.19	39.38	4.47	4.49	0.86
4	2.22	2.77	2.83	48.09	7.60	4.22	0.52
5	4.22	6.73	6.84	41.73	4.37	4.21	0.56
6	2.00	3.08	3.10	48.13	7.70	4.52	0.78
7	2.56	8.99	4.86	69.70	4.18	4.32	0.65
8	2.56	5.56	5.40	62.26	4.78	4.37	0.64
9	2.00	4.21	4.17	44.09	3.77	4.41	0.64
10	2.11	4.93	3.69	44.43	4.20	4.33	0.58
11	6.78	7.46	8.44	49.48	3.90	4.47	0.46
12	2.44	3.53	3.16	53.90	4.42	4.39	0.66
13	4.78	5.69	5.55	47.27	3.80	4.32	0.49
14	2.11	3.49	3.61	47.69	4.37	4.33	0.73
15	2.22	5.02	4.26	60.54	4.55	4.33	0.56
16	2.22	4.48	4.90	59.02	4.77	4.20	0.71
17	2.11	4.29	4.40	58.17	4.70	4.39	0.60
18	5.33	4.74	6.82	49.43	3.35	4.33	0.48
19	2.00	4.97	4.51	57.67	4.02	4.39	0.56
20	2.22	4.97	5.78	61.46	4.98	4.34	0.74
21	2.11	4.42	4.47	53.80	4.72	4.61	0.52
22	2.11	4.59	4.07	55.54	4.85	4.38	0.72
23	2.00	4.60	4.86	63.94	4.55	4.45	0.49
24	2.22	4.43	4.42	51.81	5.23	4.25	0.67
25	2.00	3.75	3.37	52.56	4.48	4.30	0.61
26	2.00	4.69	4.71	68.71	3.27	4.46	1.13
27	2.78	5.72	5.03	64.32	4.27	4.32	0.54
28	2.50	5.62	5.32	67.85	4.63	4.39	0.72
29	2.33	4.30	3.81	45.56	4.85	4.37	0.69
30	2.22	5.04	4.80	65.37	4.40	4.22	1.92
31	2.33	3.84	4.30	67.42	4.67	4.46	1.97
32	2.00	4.69	4.06	42.76	4.75	4.42	0.56
33	2.00	3.68	3.66	50.84	4.67	4.32	0.60
34	2.00	7.67	6.32	51.96	4.75	4.26	0.73

To perform the PCA analysis, we selected the parameters summarizing the greatest variability among the different tested accessions, basing on the bivariate Pearson's correlation. The selected traits were RDMR, LSI, LFl, FTD, FD, FUL*, FUa*, FpH, which adsorbed the 61.95% of the total variability. Subsequently, the correlations of the selected traits with the axes related to the three principal components were analysed. The

correlation between the traits and the three axes is reported in the Table 23. The first axis of the correlation between the selected traits and the three components showed a positive correlation with FUL*, FD, LFl, and a negative one with FUa* (Table 23). The second axis, it was positively correlated with FpH and negatively with RDMR. The third one, was positively correlated with FTD and negatively with LSI (Table 23).

Table 23. Matrix of the three extracted components, resulted selecting the most correlated traits basing of the Pearson's correlation.

Trait	PC1	PC2	PC3
RDMR	0.370	**-0.658**	-0.298
LSI	0.342	0.130	**-0.673**
LFl	**0.530**	0.461	-0.112
FTD	0.324	-0.443	**0.593**
FD	**0.668**	0.200	-0.037
FUL*	**0.883**	-0.089	0.284
FUa*	**-0.801**	-0.164	-0.049
FpH	-0.148	**0.597**	0.38
Var (%)	31.610	16.120	14.220

Through the PCA plot, it is possible to observe the distribution of the analysed genotypes in relation to the correlation of the parameters with each axis. As can be seen from the PCA plot, the genotype 26 (PS47) is distinct from the others due to its high values of fruit hardness FD (which was the highest one registered) and FUL*, in contrast to the reduced value of the chromatic component a* (FUa*) in the first axis of the plot. Additionally, the position of the genotype 26 was higher than the other genotypes, for the intermediate values of the traits FpH and RMDR, which were absorbed by the second axis represented by the second extracted component (PC2). Genotype 30 also appears well differentiated due to the high value of SPAD (LSI) recorded, which was negatively correlated with the third axis of the graph. Genotype 30 also appears to be biased towards axis 1 due to the highest value of FUL* recorded and the low value of FUa*, which is inversely correlated with the first extracted component. Regarding genotypes 3, 7, 8, 9, 10, and 11, they are inversely correlated with the first extracted component (PC1), therefore showing low values of LSI, FD, and high values of FUa*. Genotype 5, on the other hand, is disposed towards the first axis because of its reduced values of fruit hardness (FD) and chromatic component a* (FUa*) and high value of FUL*. Conversely, the genotypes 18 and 34, due to the high divergence of the FUL* and FUa*values of are located towards axis 1.

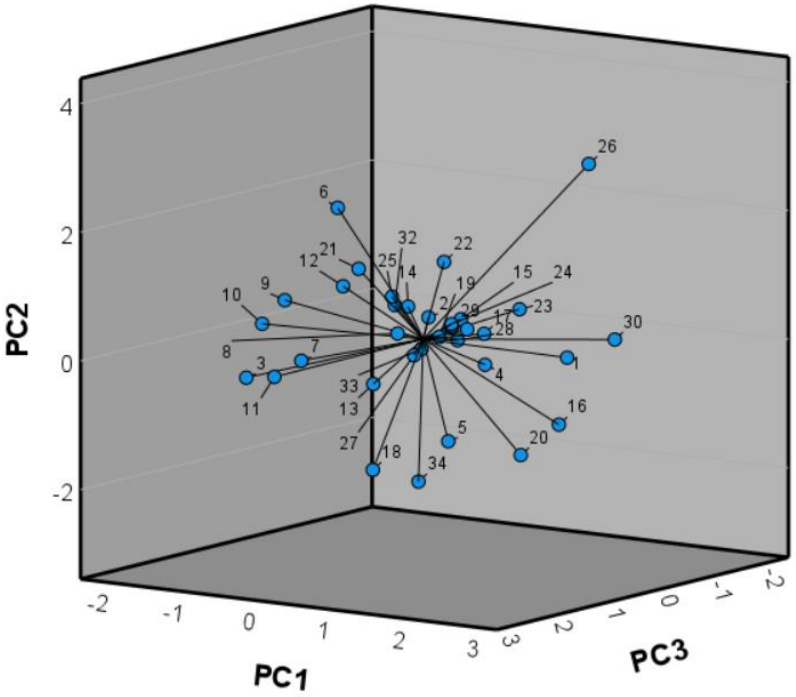


Figure 3. PCA plot for study the genotypes distribution within the three components extracted.

The SPET analysis was carried out for a subset of 13 samples, and the data of the filtered set of 460 molecular markers were elaborated generating a principal component analysis (PCA) with two components extracted. The first axes resumed the 24.50% of the total variance between the examined samples with the selected SNPs, while the second component resumed the 21.15% of the total variance. PCA analysis allowed to calculate the genetic distance, in terms of the proportion of *loci* that are different, three indicated that genotypes 23 (PS35) and 25 (PS44) were quite isolated from all the others. Additionally, also the genotypes 3 (PO163) and 14 (PS1/18) were in the same cluster, basing on the 460 molecular markers selected. With regards to the genotypes 1 (A-RC), 15 (PS12), 17 (PS15-8), 29 (PS59), 31 (PS7) and 34 (SAL), they were clustered in together due to their reduced genetic distance. An additional cluster was represented by the genotypes 27 (PS51), and 28 (PS53) which also exhibited a reduced genetic distance. The hierarchical dendrogram confirm the genetic distance observed for the examined subset of 13 samples. The analysed dendrogram allowed the quantification the degree of genetic differentiation or similarity based on the observed genetic variation in the set of 460 molecular markers. Genetic distance can be quantified in different ways, one of which is as the proportion of loci that are different between two populations or individuals. This measure of genetic distance is also known as the "proportion of differences" or "p-distance. The genetic distance between the genotypes 23 and 25 was about 0.03, it means that the 3% of the examined loci examined by the SNPs markers were different.

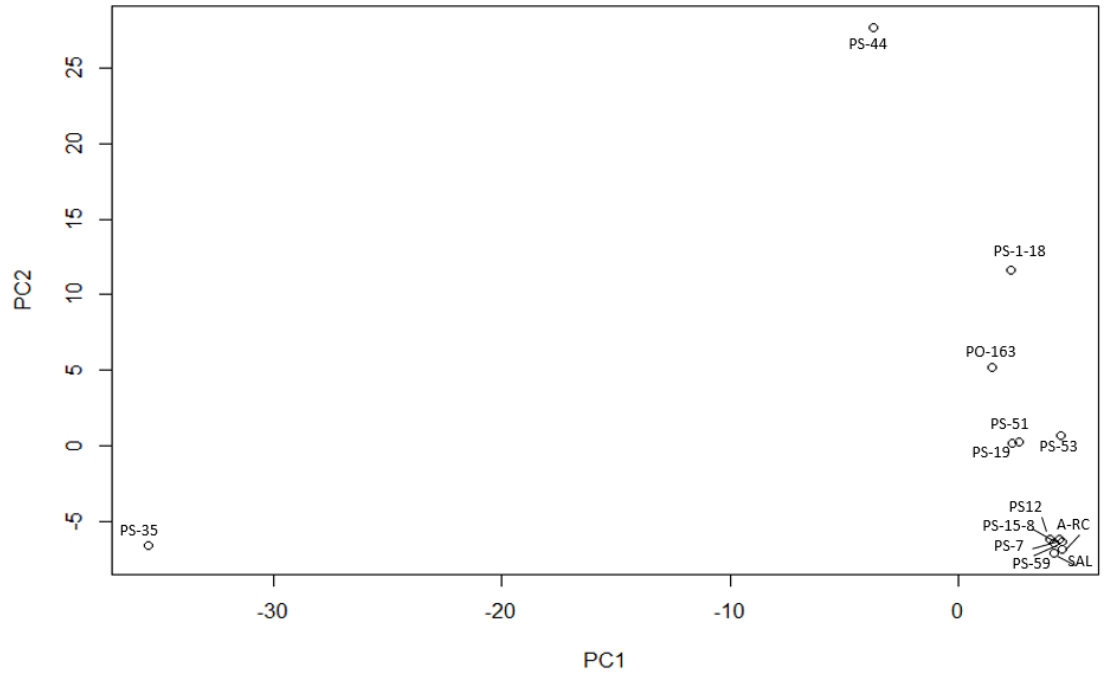


Figure 4. PCA component analysis showing the distribution of the selected genotypes basing on the 460 primers filtered in the SPET analysis.

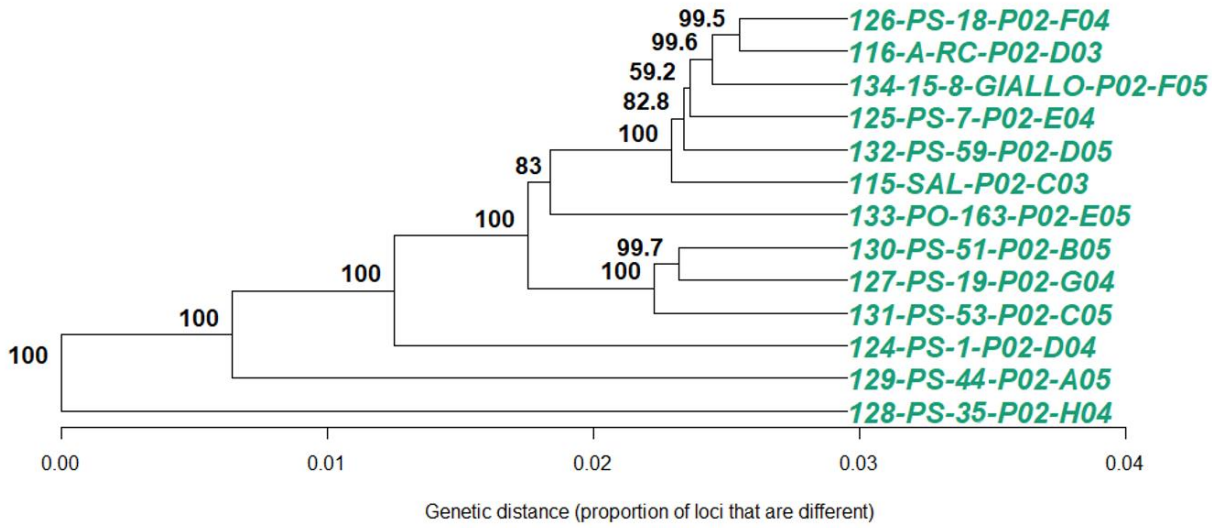


Figure 5. Hierarchical dendrogram based on the genetic distance (proportion of loci that are different) among the examined accessions.

2.3.4. Conclusion

TRIAL A

The comprehensive analysis of the Brassica core collection encompassed all the three PhD years. The activities were performed in the framework of the BRESOV project at the University of Catania. This study successfully differentiated all the studied accessions of Brassica oleracea, identifying 23 morphological traits that exhibited significant variation among these accessions. These traits hold promise for further characterizing Brassica crops. Additionally, the study identified highly diverse accessions, offering valuable opportunities for optimizing parental sources in future breeding programs aimed at developing new and more productive B. oleracea varieties. The utilization of morphological traits proved effective in assessing the diversity and relationships within B. oleracea germplasm.

Furthermore, this morphological characterization of B. oleracea wild relatives (CWRs) proved indispensable, shedding light on a noteworthy level of diversity and aiding in the estimation of phylogenetic relationships. In conclusion, this study marks the initial step toward a comprehensive conservation and improvement plan for Brassica oleracea crops in Italy.

These findings emphasize the importance of incorporating morphological data into conservation planning, while also suggesting the potential utility of further investigations using molecular markers in the plant improvement process.

TRIAL B

The analysis reported in the present chapter of my PhD thesis were carried out during my Erasmus+ Traineeship at the Polytechnic University of Valencia (UPV) in the academic year 2021-2022, aimed to evaluate the high varietal diversity of *S. lycopersicum* L. accessions belonging to the gene bank of the Horticulture and Floriculture Section of the Di3a of the University of Catania. The characterization of the 34 tomato accessions allowed to highlight on the remarkable diversification for the main morphological traits of the plant, the qualitative traits of the fruits. The previous cited analysis was supported also by the study of the genetic distance in a selected subset of accessions.

Regarding the plant, we focused on the variability of the size and structure of the root system, which are important traits in relation to the ongoing climate changes and the reduction of the water resources. A larger root system would allow for a deeper exploration of soil, determining a greater water and mineral absorption, as well as better anchoring the plant to the soil substrate. In the present work, we observed crucial changes in the structure of the plant epigeal part in relation to the vegetative biomass of the stem and leaves. Furthermore, we ascertained significant variations for the SPAD index (LSI), representing a useful measurement for evaluating the nutritional status of the plant. The highest LSI value was observed for genotype 23 (PS35), which showed a value of 57.78. The accessions that showed LSI values higher than 50.0 were the genotypes 1 (A-RC), 15 (PS12), 22 (PS28), 23 (PS35), 26 (PS47), 28 (PS53), 29 (PS59), 30 (PS6), and 32 (PS7/10). As concern the characteristics of the reproductive organs, significant differences were observed in relation to the FlSP descriptor (style position), an important trait that can determine a significant increase in terms of allogamy, which was found to be inserted for all genotypes, except for accessions 1 (A-RC), 6 (PO166), 11

(PO5), 25 (PS44), and 28 (PS53), which showed the same level as the stamens. The fruit characteristics appeared diversified in both type and organoleptic quality, and the most interesting resulting descriptors were FEC (external colour of the fruits), FPS (predominant fruit shape), and FD (fruit hardness). The most interesting genotypes regarding FEC were genotypes 1 and 16, which had a pink colour, genotypes 17 (PS15/8), 18 (PS20), 20 (PS26), 30 (PS6), and 31 (PS7) for orange colour, and genotypes 5 (PO164) and 26 (PS47), which had a yellow colour, while all other accessions showed a red colour. Regarding the FPS qualitative descriptor, which demonstrates the great varietal multiplicity of the tested accessions, different shapes of tomato berries were observed, such as the oval shape for accession 32, the heart shape for accessions 8, 9, 10, 15, 19, and 25, the ellipsoid shape for accessions 3, 7, 12, 22, 27, and 34, a cylindrical shape for accessions 5, 6, 13, 20, 23, 24, 26, and 30, a rectangular shape for accessions 2 and 29, a circular shape for accessions 4, 14, 16, 17, 21, 28, 31, and 33, or a slightly flattened shape for accessions 1, 11, and 18. Regarding fruit hardness (FD), a high variability was observed among the accessions, with the maximum value of 72.56 shore being recorded for genotype 1 (A-RC).

The highest fruit yield per plant (PFP) was calculated for the genotype 8 (PO226) with 10313.7 g followed by the genotypes 1 (A-RC), 3 (ITALIAN LINE COR B), 5 (PO164), 13 (PS05), and 28 (PS53), respectively, which showed PFP values higher 5000.0 g.

With regard to the traits determining the tomato organoleptic profile, such as the sweetness (SSC) and the acidity (FpH and FAC), expressed by the °Brix and through the pH index and titratable acidity respectively, genotypes 6 (7.70 °Brix) and 2, 4, 8, 15, 16, 17, 20, 21, 22, 23, 24, 28, 29, 31, 32, 33, and 34 (SSC values higher than 5.50 °Brix) and genotypes 16 (PS13), 21 (PS27), and 31 (PS7) were selected for future evaluations in relation to the sensorial traits of the fruit.

With regard to the examined genetic traits such as the *Sw-5* and *Tm2* loci, we didn't observe high variability among the examined accessions, except for the accession 6 (PO166), for which we detected the heterozygosity of the Tm2 locus, denoting that the previous-mentioned genotype was object of breeding program for the introgression of this gene, due to represent a Californian tomato commercial variety. Additionally, the Single Primer Enrichment (SPET) analysis carried out in Valencia, allowed the differentiation of the accessions 23 and 25, basing on the proportion of different loci.

Overall, this study showed the high variability of *S. lycopersicum* L. accessions belonging to the Vegetable Crops and Floriculture Section of the Di3A of the University of Catania, with significant differences observed for various morphological and qualitative characteristics of the plant and fruit. These findings provide useful information for the selection of suitable tomato varieties for specific environmental conditions and market demands, and for the development of breeding programs aimed at improving the yield, quality, and adaptability of tomato crops.

2.4. Research line II

INDIVIDUATION OF THE SOURCE OF RESISTANCE
IN B. OLERACEA COMPLEX SPECIES (n = 9) L.
AND S. LYCOPERSICUM L. CROPS.

2.4.1 Introduction

EXPERIMENTAL TRIAL A

Water deficiency represents an increasingly urgent worldwide issue, particularly in areas where agriculture is a major economic activity. The increasing global population exerts a mounting pressure on the agricultural sector for producing more food using less water (Hussain *et al*., 2019; D'Odorico *et al*., 2020). The reduction of water uses in agriculture, the strategies for conserving water resources, improving agricultural productivity and enhancing drought stress resistance, are the new frontier of agriculture, mostly in relation to the incoming climatic changes (Parkash *et al*., 2020).

Through the adoption of innovative water management techniques and the use of drought-resistant crop varieties, it is possible to significantly reduce water use in agriculture while maintaining or even increasing crop yields. This approach can help to build resilience in the face of increasingly unpredictable weather patterns and help farmers adapt to the challenges of climate change (Ricart *et al*., 2019). Nowadays, the crops used for modern agriculture show a limited genetic diversity due to the strong selection occurring for the domestication process, resulting with a less adaptation to the environmental factors and to the new extreme climatic conditions (Zhang *et al*., 2017; Gaut *et al*., 2018).

Brassica oleracea L. complex species (n = 9) is a worldwide vegetable spread all over the world for their nutraceutical activities. Through its crops, it represents a source of biochemicals compounds suitable for the human health, such as glucosinolates (GLSs), polyphenols (PPs) and ascorbic acid (Asa) which determinate high antioxidant capacity (Picchi *et al*., 2020; Arena *et al*., 2022; Di Bella *et al*., 2022). In several *Brassica oleracea* crops such as broccoli (*B. oleracea* var. *italica*), cauliflower (*B. oleracea* var. *botrytis*) and kale (*B. oleracea* var. *acephala*), occurred the natural introgression of different traits from the different crop wild relatives (CWRs) species and this was the case of Sicily, which represents a hotspot for their diversification (Maggioni *et al*., 2010).

Brassica CWRs, in contrast to the cultivated crops, represent a source of resistance for abiotic and biotic stresses due to their several alleles lost during the domestication process, and they can be useful in breeding for transferring adaptive traits to the cultivated ones (Treccarichi *et al*., 2023).

As reported by several authors, the bioactive compounds responsible of the above-mentioned properties, such as GLSs and PPs, have been found in high percentage in the CWRs species, and their exploitation can be useful for transferring high nutraceutical traits to the cultivated species, generating lines having added values (Branca and Maggioni, 2020). In *Brassica oleracea* crops, GLSs and PPs change in the different organs as a consequence of environmental stresses such as drought, as reported by different works (Ben Ammar *et al*., 2022; Ben Ammar and Arena *et al*., 2023). *B. macrocarpa* Guss is a rare Brassica CWR

which grows exclusively in the Mediterranean cliffs and in the rocky slopes of the Egadi Islands of Marettimo (TP) and Favignana (TP). It usually grows near the sea, and it is endangered by the fire and the wild animal grazing activities (Branca *et al*., 2012). *B. macrocarpa* was used by Argento *et al*. (2019) for the agroecological management of soilborne disease, in particular against *Meloidogyne* spp., due to its high amount of sinigrin, usually used as the basis for the biofumigant products.

Zhang *et al.* (2016) performed a comparative transcriptomic analysis with broccoli to detect resistance against *Plasmodiophora brassicae*, and they found differentially expressed genes associated with cell wall and GLSs biosynthesis which were involved in *P. brassicae* resistance. Plants are constantly exposed to various environmental stresses such as drought, heat, cold, salinity, and pathogens, which can have a significant impact on their growth and productivity. Transcriptomic is a biotechnological analysis that allows researchers to identify the genes and pathways that are activated or repressed in response to these stresses, and to elucidate the complex regulatory networks that underlie these responses (Imadi *et al*., 2015; Sicilia *et al*., 2019; Santoro *et al*., 2022). Moreover, transcriptomic analysis can also be used to identify molecular markers that are associated with stress tolerance and to develop crops with improved stress tolerance through conventional or traditional breeding programs (Agarwal *et al*., 2014). The previous mentioned aspects could have important implications for food security, as climate change and other environmental challenges continue to threaten global crop production. Overall, transcriptomic analysis is a powerful tool that has revolutionized our understanding of plant stress responses and has the potential to drive the development of stress-tolerant crops, which is essential for sustainable agriculture and food security in the face of global environmental change. Transcriptomic analysis was performed in Brassica crops by several authors, performing RNA sequencing techniques or developing a microarray chip, to detect the genes involved in different stress responses (Lee *et al*., 2008; Eom *et al.*, 2018*;* Dai *et al*., 2022).

In the present work, a comparison between the differentially expressed genes has been performed in *B. oleracea* var. *italica* and *B. macrocarpa*, in relation to the drought stress application. The evaluation was done with the whole leaf transcriptome of the previously cited species by using RNA sequencing approach to investigate among the different pathways involved in the drought stress response.

EXPERIMENTAL TRIAL B

Grafting is widely recognized as an effective technique for improving horticul-tural crop production. It represents a powerful tool for controlling pests and diseases that are otherwise difficult or costly to manage (Maurya *et al*., 2019; Thies *et al*., 2021). This is particularly relevant for soil-borne pests and diseases, where genetic resistance or tolerance of cultivars may not be a viable solution for commercial or agronomic reasons (Argento *et al*., 2019; Ayala-Doñas *et al*., 2020). The use of grafted plants is therefore a promising and eco-friendly alternative for managing biotic stressors in ag-riculture, without relying on chemical inputs that can cause harmful effects to the soil health (Suansia and Samal, 2021). Therefore, grafting has the potential to be a valuable tool for organic agriculture in which, the use of chemical products is totally denied (Moreno *et al*., 2019; Caradonia *et al*., 2023).

This technique is particularly beneficial for high-value Solanaceae and Cucurbita-ceae cultivars that are sensitive to the pathogens affecting the root system (King *et al*., 2010; Keatinge *et al*., 2014; Cardarelli *et al*., 2020; Attavar *et al*., 2020). The plantlet used as scion is selected for its desirable merceological traits while the plantlet used as rootstock is selected for its strong root system. The scion plantlet is then cut and attached to the rootstock one, allowing the two plants to grow together as a single, grafted plant (Johnson *et al*., 2020). This technique enables growers to combine the desirable traits of the scion plantlet with the strong root system of the rootstock plantlet, resulting in a stronger and more resilient plant overall.

For tomato production, grafting has become increasingly important due to it can provide several benefits to growers. Specifically, by grafting a susceptible scion onto a resistant rootstock, it is possible protect the crop from a range of soil-borne diseases, such as Fusarium wilt, Verticillium wilt, and bacterial wilt (Acharya *et al*., 2020; Testen *et al*., 2021; Chitwood-Brown *et al*., 2021). Grafting can also improve the overall vigor and productivity of tomato plants. This is because the rootstock can provide a stronger and more extensive root system, which in turn can support better plant growth and development (Rahmatian *et al*., 2014). The selection of the proper rootstocks can also play a role in nutrient and water uptake and use efficiency in grafted tomato plants. Several studies have shown that grafted tomato plants may have an improved ability to take up and utilize nutrients, particularly nitrogen, compared to non-grafted plants (Albornoz *et al*., 2020; Zhang *et al*., 2020). This improvement in nutrient uptake and use efficiency is thought to be due to several factors, including the stronger and more extensive root system provided by the rootstock, as well as potential changes in the physiology and metabolism of the scion cultivar. The optimization of the nutrient management by the rootstocks could result in cost savings for fertilizers, particularly given the significant price increases that have resulted from the Ukrainian war (Shahini *et al*., 2022; Arndt *et al*., 2023). Furthermore, some rootstocks may be better suited to specific soil conditions, such as high salinity or alkalinity, which can also affect nutrient availability and uptake (Kumar *et al*., 2016; Ribelles *et al*., 2019; Singh *et al*., 2020; Kalozoumis *et al*., 2021). In addition, grafting can enhance the quality and flavor of tomato. Several authors focused on the effects of the grafting technique for enhancing the quality of the tomato fruits, in-creasing the soluble solid content, the titratable acidity, and the enhancing the phenol-ic compounds profile (Flores *et al*., 2010; Sánchez-Rodríguez *et al*., 2012; Ozturk *et al*., 2019).

The use of rootstocks can be particularly important in winter tomato cultivation, as the lower temperatures and reduced sunlight can make the plants more susceptible to cold stress and disease. Although a tomato cultivar or rootstock that can withstand cold temperatures is not available, growers must rely on currently available commer-cial tomato rootstocks (Suchoff *et al*., 2018). However, these rootstocks have typically been developed to resist pathogens and tolerate high temperatures and salinity, rather than low temperatures. There is limited information available on how these rootstocks perform under suboptimal growing conditions (Venema *et al*., 2008; Riga *et al*., 2015).

The use of interspecific rootstocks is more common in tomato grafting, as they can provide high disease resistance by the introgression of several resistant genes, in addition to enhance the plant vigor. However, intraspecific rootstocks can also be used in some cases, particularly if the goal is to improve specific

traits such as yield or fruit quality. The identification of the genetic material resistant to biotic stress or having multiple traits suitable for organic agriculture can be facilitated using molecular markers. These markers represent a powerful tool for breeding and can be a helpful technique for identifying desirable genetic traits (Esposito *et al*., 2020; Tripodi *et al*., 2021; Treccarichi *et al*., 2021; Prohens *et al*., 2021; Fonseca *et al*., 2022; Treccarichi *et al*., 2023). Nowadays, tomato breeding activities have benefited from the use of several molecular markers such as *Sw-5*, *Tm-2*, *I-2*, *V3*, and *Py1*. These markers enable the se-lection of genetic material that is resistant to both air-borne and soil-borne diseases (Arrones *et al*., 2020; Andolfo *et al*., 2021; Vanlay *et al*., 2022).

The aim of the present study is to investigate the behavior and effects of both in-ter- and intraspecific rootstocks on tomato plant growth and development. Specifically, this study aims to analyze plant bio-morphometric traits and assess fruit produc-tion and quality. The rootstocks used in this study were developed through traditional breeding techniques, which were supported by the detection of several resistant genes using molecular markers. The scions used were three commercially available F1 hybrids, namely Barbarela, Cherry, and Vittorio, which are widely adopted by the tomato growers in all the European continent. The study was conducted in a Mediterranean greenhouse in Sicily, in accordance with organic farming protocols.

2.4.2. Materials and Methods

EXPERIMENTAL TRIAL A

Plant material includes 89 Brassica genotypes used for the preliminary screening belonging to the active Brassica collection of the Department of Agriculture, Food and Environment (Di3A) of the University of Catania (UNICT), as shown in Table 24. The trial was performed at the University of Catania during summer 2020, in organic farming conditions. Plantlets were sown in cellular trays collocating one seed per hole, using the organic soil substrate for sowing BRILL®semina bio (Geotec, Italy). Plantlets were well irrigated from the sowing date until the emission of the third true leaf. Then, irrigation was stopped for five days, with the exception of the control blocks. Plantlets were characterized for their morphometric traits which were the number of total, chlorotic and dry leaves and for their chlorophyll content by the SPAD-502 (Konica Minolta Optics, Japan). In addition, plants were scored for their drought stress resilience applying a numerical score attributing 0 for the most sensitive plantlets, 1 for the intermediate resistant ones and 2 for the highly resistant ones.

Table 24. List of the accession used for the drought stress screening.

Species	Common name	Number of accession	Provider
B. oleracea var. italica	Broccoli	33	29 from UNICT 4 from UNILIV
B. oleracea var. botrytis	Cauliflower	13	UNICT
B. oleracea var. italica x botrytis	Composite cross population	10	From CREA and UNICT
B. oleracea var. capitata	Cabbage	2	1 from UNICT 1 from UNILIV
B. oleracea var. acephala	Kale	12	7 from UNILIV 5 from UNICT
B. oleracea var. gongylodes	Kohlrabi	8	5 from UNILIV 2 from VURV 1 from UNICT
B. oleracea var. alboglabra	Chinese kale	1	UNILIV
B. oleracea var. gemmifera	Bruxell sprouts	1	UNILIV
B. rupestris	CWR	1	UNICT
B. incana	CWR	4	UNICT
B. villosa	CWR	1	UNICT
B. macrocarpa	CWR	4	UNICT
Total number		**89**	

After the preliminary screening, four genotypes were selected: two resistant and two sensitive to water stress, and they are listed in Table 25. The selection was carried out on the basis of their variation of SPAD index and total leaves number values. The stressed plantlets were irrigated until the total soil saturation, and they were recovered for 18 days irrigating the trays daily. The selected genotypes were transplanted from the alveolar trays and placed into 20 cm diameter pots having the hydraulic capacity of 4,5 L for the further stress application, which was applied ten days after the transplanting (Figure 4). The tested genotypes were underlay under two different irrigation conditions (IR). The irrigated control genotypes represented the IRR condition, while the stressed ones were the not irrigated (NIR) ones. The drought stress was applied for seven days, while the control genotypes were well irrigated, applying 50 cl of water per pot, per day. During the stress application

period, the mean, maximum and minimum temperatures were recorded by Servizio Informativo Agrometereologico Siciliano (SIAS). At the seventh day, plants were characterized for their total, chlorotic and dry leaves in addition to their SPAD index and to the morphometric traits such as the leaf lamina area (cm^2), the leaf length (cm) and the leaf width (cm). Fresh samples were collected and stored at -80°C for further analysis.

Table 25. Subset of the accessions resistant and sensitive to the drought stress.

Accession	Crop code	Species	Origin
UNICT 5088	BM	B. *oleracea* var. *italica*	Modica (RG)
UNICT 5081	BS	B. *oleracea* var. *italica*	Modica (RG)
UNICT 3365	MF	*B. macrocarpa*	Favignana (TP)
UNICT 5124	MM	*B. macrocarpa*	Marettimo (TP)

Figure 4. Control(a) and stressed (b) accession analyzed for the drought stress trial.

For assessing the drought stress tolerance or sensitivity, the selected set of plants was analyzed for their Malonaldehyde (MDA) and hydrogen peroxide (H2O2) amount, which represent metabolites related to the oxidative stress response. The amount MDA was calculated following the protocol provided by Lopez-Hidalgo *et al*. (2021), while the H2O2 was calculated by the protocol of Velikova *et al*. (2000).

Leaves, kept frozen by continuously liquid nitrogen adding, were ground using precooled mortar and pestle. Total RNA was isolated by using the extraction protocol Spectrum Plant Total RNA kit (Sigma-aldrich®, Saint Louis, MO, USA). RNA degradation and contamination were monitored by electrophoresis with 1% agarose gel. RNA purity and concentration were assayed using the NanoDrop spectrophotometer (TermoFisher Scientifc, Waltham, MA, USA). Before being sequenced, the RNA samples were subjected to quality parameter evaluation. RNA integrity was assessed using the Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA, USA).

One µg of RNA was used as input material for library preparations (eighteen libraries: three biological replicates x two varieties x three sites). Sequencing libraries were generated using NEBNext ®Ultra ™ RNA Library Prep Kit for Illumina® (New England Biolabs, Ipswich, MA, USA) following manufacturer's recommendations26. Briefy, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Bufer (5X). First strand cDNA was synthesized using random hexamer primer and M-MuLV Reverse Transcriptase (RNase H) as synthesizing enzyme. Second strand cDNA synthesis was subsequently performed using RNase H to insert breaks into the RNA molecule and DNA Polymerase I as synthesizing enzyme. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities. After adenylation of 3′ ends of DNA fragments, NEBNext Adaptor with hairpin loop structure were ligated to prepare for hybridization. In order to select cDNA fragments of preferentially 150~200 bp in length, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, MA, USA). Then 3 µl USER Enzyme by NEB were used with size-selected, adaptor-ligated cDNA at 37 °C for 15 min followed by 5 min at 95 °C before PCR. Ten PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer. Finally, PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system.

Cluster generation and sequencing were performed by Novogene (UK) company Limited (25 Cambridge Park, Milton Road, Cambridge, CB4 OFW, United Kingdom). The clustering of the index-coded samples was performed on a cBot Cluster Generation System using a PE Cluster kit cBot-HS (Illumina). After cluster generation, the library preparations were sequenced on Illumina HiSeq2000 platform to generate paired-end reads whose size was paired-end 2×150 bp reads.

For each cultivar we had three stressed and three control replicates. For each of them we employed a custom pipeline for plant RNAseq analysis. First of all, we used Trimmomatic 28 v. 0.39 to filter poor quality reads and adapters. After that, we have chosen STAR29 as our gene-level aligner which also provides raw counts without the need of an intermediary program to analyze the BAM files. The STAR indexes have been built using Brassica Oleracea TO1000DH3 as a reference genome. The differential expression analysis has been carried out using R(v. 4.2.2.) with the packages edgeR30 and limma31. The genes have been considered significantly deregulated only if the adjusted pvalue was < 0.05. Each gene has been annotated using Uniprot32 through the R package UniprotR33 (v. 2.2.2). When the annotation was not available in Uniprot, BlastKoala34 was used to input the gene function searching for a high similarity percentage with other plants. The enrichment analysis has been carried out employing clusterProfiler35 (v. 4.6.0), AnnotationHub36 (v.3.6.0), Biomartr37 (v.1.0.2), and AnnotationDbi38 (v. 1.60.0). Moreover, we conducted Principal Component Analysis (PCA) on phenotypical and physiological characteristics employing the R package Factoextra39 (v.1.0.6).

EXPERIMENTAL TRIAL B

The experimental trial deals with the evaluation of tomato rootstock started in September 2022, grafting the scions onto the experimental rootstocks. The rootstock evaluated were the interspecific cross BT02220 x BT00230, and the intraspecific BT04060 x BT02310 and BT10170 x BT00120 while the

interspecific commercial rootstock named Optifort was used as control. The three experimental rootstocks were provided by the Polytechnical University of Valencia (UPV) which developed the fol-lowing hybrids showing deep and vigorous root system (Table 26).

Table 26. List of the experimental rootstock evaluated for the trials.

Name	Code	Female parent	Male parent
BT02220 x BT00230	A	De colgar tomato	*Solanum pimpinellifolium*
BT04060 x BT02310	B	Tomata Valenciana	De colgar tomato
BT10170 x BT00120	C	Tomato molese	*Solanum habrochaites*

Additionally, the rootstock hybrids were genotyped and selected at the UPV for the resistance genes for the most important tomato massive diseases, which were the *Tm-2* gene for the resistance against the, furthermore the *Sw-5* for the resistance against the tomato spotted wilt virus (TSWV) and finally for the *I-2* gene for the resistance against the soilborne Fusarium oxysporum f. sp. lycopersici. The molecular markers used for the genotyping is listed in Table 27.

Table 27. List of the molecular markers used for selecting the rootstocks by the presence of the resistant genes.

Name	Primer forward	Primer revers
Tm2_SNP2	CAAGCATGTAACAGTTGCTTTTC	CAGGTATCCACATCAAGGTTTG
Sw5(2)-	AATTAGGTTCTTGAAGCCCATCT	TTCCGCATCAGCCAATAGTGT
I-2	CAAGGAACTGCGTCTGTCTG	ATGAGCAATTTGTGGCCAGT

All the experimental rootstock uti-lised were developed in the frame of the H2020 Breeding for Resilient, Efficient, Sus-tainable Organic Vegetables production (BRESOV) project.

The rootstocks used in this study were carefully chosen for their strong agronomic performance, following an evaluation cycle conducted in 2021 in Pachino (SR, Sicily) and Almeria by the University of Catania (UNICT) and University of Almeria (UAL), respectively. The selection process involved 21 inter and intraspecific hybrids developed by the Polytechnical University of Valencia (UPV). The evaluation cycle carried out in Sicily in 2021 was thoroughly documented in the work of Treccarichi *et al*. (2021).

Plant material includes three tomato commercial cultivar utilized as scion, which were: Cherry F1 (Green seeds), Vittorio F1 (Blumen) and Barbarela F1 (Vilmorin). Sowing was carried out the 6th of September 2022 in a nursery called Area Verde Vivai (36°42’02’’ N, 15°04’28’’ E). The grafting activity for combine the three experimental rootstock and the control Optifort with the scions was carried out in the same nursery the 22nd of September 2022. Additionally, the non- and self-grafting plants (NI and AU) were included in the trial.

The plantlets were transplanted the 3rd October 2022 in a cold greenhouse in the organic farm named ECONATURA S.S. Agricola (36°48’04’’ N, 14°35’09’’ E). The plants were transplanted at the crop density of 0.30 m x 1.5 m (about 3 plants m^{-2}) and a plastic mulching was applied for the weed management. The growing cycle was carried out in organic farming conditions, including the phytosanitary and nutritional treatments. Plants were grown pruned at single stem.

During the growing cycle the date of the third flower in anthesis of each truss, un-til the eight one, were registered. Additionally, the morphometric traits which are listed in Table 28, were analyzed for each grafting combination.

Table 28. List of descriptors used for the trial with their relative codes.

Code	Descriptor
PV	Plant vigour (1-9)
PH	Plant height (cm)
PSD	Plant stem diameter (cm)
PBD	Plant basal diameter (cm)
PLL	Plant leaf length (cm)
PLW	Plant leaf width (cm)
PSDM	Plant stem dry matter (%)
RWE	Root weight (g)
RWI	Root width (cm)
RMA	Root main angle (°)
RMD	Root diameter of the first main root (mm)
R2MD	Root diameter of the second main root (mm)
R3MD	Root diameter of the third main root (mm)
RNS	Nematodes score (0-5)
RDM	Root dry matter (%)
FTW	Fruit truss weight (g)
FS	Fruit shape (1-9)
FW	Fruit weight (g)
FLD	Fruit longitudinal diameter (cm)
FTD	Fruit transversal diameter (cm)
FUL*	Fruit chromatic parameters (CIEL*)
FUa*	Fruit chromatic parameters (CIEa*)
FUb*	Fruit chromatic parameters (CIEb*)
FLN	Fruit locules (n)
FPT	Fruit peel thickness (mm)
FFN	Fruit firmness (N)
FSSC	Fruit soluble solid content (°Brix)
FPh	Fruit acidity (pH)
FT	Number of fruits per truss (n)
FGP	Green fruits per truss (%) (for the 5^{th} trusses)
FPP	Fruit production per plant

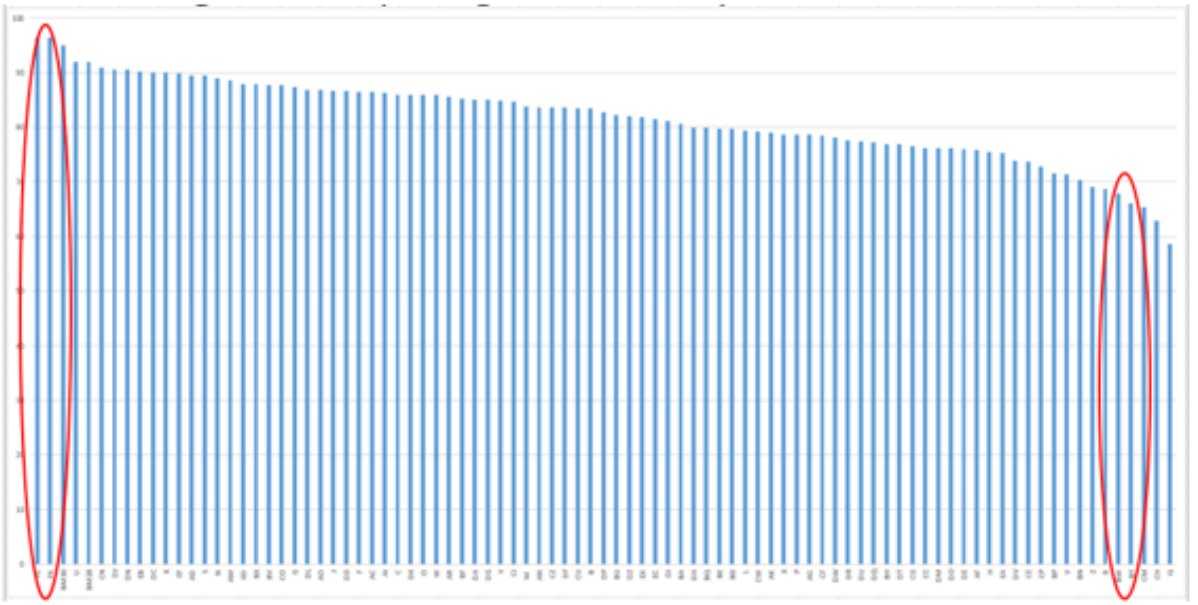
For the analysis of the fruit traits, the chromatic parameter was registered by the colorimeter (Chroma meter CR-200, MINOLTA, Japan), while for the detection of the fruit firmness we used the penetrometer (FT 327, QA Supplies, USA) by manually punching the analyzed fruits in correspondence to the fruit equatorial line and re-cording the maximum force of resistance to pressure applied. As concern the qualita-tive analysis of the fruits, for the fruit soluble solid content (FSSC) was used the digital refractometer (DBX-55A, ATAGO, Italy), while for the fruit acidity (FpH) was assessed the hydrogen-ion activity by the pH meter (inoLab level 3 with level 3 terminal, WTW group, Xylem analytics).

Data were analysed by the analysis of variance (ANOVA) by the Newman-Keuls method, and it was performed by the CoStat software version 6.4, considering the three repetitions per each rootstock-scion combination. Subsequently, the means for each repetition were used for carrying out the Pearson's correlation among all the ex-amined traits, and the Principal Components Analysis (PCA) by the extraction of the three main components. Pearson's correlation and PCA were performed by IBM SPSS version 27 software.

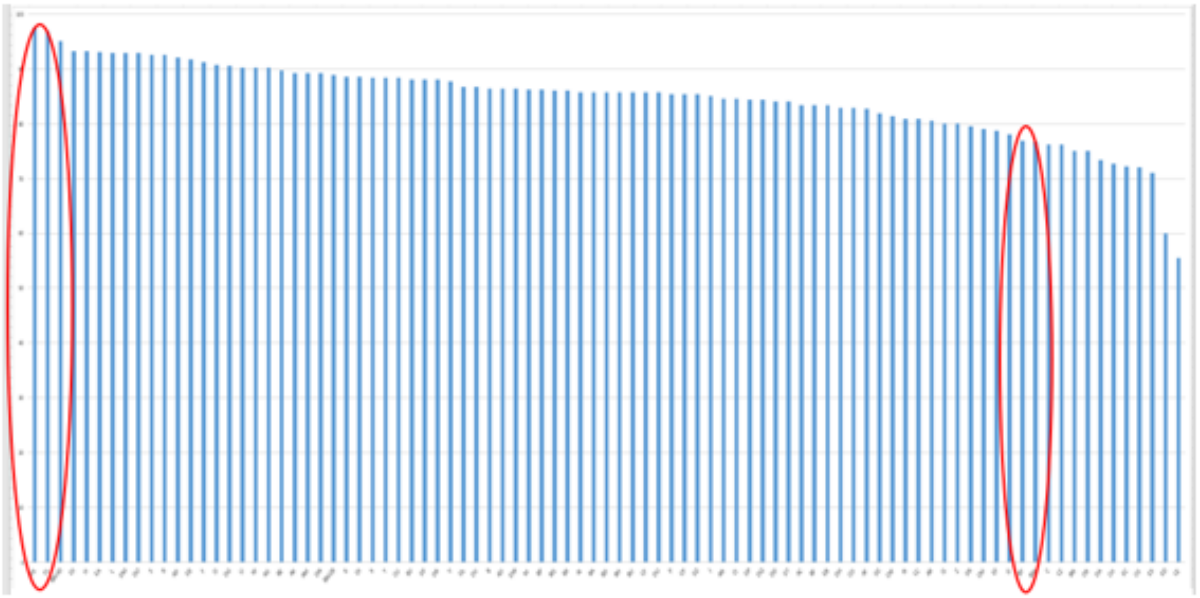
2.4.3. Results and Discussion

EXPERIMENTAL TRIAL A

The selection of the most resistant and susceptible genotypes was carried out on the basis of the SPAD index and the total leaves values (Figure 5a; Figure 5b).



a



b

Figure 5. Selection of the genotypes basing on the drought stress trial on the basis of the SPAD (a) and the total leaves number (b).

the number of total leaves ranged from 8.0 for MM to 9.67 for BS and MF grown in irrigated conditions (IRR), while it varied from 7.0 to 8.33 for BM and BS grown both in not irrigated conditions (NIR), respectively (Table 29). The variation observed for the total number of leaves was significant for the different irrigation applied (IR), but not for the genotype. With regards to the number of chlorotic leaves, it showed a reduced variance in IRR condition, varying among the examined genotypes from 0.0 to 0.33 but in contrast, it showed the largest variation in NIR condition, fluctuating from 2.0 to 3.67 leaves. We ascertained a significant variation of the number of chlorotic leaves for the IR applied and the genotype tested. Concerning the number of dry leaves, it varied in both the irrigation conditions (IRR and NIR), from 0.0 for the irrigated BM and MF, to 1.33 leaves for BM grown in NIR condition. In relation to the IRR set of genotypes, the SPAD index showed a not significant variation, ranging from 47.87 to 48.47 for MM and BM, respectively. In the NIR accessions, higher variation was observed for the SPAD index, and its value varied from 32.37 for the drought stress sensitive BS, to 53.93 for the tolerant MF (Table 29). We observed a significant variation among the tested accessions in relation to the SPAD index, both for the IR applied and to the genotyped tested. The leaf area exhibited a significant variation among the tested genotypes and among the irrigation applied. In the control genotypes fluctuated from 40.03 cm^2 to 58.40 cm^2 for MM and BM, respectively, while it varied in the stressed genotypes (NIR) from 38.33 cm^2 to 47.55 cm^2 for MF and BS, respectively. With regards to the leaf lamina length, it ranged from 7.73 cm to 9.70 cm for MM to BS grown both in IRR conditions (Table 29). On the other hand, the above-mentioned parameter, in the NIR conditions, varied from 7.68 cm to 8.59 cm for MM and BM, respectively (Table 29). Concerning the leaf width values, they fluctuated from 5.24 cm to 8.06 cm for MM and BM both in IRR condition, while it ranged from 5.20 cm to 6.15 for MM and BS grown both in NIR condition (Table 29).

Table 29. Variation of the morphometric data related to the tested genotypes (GE) both in irrigated (IRR) and not irrigated (NIR) conditions.

GE	Total leaves (n)		Chlorotic leaves (n)		Dry leaves (n)		SPAD index		Leaf area (cm^2)		Leaf lenght (cm)		Leaf width (cm)	
	IRR	NIR	IRR	NIR	IRR	NIR	IRR	NIR	IRR	NIR	IRR	NIR	IRR	NIR
BS	9,67	8,33	0,33	3,33	0,33	0,33	48,10	32,37	52,51	47,55	9,70	8,19	7,18	6,15
BM	8,67	7,00	0,33	3,67	0,00	1,33	48,47	35,53	58,40	45,91	9,38	8,59	8,06	5,54
MF	9,67	8,00	0,00	2,00	0,00	0,33	48,43	53,93	40,90	38,33	7,88	7,82	5,47	5,72
MM	8,00	8,00	0,00	3,00	0,33	0,33	47,87	42,90	40,03	38,83	7,73	7,68	5,24	5,20
Significancy of the differences by ANOVA Student-Newman-Keuls														
	Total leaves		Chlorotic leaves		Dry leaves		SPAD index		Leaf area		Leaf lenght		Leaf width	
IR	**		***		ns		**		**		**		**	
GE	ns		*		ns		*		***		***		***	
IR x GE	ns		ns		ns		*		ns		ns		**	

The content of the malondialdehyde compound varied among the tested genotypes between the IRR and NIR conditions. The two B. oleracea genotypes showed the highest MDA variation between the IRR and NIR conditions. The genotype BM exhibited a MDA content of 1.63 ng mg^{-1} in irrigated condition while it registered 2.02 ng mg^{-1} in absence of watering. With regards to the BS landrace, it showed an amount of MDA of 2.13 ng mg^{-1} for the control, while, as consequence of the application of the drought stress, the MDA content increased, and it was 4.7 ng mg^{-1}. BS showed the highest variation in terms of the MDA, from the control irrigated (IRR) to the stress condition (NIR). The two B. macrocarpa genotypes showed the lowest variation

between the IRR and NIR conditions, in comparison to the two B. oleracea landraces. The total amount of the malondialdehyde metabolite for MM grown in IRR was 1.593 ng mg^{-1} while the same genotype grown in NIR condition registered 1.815 ng mg^{-1} of MDA metabolite. With regards to the MF genotype, it showed a MDA level of 1.16 ng mg^{-1} in well irrigated condition in contrast to the 1.63 ng mg^{-1} registered in water deficiency condition.

Concerning the hydrogen peroxide (H2O2) amount expressed in percentage, it varied in the BM genotype from 0.57% to 0.92% from the control to the drought stress conditions, respectively. The landrace BS registered the highest variation from the control to the stress conditions, showing 0.53 % in IRR conditions, and 1.28 %, grown in NIR condition (Figure 6). The two B. macrocarpa genotypes showed the lowest variation of the percentage of hydrogen peroxide, and the content of the above-mentioned metabolite was of 0.13 % and 0.19 % for MM grown in IRR and NIR conditions, respectively, while it was of 0.35 % and 0.23 % for MF in IRR and NIR conditions, respectively.

Taking into consideration physiological and phenotypic characteristics together we compared the different cultivars using a PCA. As it can be seen in Figure 7 using these traits it has been possible to cluster the plants with their response characteristics to the drought stress. Moreover, from Figure 7a it is possible to notice how the tolerant plants clustered near the control ones. In Figure 7b instead we can observe that the variables “Leaf Area”, “H2O2” and “Leaf Lamina Length” are the ones that contribute more to this plants’ classification.

Figure 6. Barplots of the physiological analysis performed in the selected subset. The graphics encompasses the MDA (a) and H2O2 (b) analyses.

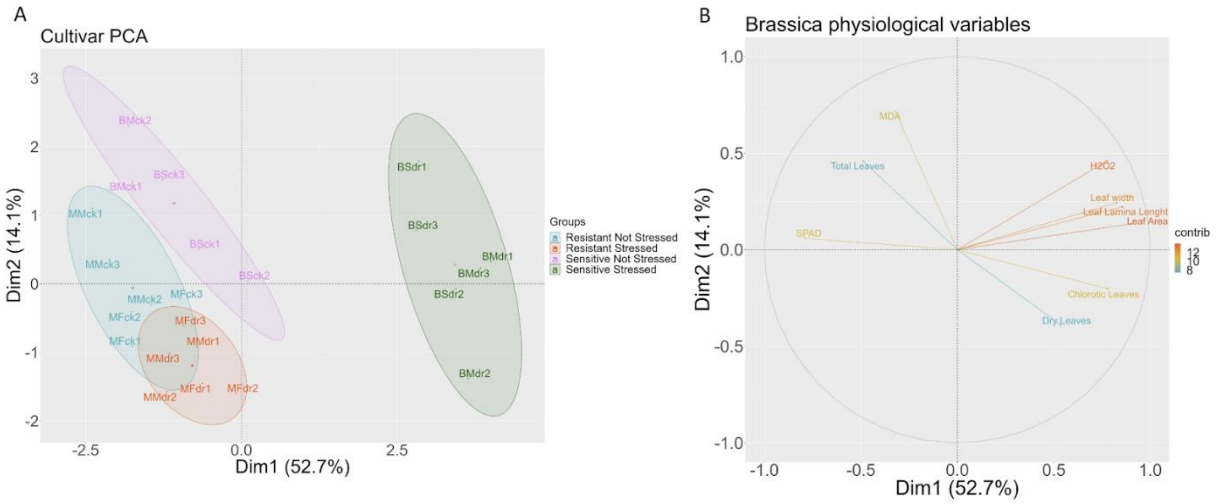


Figure 7. Principal components analysis for differentiate sensitive and tolerant accessions.

Transcriptomic analysis enables the identification of the DEGs between drought stress sensitive and tolerant of plants. The percentage of reads with q30 were between 85% to 92% showing a good sequencing quality (Table 30).

Table 30. Summary statistics of the RNA quality and sequencing results

Sample	Total bases	Lenght	GC (%)	Mean bases	q30(%)
MF	56802287700	150	46,42	4733523975	92
MM	58739441400	150	46,17	4894953450	92
BM	54967540800	150	45,33	27483770400	92
BS	45155147400	150	45,5	3762928950	85

Comparing the stressed plants with the control ones we can observe (Table 31 and Figure 8) that sensitive plants (BM and BS) possess a higher number of DEGs with respect to the resistant plants MF and MM.

Table 31. Differentially Expressed Genes (DEGs) in relation to the resistant and sensitive accessions.

Cultivar	Resistance	Number of DEGs in reference genome analysis
MF	resistant	514
MM	medium resistant	383
BM	medium sensitive	912
BS	sensitive	1147

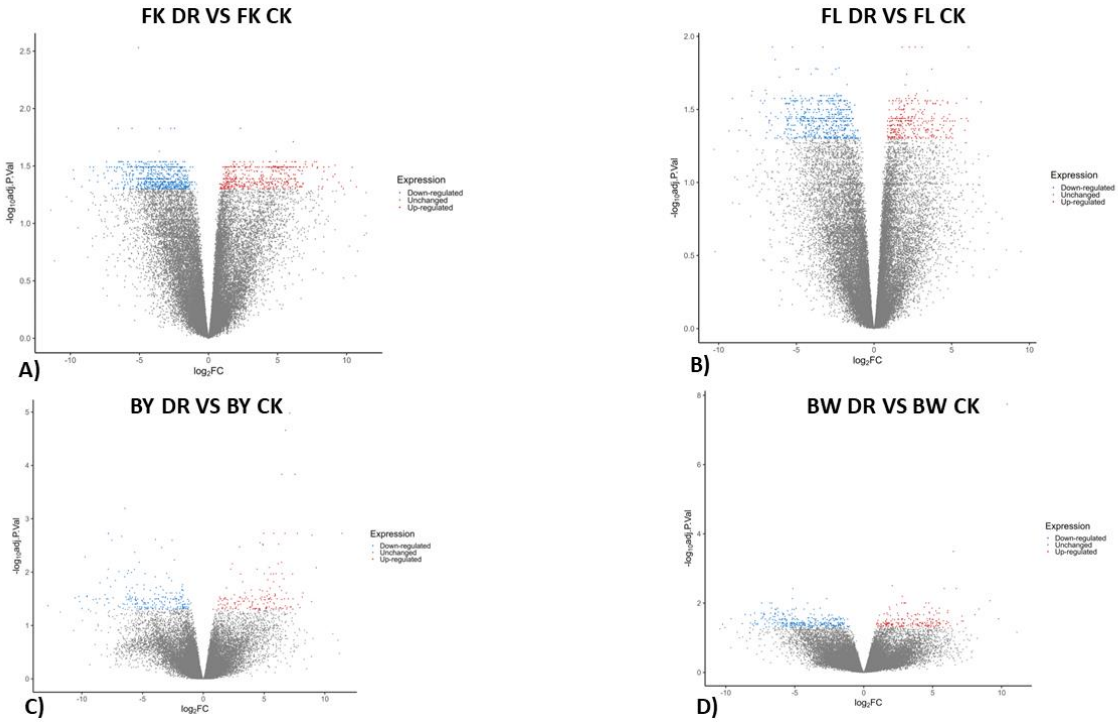


Figure 8. Volcano Plots show the DEGs between a stressed sample and a not-stressed sample for each cultivar. A) FK DR vs FK CK. B) FLDR VS FL CK. C) BY DR VS BY CK D) BW DR VS BW CK.

Trying to select a signature of tolerance and a signature of sensitivity among cultivars, we investigated which genes were in common between the cultivars through Venn Diagrams. It can be observed that only 9 genes are in common between the four cultivars. It is possible to presume that, if the regulation direction of those genes is the same, they are not involved in drought stress response. In contrast we can observe a close up to the genes in common between resistant cultivars (Figure 8) and sensitive cultivars (Figure 8). In this case it is possible to suppose that those genes, excluding the 9 in common between all cultivars, can be considered a tolerant and sensitive signature respectively.

To create the sensitive signatures, DEGs inferred from BS cultivar (DR vs CK) with the DEGs inferred from BM cultivar (DR vs CK) were compared. The same intersection was accomplished to create the tolerant signature using MM and MF cultivars. For tolerant signature 498 genes were obtained from de novo analysis and 27 with reference analysis. For sensitive signature we found 545 genes employing the de novo analysis and 320 with the reference genome analysis (Figure 9).

Furthermore, an intersection signature of 356 genes was built in the reference genome analysis and 658 in de novo analysis. This signature enabled the comprehensive comparison between tolerant cultivar versus each sensitive cultivar both for drought stressed plants and for control plants. The results of cultivar plants are subtracted from the results of their respective stressed plants. At the end the four lists of genes are intersected, and the common genes are retained as signature.

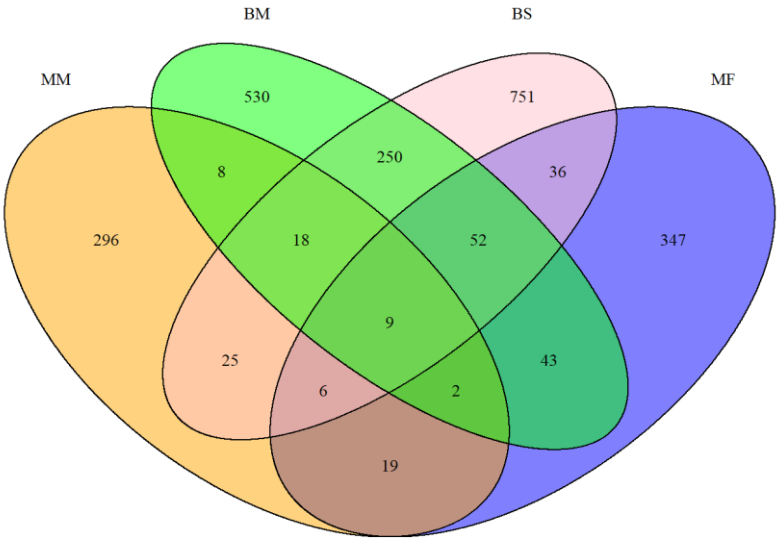


Figure 9. Venn diagram that described all genes in common for each cultivar.

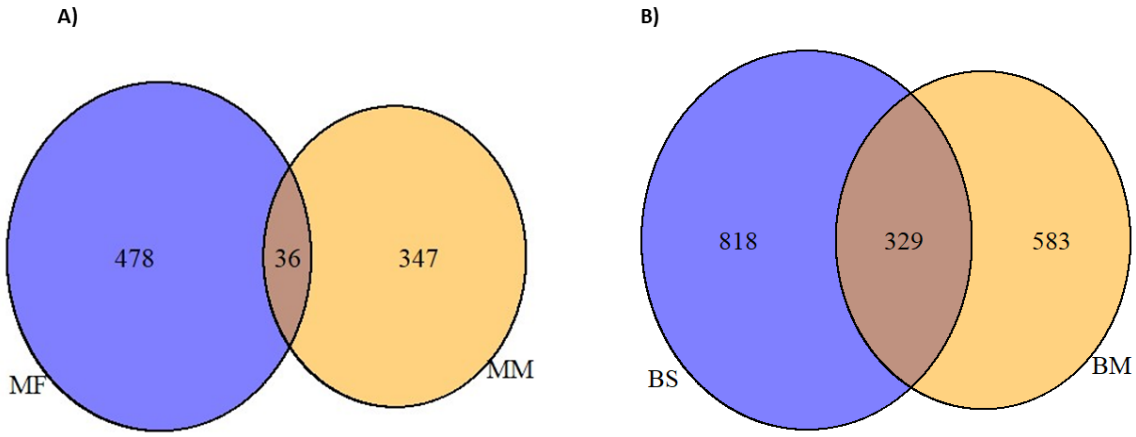


Figure 10. Venn diagrams that describe common genes between **A)** MF and MM in left **B)** BS and BM couples.

Gene Bo9g041010 appears to be common in both the tolerant and sensitive cultivars, but with opposite regulation. In fact, by analyzing its trend we can observe its downregulation in the sensitive and its upregulation in the resistant cultivars (Figure 10). In the sensitive cultivars (BS and BM) and in the tolerant ones (MF and MM) the gene results respectively downregulated and upregulated. However, this regulation results significative for the adjusted pvalue only for BS and MF (Figure 11).

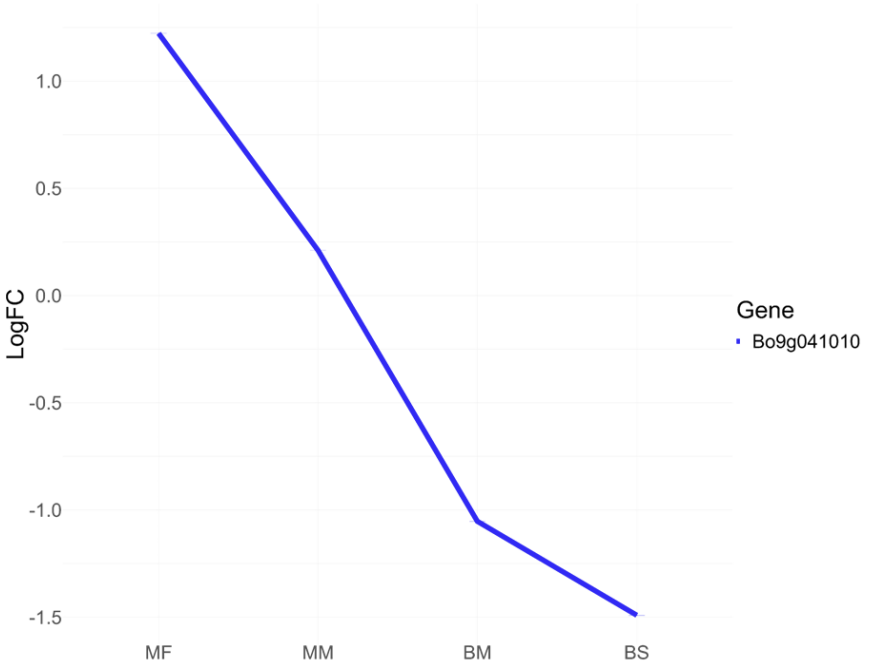


Figure 11. Line plot that describes the behavior of gene Bo9g041010 between cultivars

In Figures from 7 to 10 we can observe the Gene Ontology (GO) assignment for each gene found in our analysis. In particular in Figure 12a, the cellular component includes 20 GO and 159 genes with uncharacterised GO. While, in figure 7B, the number of genes uncharacterised is 133. In Figure 11b it is shown the biological process with 185 uncharacterised genes and 36 GO. Among them we can recognize GO:0009414 (response to water deprivation) and GO:0009737 (response to abscisic acid). Regarding figure 8 A, there is a large variation of cellular components, twenty in total. Nonetheless, we also found 159 genes for which GO was uncharacterised. Figure 8B shows the molecular function with 47 GO and 246 genes with uncharacterised GO. Considering biological processes in Figure 11c we can observe 37 GO with 185 genes which give uncharacterised GO. Moreover, we can also catch GO associated with water and auxin response. ss, which includes 37 pathways, there are 185 of uncharacterised genes.

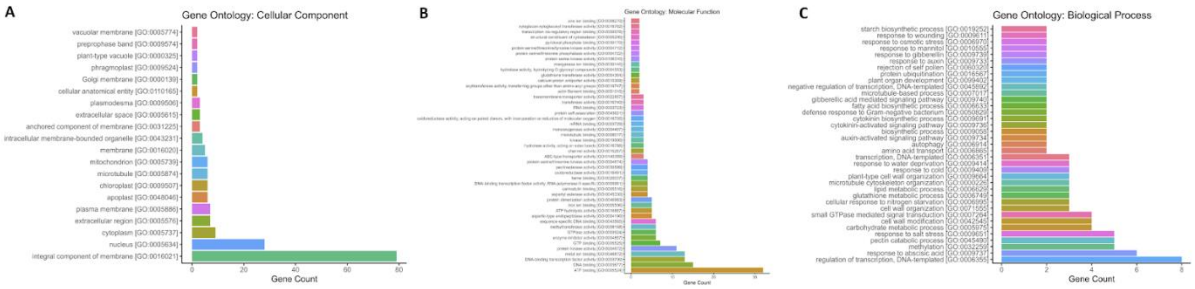


Figure 12. GO Barplot of sensitive signature (BM and BS) with the reference genome.

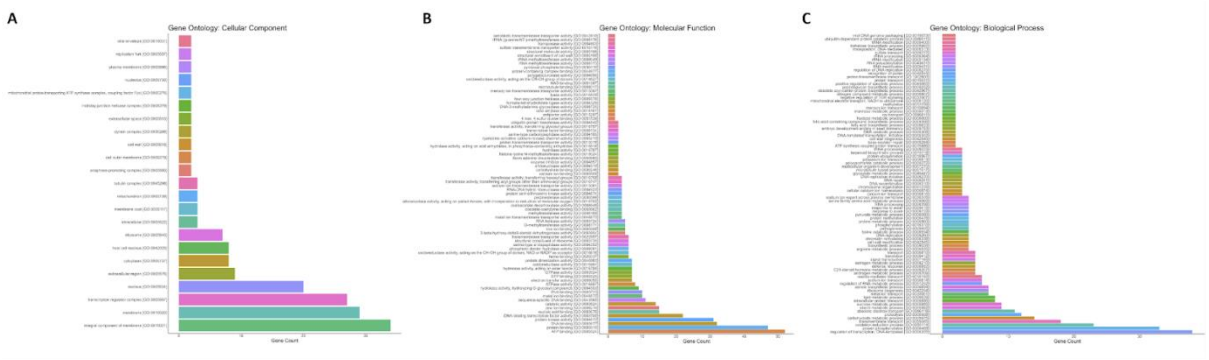


Figure 13. GO Barplot of sensitive signature (BM and BS) with de novo analysis.

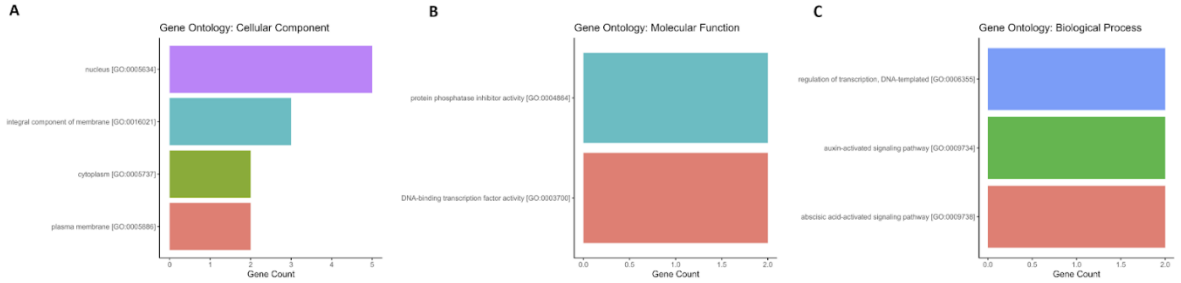


Figure 14. GO Barplot of tolerant signature (MF and MM) with the reference genome.

Figure 14 depicts the barplot generated from the tolerant signatures found with the reference genome. We can observe all the three components of Gene Ontology (GO): Cellular component (Figure 14), Molecular Function (Figure 14) and Biological Process (Figure 14). Unfortunately, because of the lack of complete annotation for *B. oleracea* we have found 15, 14, and 14 uncharacterized GO for this signature. Biological processes shown in Figure 15 describes functions that characterize tolerant plants, for example auxin- signaling pathway.

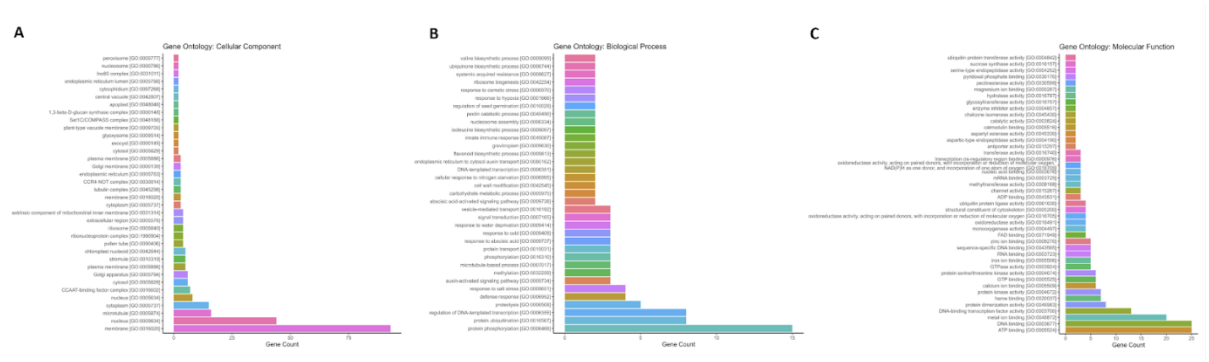


Figure 14. GO Barplot of intersection signature with the reference genome.

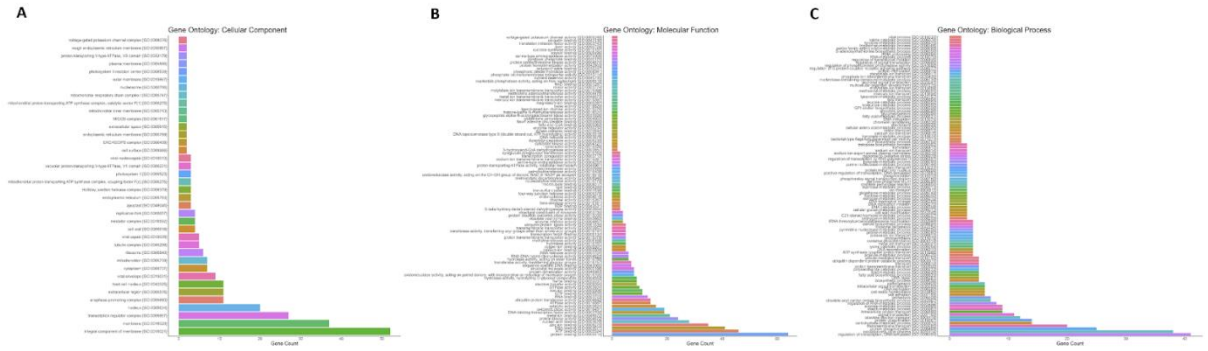


Figure 15. GO Barplot of intersection signature with de novo analysis.

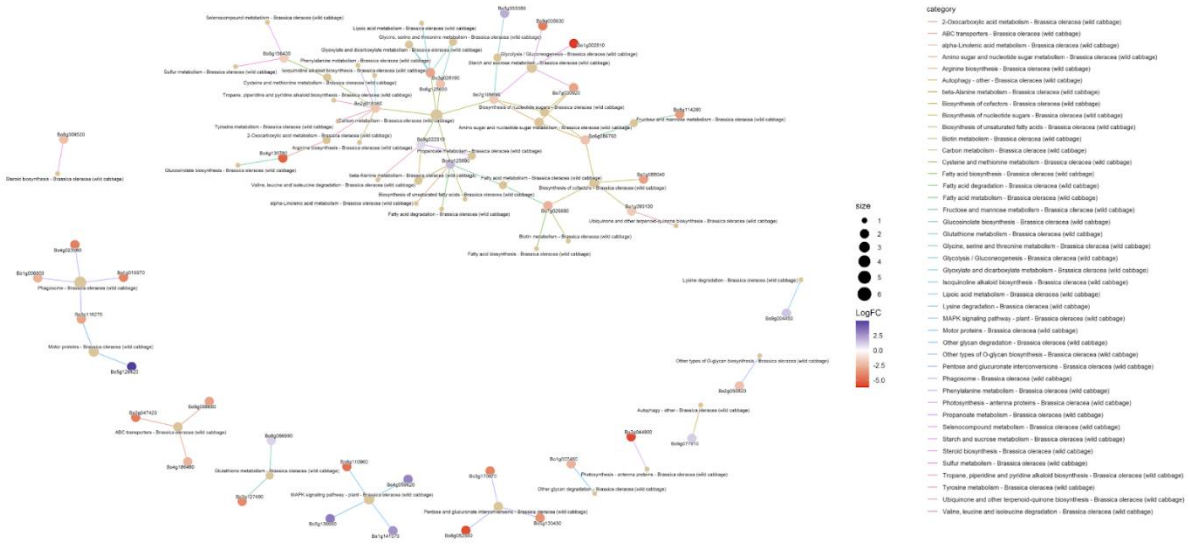


Figure 16. Pathways network of sensitive signature with reference genome

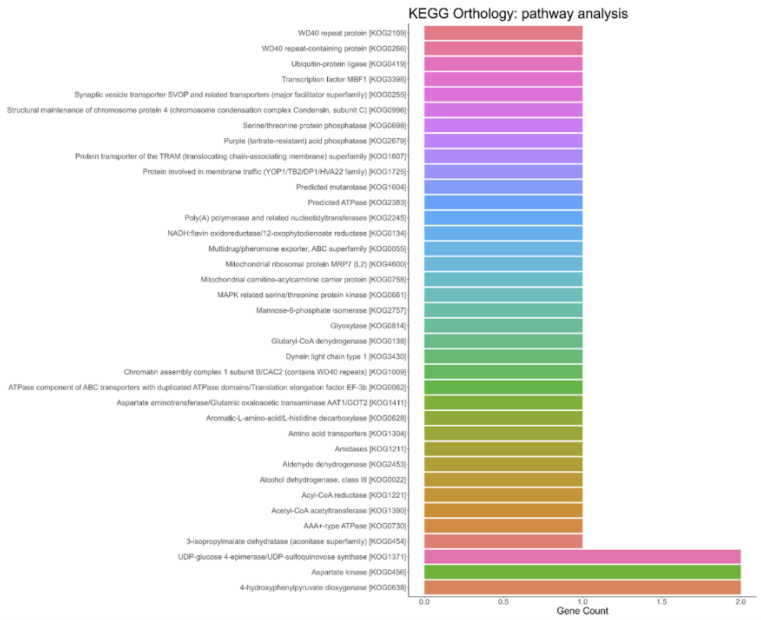


Figure 17. Pathways network of sensitive signature with de-novo analysis**.**

Figure 18. Pathways network of tolerant signature with reference genome

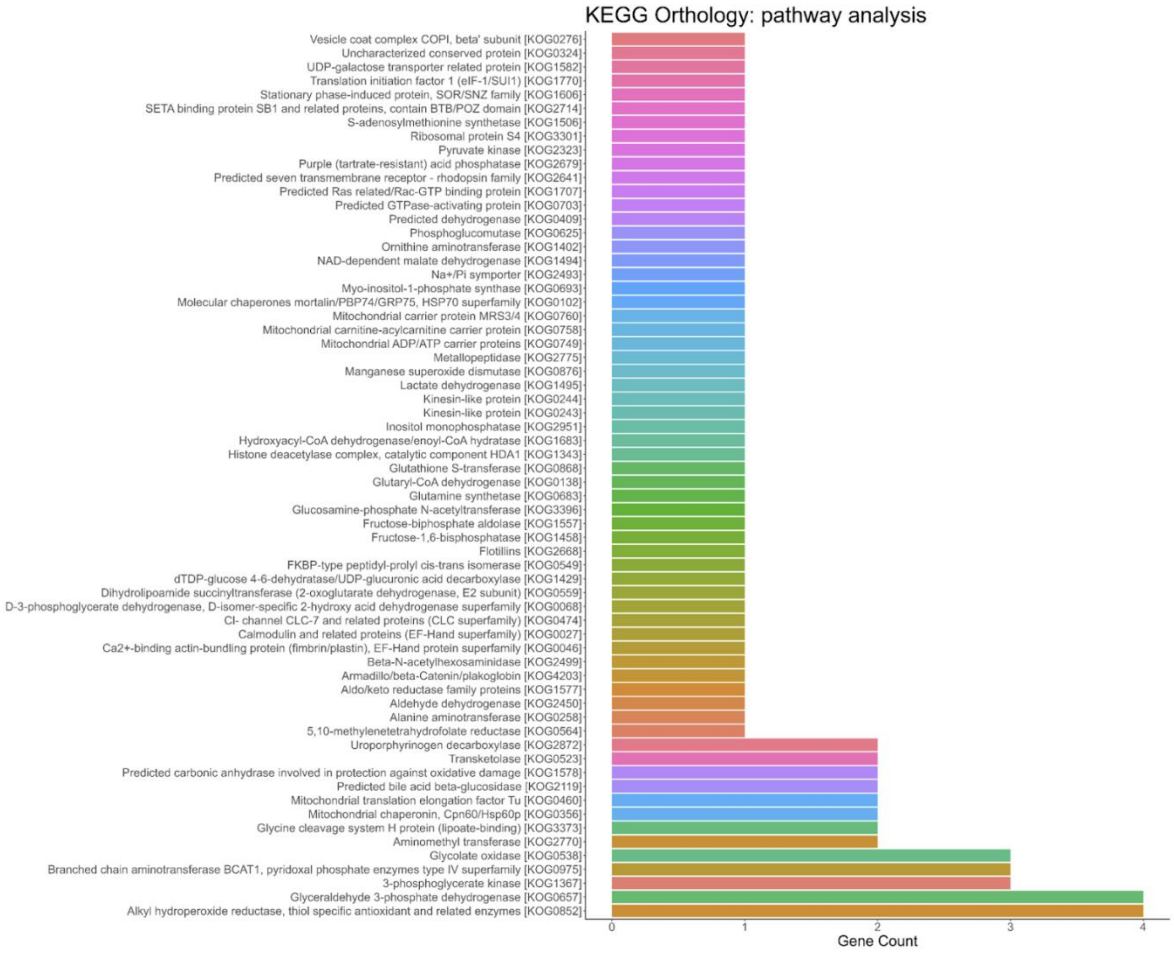


Figure 19. Pathways network of tolerant signature with de novo analysis.

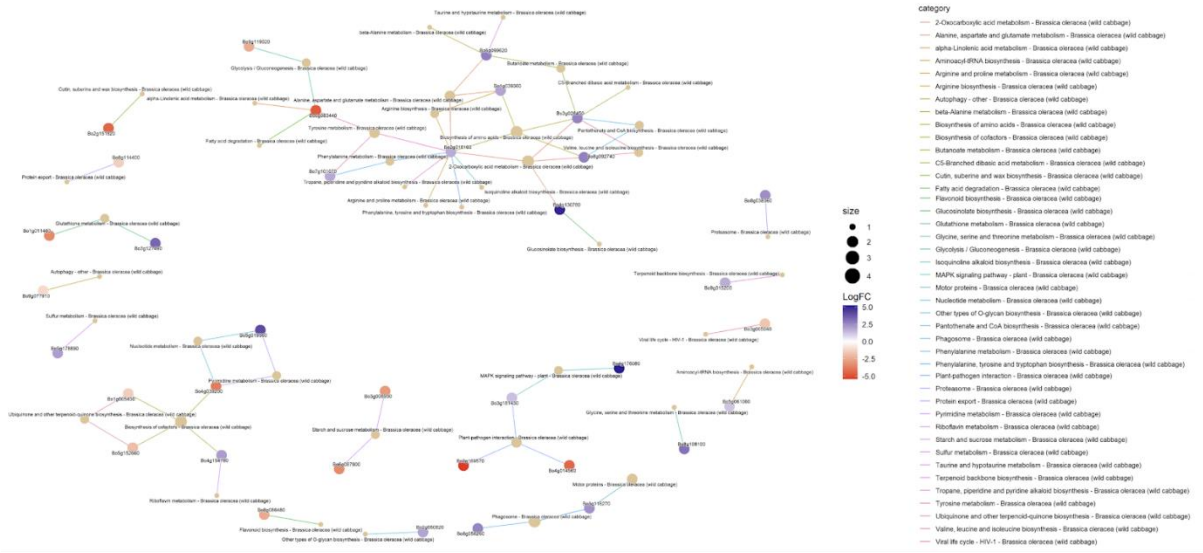


Figure 20. Pathways network of intersection signature with reference genome.

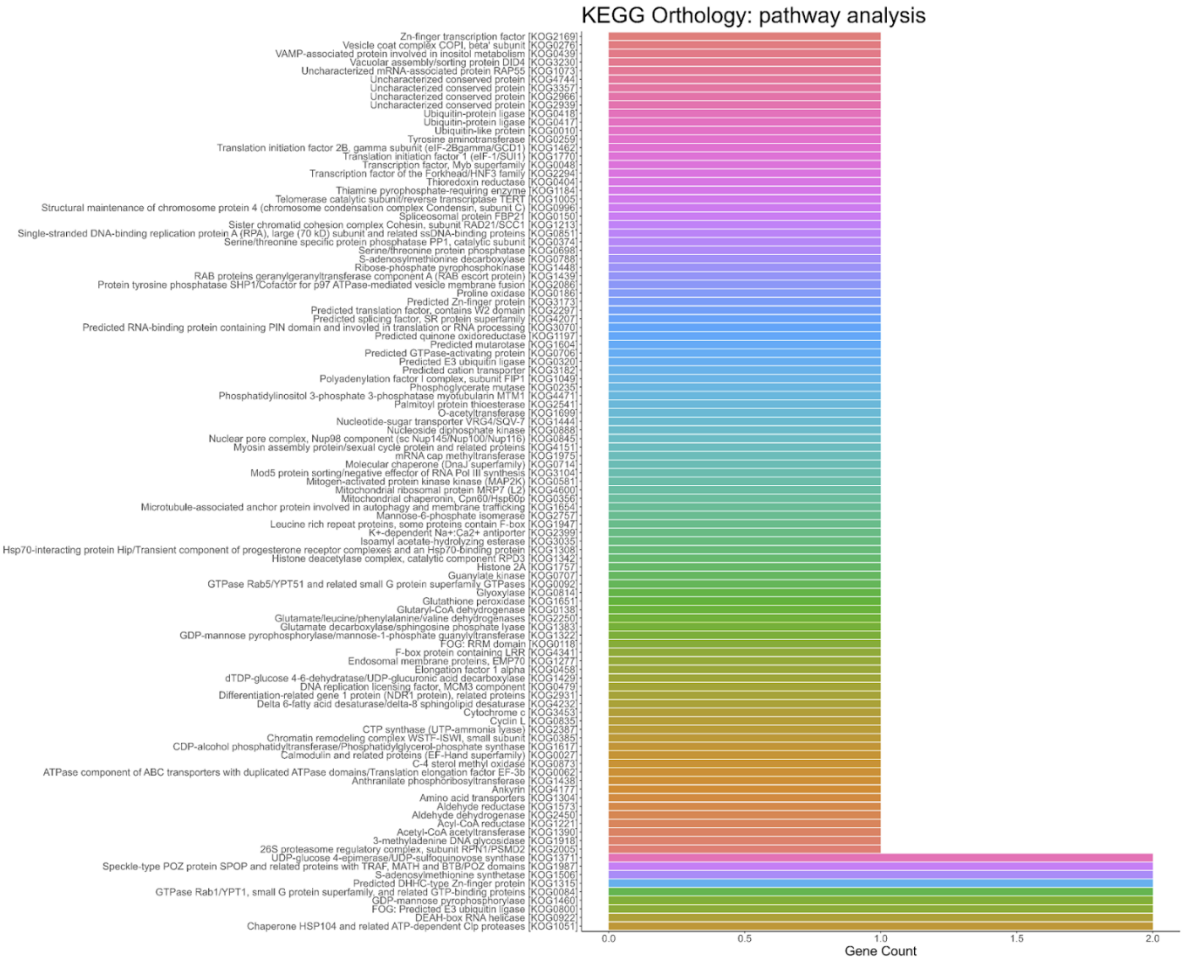


Figure 21. Pathways network of intersection signature with de-novo analysis.

EXPERIMENTAL TRIAL B

For the plant vigor (PV), we observed a significative interaction between the two experimental factors, which were the rootstock (RS) and the scion (SC) tested. PV was evaluated by a numerical score and its value varied from 4.5 for the non-grafted (NI) cultivar VI, to 8.5 for the rootstock used as control O combined with the scion BA, as well for the experimental rootstock C with the scion CH, and finally for the experi-mental rootstock B with the scion VI (Table 32). Notably, in all the different root-stock-scion combinations, the non- and self-grated plants exhibited a reduced vigor compared to the grafted ones. With regards to the plant height (PH), a significative in-teraction RS x SC was detected, with values ranging from 215.00 cm to 289.33 cm, for the rootstock used as control O with the scion CH, and for the experimental rootstock A with the genotype BA, respectively (Table 32).

As concern the plant stem diameter (PSD), it varied significantly among the dif-ferent rootstock tested, ranging from 1.76 cm for the non-grafted (NI) genotypes, to 2.22 cm for the rootstock C. PSD value varied among the different rootstock-scion combinations from 1.65 cm for the non-grafted CH, to 2.40 cm for the genotype BA grafted in the experimental rootstock C. Conversely, for the plant basal diameter (PBD), its value varied significantly in relation to the rootstock tested, varying from 1.68 cm for the non-grafted (NI) genotypes, to 2.17 cm for the rootstock used as control, O. In relation to the different rootstock-scion combinations, PBD value ranged from 1.65 cm for the non-grafted CH, to 2.35 cm for the rootstock O with the scion VI (Table 32).

With regards to the plant stem weight (PSW), we ascertained a significative variation among the different rootstock tested, and its value varied from 428.22 g for the non-grafted genotypes (NI), to 636.72 for the experimental rootstock C. In relation to all the combinations tested, we detected PSW values fluctuating from 394.67 g for the non-grafted (NI), to 690.83 g for the tested rootstock C with the genotype BA (Table 32). PSW values higher than 550.00 g were registered for BA grafted onto A and C, as well for CH grafted onto A, C and O, and for VI grafted with C and O (Table 32). Concerning the plant stem dry matter (PSDM), its value varied from 11.13 % for BA grafted with the rootstock C, to 23.78 % for the scion CH grafted with O (Table 32).

For the plant leaf lamina length (PLL), we registered a significative interaction RS x SC, and its value varied from 47.17 cm to 59.67 cm for the genotypes CH and BA, re-spectively, both grafted onto the experimental rootstock A (Table 32). On the other hand, also the plant leaf lamina width (PLW) varied significantly owing to the interac-tion between the two experimental factors evaluated. Its value varied from 41.83 for the rootstock A combined with the scion CH, to 59.50 for the rootstock C with the gen-otype BA (Table 32).

Table 32. Variation of the plant morphometric traits in relation to the different rootstock-scion combination analyzed. The analyzed traits were the plant vigor (PV), the plant height (PH), the plant stem diameter (PSD), the plant basal diameter (PBD), the stem weight (PSW), the stem dry matter (PSDM), the leaf length (PLL), and leaf width (PLW).

	BA							CH							VI						
Trait	A	B	C	O	AU	NI	**Mean**	A	B	C	O	AU	NI	**Mean**	A	B	C	O	AU	NI	**Mean**
PV	5.5	8	8	8.5	6	5.5	6.92	8	7.5	8.5	7.5	6	5.5	7.17	8	8.5	8	8	6	4.5	7.17
PH	289.33	229.8	239.2	221.7	231.8	222.0	238.97	224.7	219.2	217.2	215.0	253.5	249.7	229.9	227.3	232.8	242.5	231.2	236.2	235.0	234.1
PSD	2.02	2.00	2.40	2.02	2.08	1.75	2.04	2.17	2.17	2.28	2.13	1.93	1.65	2.06	1.98	2.07	1.97	2.12	1.88	1.88	1.98
PBD	2.15	1.85	2.10	2.08	1.98	1.77	1.99	1.88	2.07	2.15	2.07	1.71	1.65	1.92	1.97	2.08	2.17	2.35	1.93	1.62	2.02
PSW	558.5	510.3	690.8	506.6	494.0	432.8	532.19	543.3	525.8	579.7	639.7	398.8	394.7	513.7	500.8	531.3	639.7	572.5	517.2	457.2	536.4
PSDM	11.87	11.13	12.69	13.25	12.94	13.15	12.51	14.14	11.81	11.78	23.74	12.31	13.71	14.58	12.20	11.59	12.66	12.77	12.69	11.72	12.27
PLL	59.67	49.33	58.33	56.00	57.00	54.50	55.81	47.17	48.17	47.67	49.83	48.50	47.83	48.19	50.00	51.83	49.67	51.50	48.17	46.50	49.61
PLW	56.17	49.67	59.50	52.83	52.67	47.67	53.08	41.83	44.67	48.00	50.33	42.00	48.67	45.92	45.00	47.83	48.33	50.67	45.00	43.67	46.75

Mean per Roostock

Trait	**A**	**B**	**C**	**O**	**AU**	**NI**
PV	7.17	8.00	8.17	8.00	6.00	5.17
PH	247.11	227.28	232.94	222.61	240.50	235.56
PSD	2.06	2.08	2.22	2.09	1.97	1.76
PBD	2.00	2.00	2.14	2.17	1.88	1.68
PSW	534.22	522.50	636.72	572.94	470.00	428.22
PSDM	12.74	11.51	12.38	16.59	12.65	12.86
PLL	52.28	49.78	51.89	52.44	51.22	49.61
PLW	47.67	47.39	51.94	51.28	46.56	46.67

Significancy of the differences by ANOVA

	PV	**PH**	**PSD**	**PBD**	**PSW**	**PSDM**	**PLL**	**PLW**
RS	***	*	**	***	***	**n.s.**	**n.s.**	***
SC	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	***	***
RS x SC	***	***	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**	***

For the main root length (RML), we ascertained a significative interaction RS x SC, with values ranged from 23.67 cm for the non-grafted (NI) VI, to 42.62 for the root-stock C with the genotype CH (Table 33). RML values higher than 35.00 cm were registered for the non-grafted BA, as well for A and C combined with the scion CH, and fi-nally for the rootstock O and the self-grafted VI (Table 33). With regards to the main root diameter (RMD), we detected a significative variation in relation to the different scion adopted. In relation to the different scions, RMD value ranged from 0.59 mm for BA, to 1.14 mm for CH, while for the hybrid VI we registered the intermediate value of 0.75 mm (Table 33).

In relation to all the rootstock-scion combinations tested, RMD value varied from 0.38 cm for the non-grafted VI, to 1.55 cm for the rootstock O with the genotype CH as graft (Table 5). RMD values higher than 1.00 cm, were observed for all the rootstocks adopted for the scion CH, including the self-grated one, with the exception to the non-grafted one (Table 33). Additionally, the second and third main root diameters (R2MD and R3DM, respectively) varied significantly in relation to the scion grafted. For the R2MD were registered values varying from 0.26 cm for the non-grafted VI, to 1.13 cm for the rootstock O combined with the scion CH. Conversely, the R3MD value ranged from 0.17 cm for the non-grafted VI, to 1.02 cm for the rootstock O combined with the scion CH (Table 33).

Concerning the main root angle (RMA) value, it ranged from 92.33 ° to 145.00 ° for the experimental rootstocks A and C, respectively, both with the scion BA (Table 33). For the roots width (RWI), we observed a significative interaction between the two ex-perimental factors RS and SC, with values varying from 29.50 cm for the non-grafted VI, to 55.50 cm for the rootstock used as control O, combined with the genotype BA. For the root weight (RWE), we also registered a significative interaction RS x SC, with values ranging from 56.00 g for the non-grafted VI, to 288.50 for the rootstock O with the scion VI (Table 33).

With respect to the root ramification score (RRS), it varied in relation to the rootstock applied, registering values from 4.50 for the non-grafted genotypes (NI), to 6.83 for the rootstock O, used as control (Table 33). On the other hand, for the root nema-todes score (RNS), we have observed no significative variation in relation to the ex-perimental rootstock and to the scion adopted, due to the infection of the nematodes involved all the plants tested without particular effects on the fruit yield. As concern the root dry matter (RDM), we observed no significative variation as response of the RS and the SC adopted, and its value ranged from 15.06 % for the rootstock O combined with the scion BA, to 32.84 % for the self-grafted BA (Table 33).

Table 33. Variation of the root morphometric traits in relation to the different rootstock-scion combination analyzed. The analyzed traits were the root main length (RML), the diameters of the first, second and third main root (RMD, R2MD and R3MD, respectively), the main root angle (RMA), the width (RWI), the weight (RWE) the ramification and nematodes score (RRS and RNS, respectively) and the dry matter (RDM).

	BA							CH							VI						
Trait	A	B	C	O	AU	NI	**Mean**	A	B	C	O	AU	NI	**Mean**	A	B	C	O	AU	NI	**Mean**
RML	31.67	28.00	30.17	32.00	30.83	36.33	31.50	39.83	33.00	42.67	33.00	29.67	31.50	34.94	34.00	31.17	27.00	42.00	38.50	23.67	32.72
RMD	0.45	0.56	0.78	0.55	0.52	0.68	0.59	1.20	1.17	1.25	1.55	1.13	0.55	1.14	0.95	0.70	0.77	0.98	0.70	0.38	0.75
R2MD	0.29	0.36	0.40	0.45	0.33	0.63	0.41	0.88	0.98	1.03	1.13	0.92	0.47	0.90	0.80	0.58	0.58	0.63	0.52	0.26	0.56
R3MD	0.18	0.17	0.22	0.27	0.30	0.24	0.23	0.73	0.83	1.00	1.02	0.77	0.35	0.78	0.60	0.51	0.40	0.47	0.28	0.17	0.40
RMA	92.33	115.00	145.00	139.17	132.50	138.33	127.06	129.00	122.50	102.50	95.00	129.17	120.83	116.50	121.67	104.17	120.00	110.83	117.83	94.17	111.44
RWI	48.33	36.67	44.83	55.50	41.00	37.50	43.97	42.83	45.00	44.50	37.83	38.83	46.17	42.53	41.50	41.17	38.33	39.50	42.83	29.50	38.81
RWE	69.50	88.67	145.67	192.17	95.17	76.50	111.28	169.50	157.83	186.50	199.33	106.50	100.00	153.28	102.17	122.50	185.33	288.50	118.83	56.00	145.56
RRS	4	4.5	7	7.5	5	5	5.50	6	5	6	6.5	4.5	6.5	5.75	6	5.5	6	6.5	5.5	2	5.25
RNS	2.50	2.17	3.17	3.33	2.50	2.33	2.67	3.00	2.17	2.50	3.00	3.00	2.58	2.71	3.00	2.33	1.83	3.00	3.17	3.00	2.72
RDM	22.76	23.87	20.05	15.06	32.84	23.39	22.99	16.14	22.75	26.96	23.01	23.16	26.99	23.17	20.96	25.37	23.53	24.16	21.05	25.96	23.50

Means per Roostock

	A	**B**	**C**	**O**	**AU**	**NI**
RML	35.17	30.72	33.28	35.67	33.00	30.50
RMD	0.87	0.81	0.93	1.03	0.78	0.54
R2MD	0.66	0.64	0.67	0.74	0.59	0.45
R3MD	0.51	0.50	0.54	0.58	0.45	0.25
RMA	114.33	113.89	122.50	115.00	126.50	117.78
RWI	44.22	40.94	42.56	44.28	40.89	37.72
RWE	113.72	123.00	172.50	226.67	106.83	77.50
RRS	5.33	5.00	6.33	6.83	5.00	4.50
RNS	2.83	2.22	2.50	3.11	2.89	2.64
RDM	19.95	24.00	23.51	20.74	25.68	25.45

Significancy of the differences by ANOVA

	RML	**RMD**	**R2MD**	**R3MD**	**RMA**	**RWI**	**RWE**	**RRS**	**RNS**	**RDM**
RS	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	*******	*****	**n.s.**	**n.s.**
SC	**n.s.**	*******	*******	*******	**n.s.**	*****	**n.s.**	**n.s.**	**n.s.**	**n.s.**
RC x SC	*******	**n.s.**	**n.s.**	**n.s.**	**n.s.**	******	**n.s.**	**n.s.**	**n.s.**	**n.s.**

In all examined trusses, we observed a significant interaction between the two experimental factors RS x SC, except for the third truss. For the third truss, although we did not observe the RS x SC interaction, the interval of days from transplanting (DAT) to the opening of the third flowers varied significantly depending on the adopted rootstock and scion. For the first truss, the DAT for the opening of the third flower ranged from 7.0 for the hybrid CH grafted onto the rootstock B and the self and non-grafted scion VI, to 18.3 DAT for the combination of rootstock O and scion BA (Table 34). Similarly, the second truss showed variation, with DAT ranging from 13.1 days for the non-grafted VI, to 29.5 days for the hybrid BA grafted with rootstock B (Table 34). Regarding the third truss, we observed a significant variation in relation to the adopted rootstock and scion. Specifically, for the rootstock, values ranged from 26.0 DAT for the non-grafted (NI) combinations to 32.3 DAT for the experimental rootstock C. Regarding the scions, we observed values ranging from 25.1 to 39.1 DAT for CH and BA, respectively (Table 34). On the other hand, for the fourth truss, the observed variation was from 34.4 to 53.8 DAT for the non-grafted VI to the genotype BA grafted with rootstock A (Table 34). For the fifth truss, the DAT for the opening of the third flower ranged from 48.5 DAT for the non-grafted VI, to 71.0 DAT for rootstock C (Table 34). Additionally, in the sixth truss, the variation ranged from 63.9 to 85.4 DAT for the non-grafted hybrid VI and for the rootstock C with the scion BA. Furthermore, both the seventh and eighth trusses displayed variation, with DAT ranging from 79.5 to 98.1 DAT, and 91.0 to 109.8 DAT, respectively, for the non-grafted VI and the hybrid BA grafted with rootstock C.

The observed variations in DAT for the opening of the third flower in different trusses highlight on the influence of rootstock and scion interactions on truss development and flower opening. These findings contribute to a deeper understanding of plant growth and reproduction dynamics, which may have practical implications for horticultural practices.

Table 34. Variation of the time expressed in DAT (days after transplanting) for the flowering of the third flower for each truss in relation to all the rootstock-scion combinations tested.

	BA							CH							VI						
Truss	**A**	**B**	**C**	**O**	**AU**	**NI**	**Mean**	**A**	**B**	**C**	**O**	**AU**	**NI**	**Mean**	**A**	**B**	**C**	**O**	**AU**	**NI**	**Mean**
1st	14.9	15.5	18.1	18.3	14.0	13.9	15.8	7.5	7.0	7.3	7.5	7.5	7.3	7.3	7.8	7.5	7.3	7.3	7.0	7.0	7.3
2nd	26.6	29.5	27.9	27.6	23.1	21.3	26.0	15.8	16.5	16.0	16.0	15.6	13.6	15.6	16.5	16.0	16.0	16.3	14.8	13.1	15.4
3rd	41.5	40.6	41.8	39.4	36.3	34.9	39.1	25.8	26.1	26.6	27.0	23.0	22.1	25.1	26.6	27.8	29.5	27.1	24.9	21.1	26.2
4th	53.8	53.1	52.9	52.1	49.6	45.9	51.2	40.5	36.3	40.8	41.5	37.6	36.4	38.8	40.1	40.1	39.8	41.0	39.6	34.4	39.2
5th	70.4	70.6	71.0	69.3	65.3	63.0	68.3	53.3	53.1	52.9	53.0	50.4	49.3	52.0	53.0	51.5	54.0	55.1	53.8	48.5	52.6
6th	84.0	83.8	85.4	83.5	79.9	76.9	82.2	68.3	69.0	68.1	69.0	66.4	65.6	67.7	69.9	68.6	70.6	70.3	68.1	63.9	68.6
7th	95.9	97.0	98.1	95.0	93.3	89.3	94.8	84.9	85.1	85.3	85.8	82.6	81.3	84.1	84.1	83.9	85.6	86.0	81.6	79.5	83.5
8th	107.9	108.9	109.8	107.3	105.0	101.9	106.8	96.9	96.8	98.4	97.3	93.4	94.4	96.2	99.4	96.4	97.3	98.4	94.1	91.0	96.1

Means per Roostock

	A	**B**	**C**	**O**	**AU**	**NI**
1st	10.0	10.0	10.9	11.0	9.5	9.4
2nd	19.6	20.7	20.0	20.0	17.8	16.0
3rd	31.3	31.5	32.6	31.2	28.0	26.0
4th	44.8	43.2	44.5	44.9	42.3	38.9
5th	58.9	58.4	59.3	59.1	56.5	53.6
6th	74.0	73.8	74.7	74.3	71.5	68.8
7th	88.3	88.7	89.7	88.9	85.8	83.3
8th	101.4	100.7	101.8	101.0	97.5	95.8

Significancy of the differences by ANOVA

	1st	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	**8th**
RS	***	***	***	***	***	***	***	***
SC	***	***	***	***	***	***	***	***
RC x SC	***	***	**n.s.**	***	***	***	***	***

The fruit predominant shape (FS) of the examined accession varied basing on the scion grafted. For the hybrids CH and VI was detected a circular shape, in accordance with the qualitative descriptors IBPG, while for the hybrid BA, was detected the slightly flattened shape (Figure 18). We detected no significative differences in relation to the fruit shape as consequence of the different grafting combinations.
The fruit weight (FW) varied significantly in relation to the scion adopted, there-fore for BA we observed values higher about the 434% and 486% compared to the scion CH and VI, respectively.

FW value ranged from 17.02 g for the combination between the rootstock B and the scion VI, to 112.54 g for the self-grafted BA (Table 35). For the fruit longitudinal di-ameter (FLD), we ascertained a significative variation RS x SC, registering values be-tween 28.96 mm for the grafting combination between B and VI, to 51.38 mm for the rootstock C combined with BA. On the other hand, as well for the transversal diameter of the fruit (FTD), we observed a significative interaction RS x SC, with values varying from 30.57 mm for the rootstock C with the scion VI, to 64.56 for the rootstock C with BA used as scion (Table 35).

Concerning the chromatic parameter FUL*, we observed significative variation in relation to the rootstock adopted, varying from 35.78 when the rootstock B was adopted, to 49.61 for the self-grated (AU) genotypes. Among the genotypes, FUL* var-ied from 24.46 for B with the scion CH, to 66.41 for the self-grafted VI. Contrarily, for the chromatic parameter FUa*, we observed no variation among the different rootstock adopted, and its value varied from 10.82 for the genotype VI grafted onto the rootstock O, to 16.29 for CH grafted onto C. On the other hand, for the chromatic pa-rameter FUb*, we observed a significative interaction RS x SC, and its value ranged from 19.09 for CH grafted with the experimental rootstock A, to 31.15 for the non-grafted CH (Table 35).

Our analysis of the fruits number of locules (FLN) did not reveal any significant variation between the two experimental factors (RS and SC). Among all grafting combinations involving CH and VI, we observed an average FLN value of two locules per fruit. However, for the grafting combinations with BA, we noted a slightly higher mean FLN value of 2.35 locules, due to several fruits from the scion BA showed three locules. It's worth noting that this deviation in FLN was limited to the BA scions and did not extend to the CH and VI scions. With regards to the fruit peel thickness (FPT), we observed a significative variation in relation to the scion grafted with the different experimental rootstocks. For the scion BA, we observed the highest FPT value of 5.75 mm, while for the tomato cherry hybrids VI and CH we registered 4.21 mm and 4.33 mm of the peel. As concern the fruit firmness (FFN), we registered a significative interaction RS x SC, and its value varied from 1.09 N for the self-grafted BA, to 2.29 N for the non-grafted VI (Table 35).

With regards to the soluble solid content of the fruit (FSSC), we detected a significant interaction RS x SC, and its value varied from 3.63 ° Brix for the combination between the rootstock A with the scion BA, to 7.10 for the non-grafted CH. For the sci-ons CH and VI, were registered values higher than 6.00 ° Brix in all the grafting com-binations, with the exception CH grafted onto the experimental rootstock C (Table 35). As concern the fruit acidity (FPh), a significative interaction RS x SC was observed. FPh value varied from 4.17 to 4.58 for both the self-grafted CH and BA, respectively (Table 35).

Table 35. Variation of the traits related to the fruit production and quality, in relation to the different rootstock and scion combinations.

	BA							CH							VI						
Trait	A	B	C	O	AU	NI	**Mean**	A	B	C	O	AU	NI	**Mean**	A	B	C	O	AU	NI	**Mean**
FW	94.25	93.84	107.11	104.27	112.54	84.71	99.45	22.61	24.38	21.44	21.01	24.41	23.39	22.87	21.27	17.02	17.75	20.52	25.58	20.52	20.44
FLD	44.86	45.10	51.38	46.95	47.87	43.94	46.68	31.50	32.33	30.70	30.21	31.76	30.79	31.21	29.59	28.96	29.46	29.36	31.31	30.51	29.87
FTD	57.83	57.80	64.56	61.72	61.02	53.83	59.46	34.26	34.80	33.39	33.63	35.29	33.43	34.13	35.76	32.53	30.57	33.35	36.37	34.88	33.91
FUL*	43.87	43.20	42.58	40.93	42.87	44.00	42.91	38.30	24.46	25.35	38.94	39.53	32.91	33.25	54.13	39.69	53.93	38.77	66.41	52.36	50.88
FUa*	13.08	14.27	16.97	15.74	16.48	15.65	15.36	12.17	15.74	16.29	12.45	12.67	17.38	14.45	15.55	11.38	14.76	10.82	16.01	12.16	13.45
FUb*	23.11	25.28	25.14	22.88	24.75	24.36	24.25	19.09	26.12	24.92	20.23	19.87	31.15	23.57	22.62	22.09	19.91	20.21	22.41	22.26	21.58
FLN	2.23	2.40	2.13	2.50	2.40	2.40	2.34	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
FPT	5.66	5.31	5.63	7.20	4.88	5.83	5.75	7.43	3.36	3.48	3.82	3.73	4.17	4.33	4.68	3.69	4.30	3.89	4.54	4.14	4.21
FFN	1.46	1.27	1.39	1.79	1.09	1.40	1.40	1.83	2.11	1.94	1.92	1.99	1.87	1.94	2.04	1.45	1.97	1.74	2.11	2.29	1.93
FSSC	3.63	3.56	3.54	4.07	3.84	3.72	3.73	6.20	6.08	5.12	6.25	6.46	7.10	6.20	6.28	6.94	6.63	6.25	6.60	6.75	6.57
FpH	4.39	4.29	4.36	4.53	4.58	4.34	4.42	4.28	4.20	4.25	4.28	4.17	4.35	4.26	4.30	4.27	4.25	4.27	4.34	4.34	4.30
FT	9.33	7.74	7.65	7.78	7.58	6.97	7.84	13.57	15.90	12.43	13.83	15.18	14.46	14.23	13.59	13.39	14.93	13.58	17.17	14.51	14.53
FGP	54.83	30.00	33.02	42.21	29.52	51.17	40.13	18.76	15.79	26.39	34.51	38.22	31.23	27.48	17.41	11.05	16.18	38.55	13.03	15.10	18.55
FPP	6472	5236	5863	5768	6125	4232	5616	2454	3085	2140	2334	2949	2700	2610	2318	1842	2138	2234	3495	2396	2404

Means per Rootstock

Trait	**A**	**B**	**C**	**O**	**AU**	**NI**
FW	46.04	45.08	48.77	48.60	54.18	42.87
FLD	35.31	35.47	37.18	35.51	36.98	35.08
FTD	42.62	41.71	42.84	42.90	44.23	40.71
FUL*	45.43	35.78	40.62	39.55	49.61	43.09
FUa*	13.60	13.80	16.01	13.01	15.05	15.06
FUb*	21.61	24.50	23.32	21.11	22.35	25.92
FLN	2.08	2.13	2.04	2.17	2.13	2.13
FPT	5.92	4.12	4.47	4.97	4.38	4.71
FFN	1.78	1.61	1.77	1.82	1.73	1.85
FSSC	5.37	5.53	5.09	5.52	5.63	5.86
FpH	4.33	4.26	4.28	4.36	4.37	4.34
FT	12.16	12.34	11.67	11.73	13.31	11.98
FGP	30.33	18.95	25.20	38.43	26.92	32.50
FPP	3747.98	3387.84	3380.31	3445.51	4189.72	3109.03

Significancy of the differences by ANOVA

	FW	**FLD**	**FTD**	**FUL***	**FUa***	**FUb***	**FLN**	**FPT**	**FFN**	**FSSC**	**FpH**	**FT**	**FGP**	**FPP**
RS	**n.s.**	**	**	***	**n.s.**	**	**n.s.**	**n.s.**	**n.s.**	*	*	**	**	**n.s.**
SC	***	***	***	**n.s.**	**n.s.**	*	**n.s.**	**	***	***	***	***	***	***
RS x SC	**n.s.**	***	***	**n.s.**	**n.s.**	**	**n.s.**	**n.s.**	*	**	**	***	***	*

The fruits number per truss, varied significantly for the interaction between the two experimental factors, registering values ranging from 11.05 % for the scion CH grafted with the rootstock B, to 54.83 % for BA grafted with the experimental rootstock A (Table 36).

As concern the fruit production per plant (FPP), it varied significantly in relation to the scion grafted onto the different experimental rootstocks. For BA we registered the highest FPP value which was about 5616 g per plant, while the scions VI and CH produced about 2404 g and 2610 g, respectively. In relation to the different rootstock-scion combination adopted, the highest performance in terms of fruit production per plant was registered for the combination between the rootstock A with the scion BA. Contrarily, the lowest FPP value was detected for the combination between the rootstock B and the scion VI, and it was about 1842 g (Table 36).

The most correlated trait related to the plant was the leaf lamina length (PLL), which was positively correlated with the leaf width (PLW), the roots width, the fruit weight (FW), longitudinal and transversal diameter (FLD and FTD, respectively), the number of fruits locules (FLN), the fruit peel thickness (FPT), the fruit acidity (FPh), the percentage of green fruits (FGP) and the fruit per plant (FPP) (Table 37). Additionally, the PLL, was negatively correlated with the main first, second and third root diameters (RMD, R2MD and R3MD, respectively), with the fruit shape (FS), the fruit firmness (FFN), the soluble solid content (FSSC), and the number of fruits per truss (FT) (Table 37).

Regarding the root traits, the most correlated one was the R3MD, and it was posi-tively correlated with the plant stem dry matter (PSDM), with the main root length, diameters, and weight (RML, RMD and R2MD, RWE respectively), with the fruit shape (FS), firmness (FFN), soluble solid content (FSSC) and the fruit number per truss (FT) (Table S1). Furthermore, R3MD was negatively correlated with the plant height (PH), the leaf lamina length (PLL), the fruit weight (FW), longitudinal and transversal diam-eters (FLD and FTD, respectively), the chromatic component of lightness (FUL*), the number of locules (FLN), the fruit peel thickness (FPT), the acidity (FPh) and the pro-duction per plant (FPP) (Table 36).

As concern the fruit traits, the most correlated one it was the fruit longitudinal diameter (FLD), which showed a positive correlation with the leaf length and width (PLL and PLW, respectively), in addition to the main root angle (RMA), the fruit weight and transversal diameter (FW and FTD, respectively), the fruit chromatic coor-dinate a* (FUa*), the locules number (FLN), the peel thickness (FPT), the acidity (FPh), the percentage of green fruits (FGP) and the fruit production per plant (FPP). Conversely, FLD was negatively correlated with the root's parameters RMD, R2MD, R3MD, and with the fruits shape, firmness, soluble solid content and number of fruits per truss (FS, FFN, FSSC and FT, respectively) (Table 36).

Table 37. Pearson's correlation among the analyzed traits.

	PV	PH	PSD	PBD	PSW	PSDM	PLL	PLW	RML	RMD	R2MD	R3MD	RMA	RWI	RWE	RRS	RNS	RDM	FS	FW	FLD	FTD	FUL*	FUa*	FUb*	FLN	FPT	FFN	FSSC	FpH	FT	FGP	FPP
PV	1.00																																
PH	-.474*	1.00																															
PSD	.643**	-0.26	1.00																														
PBD	.652**	-0.06	.650**	1.00																													
PSW	.587**	-0.12	.762**	.761**	1.00																												
PSDM	0.05	-0.29	0.09	0.07	0.30	1.00																											
PLL	-0.02	0.37	0.22	0.39	0.27	-0.06	1.00																										
PLW	0.14	0.29	0.36	.497*	.517*	0.08	.840**	1.00																									
RML	0.31	-0.32	0.26	0.39	0.13	0.09	-0.11	-0.12	1.00																								
RMD	.452*	-.462*	.474*	0.27	0.34	.548**	-.402*	-0.31	.495*	1.00																							
R2MD	0.39	-.484*	0.30	0.16	0.14	.458*	-.478*	-.461*	.492*	.956**	1.00																						
R3MD	0.40	-.424*	0.39	0.20	0.18	.446*	-.480*	-.413*	.434*	.932**	.958**	1.00																					
RMA	0.14	-0.21	0.02	-0.13	-0.13	-0.19	0.28	0.10	0.03	-0.08	-0.07	-0.21	1.00																				
RWI	0.32	0.15	0.20	0.38	0.11	-0.08	.436*	.413*	0.29	-0.04	-0.01	0.01	0.32	1.00																			
RWE	.667**	-.405*	.494*	.712**	.549**	0.32	-0.10	0.11	.531*	.572**	.461*	.461*	0.02	0.23	1.00																		
RRS	.663**	-0.33	0.31	.493*	.414*	0.33	0.18	0.34	.447*	0.36	0.30	0.25	.435*	.606**	.668**	1.00																	
RNS	-0.04	-0.09	0.14	-0.08	-0.01	0.27	0.05	0.02	0.22	0.13	0.03	0.02	0.15	0.20	0.18	0.24	1.00																
RDM	-0.38	0.10	-0.16	-0.12	-0.22	-0.10	-0.03	0.05	-0.17	-0.20	-0.17	-0.03	-0.31	-.420*	-0.27	-0.39	-.471*	1.00															
FS	0.09	-0.19	-0.07	-0.05	-0.04	0.16	-.814**	-.696**	0.22	.527*	.574**	.617**	-0.40	-0.29	0.31	0.00	0.06	0.04	1.00														
FW	-0.09	0.18	0.12	0.05	0.06	-0.16	.818**	.713**	-0.23	-.521*	-.585**	-.603**	.437*	0.33	-0.29	0.03	0.02	-0.03	-.988**	1.00													
FLD	-0.07	0.17	0.18	0.04	0.10	-0.16	.806**	.715**	-0.23	-.474*	-.548**	-.575**	.484*	0.34	-0.28	0.05	0.03	-0.07	-.977**	.992**	1.00												
FTD	-0.07	0.18	0.15	0.05	0.07	-0.16	.817**	.714**	-0.23	-.507*	-.577**	-.603**	.443*	0.34	-0.29	0.04	0.08	-0.09	-.982**	.995**	.993**	1.00											
FUL*	-0.28	0.21	-0.36	-0.15	0.01	-0.08	0.05	-0.07	-0.25	-.418*	-.446*	-.532*	0.01	-0.30	-0.30	-0.21	0.22	-0.15	-0.04	0.04	0.01	0.06	1.00										
FUa*	-0.06	-0.11	-0.08	-0.13	-0.07	-0.17	0.18	0.26	0.00	-0.21	-0.14	-0.18	.531*	.421*	-0.23	0.31	-0.10	0.10	-0.33	0.38	.404*	0.37	-0.03	1.00									
FUb*	-0.23	0.06	-0.24	-0.29	-0.31	-0.24	0.09	0.27	-0.11	-0.37	-0.29	-0.26	0.17	0.29	-0.37	0.05	-0.26	0.36	-0.28	0.29	0.31	0.28	-0.35	.713**	1.00								
FLN	-0.06	-0.01	-0.11	-0.04	-0.16	-0.13	.641**	.480*	-0.18	-.514*	-.494*	-.547**	.409*	0.28	-0.25	0.01	-0.08	-0.03	-.914**	.897**	.856**	.875**	0.03	0.30	0.23	1.00							
FPT	0.08	0.04	0.02	-0.07	0.03	-0.04	.431*	0.24	0.06	-0.29	-0.33	-.415*	.490*	0.40	-0.09	0.22	0.29	-.621**	-.598**	.590**	.593**	.603**	0.18	0.11	-0.10	.599**	1.00						
FFN	-0.08	-0.15	-0.16	-0.17	-0.11	0.12	-.714**	-.633**	0.06	0.36	.435*	.420*	-0.28	-0.13	0.19	-0.09	0.27	-0.26	.778**	-.758**	-.737**	-.731**	0.11	-0.11	-0.22	-.681**	-0.35	1.00					
FSSC	-0.03	-0.09	-0.26	-0.17	-0.17	0.15	-.753**	-.674**	0.04	0.32	0.38	.420*	-0.34	-0.29	0.20	-0.03	0.07	0.04	.950**	-.936**	-.937**	-.933**	0.09	-0.35	-0.24	-.844**	-.538*	.733**	1.00				
FPh	-0.22	0.08	-0.09	0.00	-0.09	-0.02	.643**	.535*	-0.14	-.609**	-.635**	-.575**	0.27	0.37	-0.22	0.09	0.23	0.11	-.669**	.726**	.669**	.703**	0.21	0.36	0.26	.710**	.520*	-.484*	-.543**	1.00			
FT	-0.05	-0.02	-0.15	-0.06	-0.07	0.11	-.741**	-.663**	0.13	.409*	.457*	.489*	-0.36	-0.19	0.23	-0.07	0.09	-0.03	.942**	-.916**	-.903**	-.906**	0.09	-0.26	-0.26	-.879**	-.566**	.832**	.931**	-.600**	1.00		
FGP	-0.24	0.36	-0.11	0.07	-0.10	0.16	.627**	.529*	0.09	-0.13	-0.15	-0.22	0.13	0.29	-0.02	0.09	0.11	-0.07	-.634**	.587**	.574**	.577**	-0.21	0.02	0.10	.570**	0.34	-.451*	-.634**	0.31	-.613**	1.00	
FPP	-0.18	0.35	0.11	0.06	0.04	-0.17	.804**	.695**	-0.24	-.526*	-.595**	-.597**	0.37	.415*	-0.33	-0.03	0.06	-0.06	-.935**	.961**	.954**	.961**	0.08	0.38	0.29	.816**	.554**	-.663**	-.880**	.703**	-.788**	.575**	1.00

Principal component analysis (PCA) was utilized to extract three components and analyze the distribution of accessions in relation to different rootstock-scion combinations. Each component represented a percentage of the total variability observed, with the first component (PC1) accounting for the highest at approximately 38% (Table 38). PC1 was positively correlated with several fruit-related traits, including FW, FTD, FLD, FPP, FLN, PLL, FpH, PLW, FPT, FGP, RMA, FUa*, RWI, and FUb*, while showing negative correlation with FS, FT, FSSC, FFN, R3MD, R2MD, and RMD (Table 38). The second component (PC2) accounted for about 18% of the total variance and was positively correlated with PV, RWE, RRS, PSD, PBD, PSW, RMD, RML, RWI, R2MD, R3MD, and PSDM, while being negatively correlated with FUL* and PH. PC3 represented ap-proximately 8% of the total variability and was positively correlated with RMA and RNS, while being negatively correlated with RDM (Table 38).

In the PCA plot, all grafting combinations of the Barbarela F1 (BA) were clustered together and were close to the PCA due to their higher fruit weight and production, which differentiated them from the other scions. The cherry hybrids F1 used as scions, CH and VI, showed similar distribution between the three PCA axes due to their simi-lar values in fruit-related traits (Figure 18).

The combination of Optifort (O) rootstock with scions CH and VI, as well as the experimental rootstock C grafted with CH, were located in the upper side of the PCA plot due to their superior agronomic performance. These combinations exhibited high values of PV and RWE, surpassing all other combinations (Figure 18).

Discussion

As is well known, tomato plants can reach significant improvements in horticultural traits and disease resistance when grafted with inter and intraspecific rootstock. Specifically, grafting has been shown to enhance plant vigor and production while improving resistance to soil-borne diseases (Rivard et al., 2010; Barrett *et al*., 2012; Petran and Hoover, 2014). The tomato rootstock Optifort (O), which was adopted as control in the present work, is known for its high plant vigor when combined with tomato or eggplant scions (Kyriacou *et al*., 2020; Mozafarian *et al*., 2020). Additionally, several studies have reported that Optifort rootstock confers re-sistance to various abiotic and biotic stresses in tomato plants, which affects their growth and development (Gilardi *et al*., 2012; Kappel *et al*., 2022). In our work, the rootstock Optifort, exhibited the plant vigour (PV), and the plant stem diameter (PSD) comparable to the ones of the experimental rootstocks (A, B and C) tested. On the other hand, the control Optifort, showed the highest plant basal diameter (PBD). The results that we achieved in this work are consistent with the findings of Vanlay *et al*. (2022), who evaluated the agronomic performance of inter and intraspecific tomato rootstocks developed through traditional breeding strategies and incorporating resistant genes for soil-borne and airborne diseases. Specifically, our results regarding the key agro-nomic traits which were the plant height (PH), plant stem diameter (PSD), and root main length (RML) are consistent with their findings. Lang *et al*. (2020) also observed significant differences in plant stem diameter (PSD) in response to the rootstock used, and their results indicating lower PSD values in self and non-grafted plants are con-sistent with our own findings.

In comparison to the work of Khah *et al*. 2006, we also observed a significative in-teraction between the rootstock and the scion in relation to the plant height, also in comparison to the self-grafted and self-rooted plants. Our findings were consistent to the ones obtained in the previous-cited work, and we observed no particular difference for the plant height at the end of the growing cycle. We also evaluated the plant stem dry matter (PSDM) of the different rootstock-scion combinations and found no signif-icant variation between them, either individually or in interaction. Our results were consistent with those of Borgognone *et al*. (2013), who studied the effects of different nitrogen applications on various grafting combinations. Particularly, we observed no significant variation in PSDR content among self-grafted plants, which suggests that grafting did not influence the PSDM percentage. Additionally, similar to the findings of Borgognone *et al*. we did not observe any significant variation in root dry matter (RDM) content among the different grafting combinations tested, including self-grafted plants.

With regards to the root biomass, we ascertained that the roots weight (RWE) was significantly lower in the non-grafted plants in comparison to the grafted ones, and this result was consistent with the work of Lang *et al*. (2020) which tested the several roots' traits in relation to the different rootstock adopted.

As concern the plant earliness in terms of flowering in relation to the grafting technique, we ascertained a significant reduction of the flowering time in the self-grafted plants, but mostly in the non-grafted ones. In this frame, our results in terms of plant earliness were consistent with the work of Khah *et al*. (2006), in which they assess a significant reduction in the day after transplanting for the flowering pro-cess. Simultaneously, our results were in contrast with the work of Nkansah *et al*. (2013), in which they observed a significative reduction of the days to flowering in the grafted plants, compared to the non-and self-grafted ones.

In relation to the fruit weight (FW), the results that we achieved were not con-sistent with those of Zhou *et al*. (2022). Specifically, we did not observe a significant effect of rootstock on FW, although the values were generally higher in auto-grafting combinations.

In our study, we examined the longitudinal and transversal diameters of tomato fruits (FLD and FTD), and our results were consistent with those of Gong *et al*. (2022), who investigated variations in tomato fruit quality based on planting season, rootstock, and scion grafting. Specifically, we observed the same interaction between rootstock and scion for these traits. We also assessed the fruit firmness (FFN) of the different rootstock-scion combinations and found that there was low variation between them. Specifically, our results agreed with those of Khah *et al*. (2006), who reported no significant variation in FFN values among non-grafted plants. Additionally, in terms of fruit firmness (FFN), our findings for the F1 cherry varieties CH and VI were consistent with the results reported by Ruiz *et al*. (2015), who investigated several tomato qualitative traits, particularly related to the fruit texture, in response to different irrigation regimes, in 5 tomato cherry cultivars. However, for the F1 BA variety, which has a slightly flattened shape, we observed significantly lower FFN values compared to the afore-mentioned study. Regarding the fruit peel thickness (FPT), our findings indicate significantly higher FPT values in the tomato hybrids that we used as scions compared to those reported by Ohta *et al*. (2017), who correlated the FPT to the occurrence of radial cracking, which was not observed in our trial.

We also evaluated the chromatic parameter (CIEL*a*b*) of the cherry tomatoes tested, which was previously studied by Heredia *et al*. (2009) in relation to different osmotic dehydration techniques during the autumn-winter cycle. Our analysis revealed higher values for all chromatic coordinates, including lightness (L*), a*, and b*, compared to the findings of Heredia *et al*. Furthermore, they measured the fruits' solu-ble solid content (FSSC) as a response to osmotic dehydration at different times of ap-plication, and our results showed higher values compared to fresh fruit (osmotic de-hydration at time 0). Our findings were also in agreement with the ones achieved by de Matos *et al*. (2021), who studied the effect of chemical and organic fertigation on cher-ry tomato cultivars, and with Djidonou *et al*. (2016), who investigated the variation in tomato quality traits in relation to interspecific hybrids as rootstock. Specifically, we observed a significant interaction between rootstock and scion for FSSC and fruit acidity (FPh), which was consistent with the results of Djidonou *et al*.

The results that we registered in terms of the fruits production per plant (FPP), were consistent with the ones of the work of Borgognone *et al*. (2013), in which FPP, showed no significant variation in relation to the grafting combinations, including the self-grafting ones.

2.4.4. Conclusion

EXPERIMENTAL TRIAL A

The primary goal of the experimental trial A within the first research line, was to identify the most resilient material under drought stress conditions, with the potential for incorporation into an organic breeding program. Therefore, the chosen genotypes exhibited distinct responses to drought stress application, notably observed in the *B. macrocarpa* Guss. genotypes. These genotypes displayed a unique metabolic adaptation to overcome drought stress. Given that *B. macrocarpa* is a non-domesticated species, its natural habitat on rocky slopes along the sea cliffs of the Egadi Islands has conferred a strong adaptation to drought conditions, contributing to its resilience under such environmental challenges.

EXPERIMENTAL TRIAL B

The experimental trial B of the present PhD thesis aims to identify optimal rootstock combinations through innovative genetic improvement for organic table tomato cultivation. Statistical analysis reveals significant variation in production per plant, with the Barbarela F1 rootstock showing the highest values. Experimentation highlights substantial productivity and fruit quality differences in the F1 hybrid Barbarela compared to Cherry and Vittorio varieties, attributed to distinct vegetative-to-reproductive phase transitions. Notably, the Barbarela F1 hybrid produces fewer but significantly heavier fruits. The study lays the foundation for new organic greenhouse cultivation protocols, focusing on rootstocks selected for resistance genes, with future plans to explore resilience under abiotic stresses like water stress.

2.5. Research line III

DEVELOPMENT OF DIFFERENT AGRONOMIC AND GENETIC TOOLS FOR IMPROVING THE RESILIENCE OF *B. OLERACEA* L. COMPLEX SPECIES (n=9) AND *S. LYCOPERSICUM* L. FOR ORGANIC FARMING.

2.5.1. Introduction

EXPERIMENTAL TRIAL A

Molecular markers provide a simple, rapid, and non-destructive method of genotype selection and can be detected at any stage of the plant for reducing significantly the time, cost and other resources required for breeding programs to develop varieties (Jiang, 2013). Marker-Assisted Selection (MAS) is a technique for identifying and localizing genes associated with certain plant traits in its genome. The goal of plant breeding is to create new varieties that combine several traits defined by the breeder to meet the needs of farmers and consumers. It is also of significant interest in programs for the introgression of a gene of interest into an elite variety by backcrossing. It is an effective tool in plant breeding, especially when the target phenotypic traits are laborious or expensive to measure which can be supported by the molecular markers (Collard and Mackill, 2008).

Brassica is a genus of dicotyledonous plants of Brassicaceae family which native to Eurasia and the Mediterranean basin and includes about forty species. It is the type of genus of the Brassicaceae family (Snogerup *et al*., 1990). They are generally annual or biennial herbaceous plants, with cross-shaped flowers characteristic of Cruciferae. Cultivated species have a very diverse morphology depending on whether they have been selected for their leaves, their petioles, buds, flowers, roots, and seeds. They are grown as vegetables, condiments, oilseeds, or medicinal plants. Four species are mainly cultivated with an important role in the human diet: *Brassica oleracea* (Cole crops), *Brassica nigra* (black mustard), *Brassica napus* (rapeseed, rutabaga), *Brassica rapa* (turnip, rape, Chinese cabbage) (Katz, 2003). Among the *Brassica oleracea* crops, cauliflower (B. oleracea L. var. botrytis L.) and broccoli (B. oleracea L. var. italica) are the only two crops that offer a product represented by a hypertrophic reproductive organ, whereas all the others have constantly modified vegetative organs (Maggioni *et al*., 2010). The identification process of broccoli and cauliflower began a long time ago, however, the similar morphological structure of the edible organs of both cultures has repeatedly caused confusion to find unique descriptions of the plants, and names used in the past for today crops. The apparent similarity of broccoli and cauliflower plants and the similar morphology of the inflorescence may have influenced the scientific and common names, which are interchangeable in some cases (Gray, 1982; Bellostas *et al*., 2007; Maggioni *et al*. 2010). B. oleracea vegetables are full of bioactive compounds conferring to their products high antioxidant activity related to the richness of glucosinolates, isothiocyanates, polyphenols (Picchi *et al*., 2020; Ben Ammar *et al*., 2022).

Recent DNA analysis using molecular techniques supports a high degree of similarity between Sicilian wild Brassica species (n = 9) and B. oleracea crops (Lanner, 1998; Geraci *et al*. 2001; Choi *et al*. 2007; Maggioni *et al*., 2014). More recently, more similarities have been observed between B. oleracea crops and

wild Mediterranean Brassica oleracea complex species (n=9) than for the British wild Brassica ones (Allender *et al*., 2007; Maggioni, 2015; Maggioni *et al*., 2018; Stansell *et al*., 2018; Stansell and Björkman, 2020; Mabry *et al*., 2021).

Molecular markers proved to be important tools for assessing genetic variation and relationships in plant species above all for organic breeding (Treccarichi *et al*., 2021). Among these there are simple sequence repeats (SSRs), alternately known as microsatellite markers, which have been successfully used for evaluating the genetic variability and distinguishing among nearly related Brassica genotypes (Louarn *et al*., 2007; Ofori *et al*., 2008; Moghaddam *et al*., 2009), because of their codominance and ability to reveal an high number of alleles for locus, resulting in a high degree of reproducibility and variability (Leroy *et al*., 2000).

In plants, MADS-box genes are involved in the control of all major aspects of their development such as the differentiation between of male and female gametophytes, development of embryos and seeds, roots, flowers and fruits, and the of flowering time determination (Rounsley *et al*., 1995). Bowman *et al*. (1991) studied flower development genes, found several genes involved, like apetala 1 and cauliflower, observed also in *Arabidopsis thaliana*. These genes are closely related to members of the MADS-box gene family and a mutant copy of these is present in the *B. oleracea* genome C. Irish and Sussex (1990) characterized a lot of floral morphotypes produced by the homeotic recessive *apetala* 1 (*ap1*) mutation in *Arabidopsis* and the homozygote for this mutation demonstrated low inflorescence affecting the formation of floral buds.

Exercising the simple sequence repeat (SSR) marker *BoAP1*, advanced number of alleles were found in the wild *B. oleracea* complex species (n=9) than in cabbage and cauliflower. *BoAp1-a* locus located in a single genomic region on relation group O6 chromosome of *B. oleracea* with the other ones (*BoCAL*, *BoLFY*, *BoAP1-c*, *BoREM1*) is related to MADS-box genes (Duclos and Bjorkman, 2005). In 2000, Smith and King proposed a genetic model grounded on segregation of recessive alleles for *BoCAL* and *BoAP1* candidate genes which showed differences in the stage of flower development arrest between broccoli and cauliflower. According to Smith and King's allelic distribution genetic model, the domestication strategy reduced the allelic diversity by promoting loci affecting the arrest of floral development which determined the inflorescence hypertrophy and then the domestication for cauliflower's curd morphotype; the Sicilian Purple was indicated as an important intermediate of this domestication strategies (King, 2001). In 2004, four primers (*BoAP1*, *BoABI1*, *BoPLD1* and *BoTHL1*) were designed by Tonguc and Griffiths to investigate and amplify the genomic DNA to evaluate the genetic similarity between several Brassica oleracea cultivars belonging to three varietal groups (broccoli, cauliflower, and cabbage). Other primer PBCGSSRBo39 was designed by Burgess et al. (2006) to demonstrate a useful molecular marker for crop improvement which was derived from shotgun sequencing methods.

These five SSR primers (BoAP1, BoABI1, BoPLD1, BoTHL1 and PBCGSSRBo39) were chosen by Branca *et al*. (2018) by opting them from among others primers, for phylogenetic analysis and to evaluate genetic similarity between different *B. oleracea* accessions and wild *B. oleracea* complex species (n=9), belonging to two crops (cauliflower and broccoli) as well as to estimate genetic divergence using the FST statistical parameter, were broccoli cultivars grouped with cauliflower cultivars as expected and wild species

showed major genetic differences. Sheng *et al*. (2019) characterized and mapped 91 MADS-box transcription factors able to discern from the type I (Mα, Mβ, Mγ) and type II (MIKCC, MIKC*) genetic groups as consequence of phylogenetic and gene structure analysis: 59 genes were randomly distributed, on 9 chromosomes, and 23 were located in 19 scaffolds, while 9 of them were not located due to the lacking information on NCBI database (Sheng *et al*., 2019). Treccarichi *et al*. (2021) used the set of markers used by Branca *et al*. (2018) to calculate the genetic diversity among 9 accessions of *B. oleracea* crops and *B. oleracea* complex species (n=9) and evaluating the hypertrophic induction of the curd. The SSRs assay can be also exploited in population genetics to discover allelic variants related to interesting traits and could be also a topic for the breeders which can apply to inherit them in the F2 population (Gaebelein *et al*., 2019).

In the present work, the above cited five SSR primers based on the sequences of several MADS-box genes were used to analyse the allelic variation of different Sicilian landraces and hybrids F1 of cauliflower and broccoli, and of some B. oleracea complex species (n=9), for associating them with the inflorescence morphometric traits which have been measured among the accessions. The following manuscript aims to identify the most interesting allelic variants able to be used For MAS as a useful breeding tool for organic breeding.

EXPERIMENTAL TRIAL B

The exploitation of new genetic resources by next generation sequencing (NGS) techniques could be assessed for discovering new resistance traits suitable for more sustainable productions (Ashraf *et al*., 2022). The new technologies can enhance and shorten the process of selection of new genotypes in comparison to the normal, conventional breeding techniques by the marker assisted selection (MAS), quantitative traits loci (QTLs) mapping, and genome sequencing and assembly. All the above-mentioned techniques require the application of bioinformatic tools and molecular techniques to be performed.

Several methods have been performed to assess the genetic diversity of the tomato gene pools such as the genotyping by molecular markers (Shi *et al*., 2011; Foolad and Panthee, 2012; Zhou *et al*., 2015), the genome wide association by linkage maps (Tripodi *et al*., 2021), the construction of physical chip equipped with thousands of molecular markers such as the DNA microarray (Lievens *et al*., 2003; Sim *et al*., 2012; Tranchida-Lombardo *et al*., 2019), and the genotyping by sequencing (Carbonell *et al*., 2018; Xie *et al*., 2019). Many of the methods I previously mentioned involve analysing genetic data related to tomato plants' resistance to various viruses, including the tomato spotted wilt virus (TSWV). These methods often focus on specifical genes associated with resistance and aim to understand how different variants of those genes affect the plant's ability to resist infection. The most studied one, is the *Sw-5* gene cluster, which encodes protein receptors that are potentially able to recognize microbial products and activate signalling pathways that lead to plant cell immunity (De Oliveira *et al*., 2018). The *Sw-5* domain is a dominant resistance gene which was initially discovered in a wild Peruvian tomato (*S. peruvianum*). It has since been incorporated into tomato breeding programs with the goal of developing cultivars that are resistant to viruses such as the tomato spotted wilt virus

(TSWV). Through the introduction of the *Sw-5* gene into cultivated tomato varieties, breeders aim to confer resistance to these viruses and reduce crop losses caused by viral infections.

In addition to the TSWV, the Tomato mosaic virus (ToMV) represents another infectious virus diseases *S. lycopersicum* L. The major genes conferring the ToMV resistance were detected, and they were: *Tm-1, Tm-2* and *Tm-22* (Hall, 1980; Lanfermeijer *et al*., 2003; Shi *et al*., 2011; Nagai *et al*., 2019). The following genes were introgressed in several tomato cultivars by breeding programs and markers assisted selection (MAS) could represent the preferred way to select, among the different accessions, the populations having the above-mentioned resistance genes. In the following proposal draft, the screening of the resistant population against ToMV disease will be performed using the SNP marker Tm-2 (Table 39), which provides hight information about the disease resistance by its homozygous or heterozygous conditions. The detection will be performed among the individuals in every accession (insuring the ToMV resistance) detecting the heterozygous condition of the examined locus, which determines the disease resistance (Shi *et al*., 2011; Martinelli *et al*., 2015).

2.5.2. Materials and Methods

EXPERIMENTAL TRIAL A

Plant material includes 31 accessions of Sicilian landraces of broccoli (B. oleracea var. italica) and cauliflower (B. oleracea var. botrytis) and 8 crop wild relatives (CWRs) belonging to the Brassica active collection of the Department of Agriculture, Food and Environment (Di3A) of the University of Catania (UNICT), as shown in Table 39. Crop cultivation was carried out in an open field and the experimental design was described by Branca *et al*. (2018). Plants were characterized for their agronomical traits related to the inflorescence production following the International Board for Plant Genetic Resources (IBPGR) descriptors. Examined traits include (IW), height (IH), diameter (ID1), shape (IS), angle of curvature (IA), and inflorescence stem thickness (ID2) and were analysed by the laboratory of Biotechnology of Vegetable and Flower Crops of the Di3A department of the University of Catania (UNICT). IW was calculated using an analytical scale while the IH and ID1 traits were calculated using a meter rule and ID2 was noted using a calibre. IS parameter belonging to the ratio between IH and ID1 while curvature angle IA is the angle limited to the central vertical inflorescence axes and the tangent to the extreme part of it and was calculated using goniometer.

DNA extraction was performed using the kit GenEluteTM Plant Genomic DNA Miniprep (Sigma Aldrich Inc.) and 200 ng μl^{-1} were used for PCR reaction, as reported by Branca *et al*. (2018). PCRs were done used the primers list reported in Table 40, obtaining the flanking SSRs sequences by Tonguc and Griffiths (2004) for BoTHL1, BoAP1, BoPLD1, and BoABI1 and by Burgess *et al*. (2006). SSRs genome allocation were checked using basic local alignment search tool (BLAST) (version 1.17) and Ensembl database (release 2021, version 3) and Uniprot database (release 2021, version 3) was used to study encoding regions close to the gene of interest. DNA amplification was performed in a Perkin Elmer 9700 thermocycler (ABI, Foster

City, CA, USA) as reported by Branca *et al*. (2018). Capillary electrophoresis was carried out by ABI PRISM 3130 Genetic 191 Analyser (Applied Biosystems, Waltham, MA, USA) as described by Branca *et al*. (2013) and Branca *et al*. (2018) and GeneMapper 3.7 software (Applied Biosystems, Waltham, MA, USA) was used to note the fragments size checking manually each alleles peak.

Table 39. List of *B. oleracea* complex species (*n* = 9) accessions utilized.

Accession Code	Laboratory Code	Origin	Species
UNICT 583	BR 46	Vittoria	BR1
UNICT 658	BR 45 S1	Acireale	BR2
UNICT 658	BR 129	Roccella Valdemone	BR3
UNICT 657	BR 128	Roccella Valdemone	BR4
UNICT 655	BR 126	Adrano	BR5
UNICT 637	BR 106	Cefalù	BR6
UNICT 3675	BR 94 S1	Francavilla	BR7
UNICT 3668	BR 115 S1	Troiano	BR8
UNICT 574	BR 36	Biancavilla	BR9
UNICT 3578	BR 165 Marathon	Esasem	BRF1.1
UNICT 651	BR 122 Packman	Petoseed	BRF1.2
UNICT 4145	BR 13 S3 AC	Modica	CI1
UNICT 579	BR 41	Modica	CI2
UNICT 3190	BR 15 S 1 A	Modica	CV1
UNICT 3669	BR 17 S2	Ragusa	CV2
UNICT 3674	CV 19 S2 A	Piazza Armerina	CV3
UNICT 4137	CV 99 S2 B	Adrano	CV4
UNICT 4138	CV 76 S2	Acireale	CV5
UNICT 3652	CV 159	Catania	CV6
UNICT 3900	BR 13 A X CV98/21	Di3A	CV7
UNICT 3895	CV 98/2 X CV 136 EG	Di3A	CV8
UNICT 3089	CV 75 S3AC	Acireale	CV9
UNICT 3906	CV 24 S4	Biancavilla	CV10
UNICT 3671	CV 72 S2	Catania	CV11
UNICT 3876	CV 171 Menhir F1	ISI sementi	CVF1.1
UNICT 3878	CV 173 Freedom	3878 Royal Sluis	CVF1.2
UNICT 3902	CV 33 S1	Royal Sluis	CVF1.3
UNICT 3880	CV 175 White Flash	Sakata	CVF1.4
UNICT 3879	CV 174 Graffiti	ISI sementi	CVF1.5
UNICT 3892	CV 98/2 X BR 13 S3	DISPA 3	CVF1.6
UNICT 3893	CV 136 EG X CV98/2	DISPA 1	CVF1.7
UNICT 342	Brassica macrocarpa 1	Favignana	BM
UNICT 733	Brassica rupestris 1	San Vito Lo Capo	BU1
UNICT 3270	Brassica rupestris 2	Bivongi	BU2
UNICT 732	Brassica rupestris 3	Roccella Valdemone	BU3
UNICT 736	Brassica rupestris 4	Ragusa Ibla	BU4
UNICT 3040	Brassica villosa 1	Marianopoli	BV
UNICT 3512	Brassica incana 1	Agnone Bagni	BY1
UNICT 4158	Brassica incana 2	Sortino	BY2

Legend: CV—Cauliflower; CI-Ciurietti landrace; BR—Broccoli; BY—B. incana; BM—B. macrocarpa; BU—B. rupestris; BV—B. villosa.

Table 40. List of primers utilized with their sequences and chromosome position.

Primers name	SSR motif	Primer sequence (forward, reverse)	Chrom osome	Position (bp)	Working code
BoAP1	$(AT)_{9\text{-}1}$	GGAGGAACGACCTTGATT GCCAAAATATACTATGCGTCT	C6	33,883,667-33,887,357	P1
BoTHL1	$(CTT)_7$	GCCAAGGAGGAAATCGAAG AAGTGTCAATAAGGCAACAAGG	C9	17,254,558 - 17,255,176	P2
BoABI1	$(TC)_{16}$	TATCAGGGTTTCCTGGGTTG GTGAACAAGAAGAAAAGAGAGCC	C1	1,229,915,511-12,992,170	P3
BoPLD1	$(CT)_7(AT)_{7\text{-}1}$	GACCACCGACTCCGATCTC AGACAAGCAAAATGCAAGGAA	C5	46037340 - 46,037,606	P4
PBCGSSRBo 39	$[GGTCG]_4$	AACGCATCCATCCTCACTTC TAAACCAGCTCGTTCGGTTC	C7	50595248-50595537	P5

The Allelic data set was codified by numeric scores, distinguish from 0 (absence of any allele), 1 (heterozygosity), 2 (homozygosity). Statistical analysis was performed using SPSS software version 27 to realize Pearson's correlation among the traits and among each allele to identify the allelic variant involved in the size of inflorescence. PCA was also performed to explain the variability among genotypes by the main components.

EXPERIMENTAL TRIAL B

Plant materials includes 34 accession of *Solanum lycopersicum* belonging to gene bank of the Department of Agriculture, Food and Environment (Di3A) of the University of Catania (UNICT), as shown in Table 41.

Table 41. List of the genotypes tested for the trial with the origin or the common name.

Accession	Crop code	Working code	Origin
	A-RC	1	Reggio Calabria
	LINEA 17	2	Borghese Sluis
UNICT2009	LINEA ITALIANA COR B	3	Corbarese
UNICT1912	PO163	4	California
UNICT1913	PO164	5	California
UNICT1915	PO166	6	California
UNICT1960	PO211	7	S. Stefano di Camastra.-Azzolina
UNICT1975	PO226	8	Trapani
UNICT3301	PO264	9	T-47
UNICT3371	PO269	10	P4 - COIS94
UNICT1754	PO5	11	La rosa
UNICT2028	PS LA ROSA 12	12	La rosa
UNICT2021	PS05	13	Melfi
UNICT2017	PS1/18	14	Italsementi
UNICT2028	PS12	15	Francavilla
UNICT2029	PS13	16	Ponderosa
UNICT2031	PS15/8	17	Milazzo 1
UNICT2036	PS20	18	Montechiaro
UNICT2040	PS24	19	Zorzi
UNICT2042	PS26	20	Vibo Valentia
UNICT2043	PS27	21	Palmi
UNICT2044	PS28	22	S. Stefano in Aspromonte
UNICT2051	PS35	23	Piccolo rosso a punta
UNICT2020	PS4/20	24	Basico'
UNICT2060	PS44	25	La Rosa
UNICT2063	PS47	26	Trapani
UNICT2067	PS51	27	F2 Q53*EE12M Irene - Dr. Acciardi
UNICT2069	PS53	28	F2 20*126 Irene - Dr. Acciardi
UNICT2075	PS59	29	Enna - Prof. Noto
UNICT2022	PS6	30	S. Giorgio – Calabria
UNICT2023	PS7	31	Rizziconi – Calabria
UNICT2023	PS7/10	32	Rizziconi – Calabria
UNICT2024	PS8	33	Pizzoni – Calabria
	SAL	34	Lipari
BT05000	TDP	35	Tomate de Penjar, Valencia
BT04140	RDA	36	Rosada de Ademuz
BT05010	CT	37	Tomate comercial
ALD1	TVA	38	Tomata Valenciana

Plants were sown in March 2022 in cellular trays, and they grow until the phenological phase of four true leaves inside growth chambers at the Universitat Politècnica of València (UPV), controlling light intensity, temperature, and humidity. Plants were transplanted in April 2022 in open field, in an organic farming in

Valencia (ES) with the experimental design of three randomized blocks (Figure 19). Plants were grown at single stem by the pruning of the lateral shoots.

Figure 19. *S. lycopersicum* collection grown in Valencian field under organic conditions. The specif kind of cultivation is called "baraca" and it is represented by the plants which are supported by canes arranged in a triangular pattern.

During the growing cycles plants were characterized by the qualitative and quantitative descriptors related to the plants, leaves, inflorescence, and fruits, analysing the fruits setting, the ripening earliness and uniformity. Leaves were analysed for their chlorophyll index by the SPAD 502 (Minolta, Japan), in addition to their anthocyanin and flavanols content and their nitrogen balance index (NBI) by the DUALEX^{TM} (Force A, France). Fruits were harvested at the commercial stage and were characterized for their morphometric traits and for their soluble solid content and for acidity. Notably, all the bio-morphometric descriptors used for the trial are the same employed for the experiment of the Research line I, trial B, and so they are reported in Table 4. Genotypes were characterized following the International Board of Plant Genetic Resources (IBPGR) descriptors for *Solanum lycopersicum*.

The experimental trial B encompassed the genotyping of six genes involved in the tolerance against crucial tomato pathogens. In this framework, were employed six molecular markers which were *Tm2*^{2} for the detection of the resistance against Tomato Mosaic Virus (ToMV), *I2* against race 2 of the *Fusarium* wilt pathogen *Fusarium oxysporum* f sp *lycopersici*, *Sw5* against a wide range of (thrips-transmitted) orthotospoviruses encompassing tomato spotted wilt virus (TSWV), *Ve* involved in race-specific resistance to

infection by *Verticillium* diseases, Ty5 against Tomato Yellow Leaf Curl Virus (TYLCV) and Mi which confers resistance against three of the most damaging species of root knot nematodes (*Meloidogyne* spp). The list of the molecular markers used is listed in Table 42.

Table 42. List of the molecular markers used for the evaluation of the resistant genes.

Name	Primer forward	Primer reverse
Tm2²	CAAGCATGTAACAGTTGCTTTTC	CAGGTATCCACATCAAGGTTTG
I2	CAAGGAACTGCGTCTGTCTG	ATGAGCAATTTGTGGCCAGT
Sw5	AATTAGGTTCTTGAAGCCCATCT	TTCCGCATCAGCCAATAGTGT
Ty5	TTGTTCCTGATGGTTCTGGT	TTTCTTCATCTGGGGTTTCA
Ve	TCACGTAATGGTCTAACTGGTCTC	AAGGCTCCCGCTGAGTAAAT
Mi	TGGAAAAATGTTGAATTTCTTTTG	GCATACTATATGGCTTGTTTACCC

The detection of the presence or absence of the co-dominant resistant gene introgressed by the CWR *S. peruvianum*, was performed by the electrophoresis on agarose gel, stained with GelRed® (Biotium, USA), at the concentration of 2.5 %, at 100 V for 47 minutes. On the other hand, for the resistance marker *Tm2*, was carried out the Hight Resolution Melting (HRM) using the LightCycler® 480 (Roche Diagnostics, USA). For Tm2, the reaction was of 10 µL per each sample. The different melting temperatures will allow easily to distinguish the homozygous resistant (Tm= 59°C), homozygous susceptible (Tm= 66°C) and heterozygous resistant (Tm= 59°C and 66°C). For all the molecular markers, the DNA concentration was adjusted at 30-40 ng μL^{-1}.

2.5.3. Results

EXPERIMENTAL TRIAL A

Based on the bio-morphometric characteristics of the inflorescence analyzed were inflorescence weight (IW), height (IH), diameter (ID1), stem thickness (ID2), shape (IS) and angle of curvature (IA). With regard to IW, it varied among genotype from 1095.8 to 16.65 g, for CVF1.1 and BV, respectively (Table 43). IW showed the highest values in cauliflower F1 hybrids and landraces, followed by broccoli heirlooms and landraces. CWRs showed the lowest value of IW varying from 33.3 to 16.7 g, for BU1 and BV, respectively. With regard to IH, the CWRs group, represented by the accessions BM, BU1, BU2, BU3, BU4, BV, BY1, BY2 showed the highest IH values followed by the lowest IW, ID2, ID1 and IA due to the characteristics of their inflorescence architecture, which is large and thin, with large flower buds, low inflorescence density and low bolting resistance. IH varied for the CWRs group from 14.8 to 27.6 cm, while in cauliflower and broccoli groups, it varied from 7.5 to 22.2 cm for CVF1.4 and BR2, respectively. Concerning ID2, we observed the highest values for cauliflower morphotype varying from 28.6 to 39.8 mm for CV3 and CV1, respectively, while broccoli genotypes exhibited an average value of 3.1 cm varying from 2.6 to 3.8 mm for BR8 and BR1, respectively. The crop wild relatives group showed an average ID2 value. of 17.9 mm varying from 9.6 to 22.5 mm for BM and BY1, respectively (Table 43). Concerning ID1 it showed the highest value for the cauliflower group varying from 13.6 to 21.1 cm for CV3 and CV4, respectively, and it varied for the broccoli group from

4.7 to 12.3 cm for BR9 and BRF1.1, respectively (Table 43). IS showed the highest values for broccoli accessions varying from 1.2 to 3.6 for BRF1.1 and BR9, respectively (Table 43).

Table 43. Inflorescence morphometric characteristic in descending order, from the heaviest to the lightest. The analysed traits were the weight (IW), height (IH), stem and inflorescence diameter (ID2 and ID1), shape (IS) and curvature angle (IA).

Accession	IW	IH	ID2	ID1	IS	IA
CVF1.1	1095.8 (21.1)*	11.1 (8.4)	42.32 (8.5)	18 (8.7)	0.62 (9.6)	110 (21.9)
CV1	965.7 (37.4)	15.4 (14.6)	39.82 (16.4)	20.7 (17.4)	0.74 (16.6)	105 (19.4)
CV4	666.6 (42.5)	15.2 (13.2)	34.09 (19.6)	21.1 (15.0)	0.72 (12.1)	112 (20.4)
CI1	628.8 (33.7)	16.8 (16.6)	38.13 (18.5)	19.7 (14.6)	0.85 (14.6)	101 (22.5)
CVF1.2	605 (33.8)	8.9 (16.7)	30.99 (10.3)	16.9 (11.8)	0.53 (12.1)	113 (13.3)
CV5	567.3 (38.2)	14.5 (15.6)	36.96 (19.8)	19.5 (13.1)	0.74 (17.29)	113 (13.5)
CV6	564.9 (37.0)	14.5 (20.7)	34.55 (12.6)	20 (15.1)	0.72 (18.7)	104 (16.7)
CV7	554.5 (56.7)	18.8 (20.4)	30.84 (26.9)	19.5 (19.3)	0.96 (29.8)	107 (17.7)
CVF1.3	541.5 (54.7)	13.7 (24.4)	32.25 (21.9)	18.9 (29.6)	0.72 (18.3)	112 (22.3)
CV8	503.9 (35.4)	16.8 (28.4)	32.36 (18.1)	16.5 (17.9)	1.02 (34.4)	100 (27.4)
CVF1.4	467.09 (41.1)	7.46 (20.9)	29.97 (13.3)	14.6 (15.7)	0.51 (11.1)	101 (15.6)
CVF1.5	461.8 (47.1)	10.8 (16.1)	32.98 (13.9)	17.5 (16.4)	0.62 (17.4)	110 (19.3)
CV9	453.5 (49.7)	11 (17.2)	35.54 (17.9)	18.1 (27.3)	0.61(22.5)	117 (15.8)
CV10	443 (55.9)	12.7 (23.0)	36.41 (24.2)	16.7 (23.8)	0.76 (32.4)	91 (26.5)
CVF1.6	438.8 (84.4)	17.6 (24.4)	28.81 (28.1)	16.8 (29.4)	1.05 (34.2)	93 (17.7)
CI2	378.3 (46.2)	10.2 (21.0)	36.8 (16.9)	17.2 (19.5)	0.59 (17.8)	113 (17.5)
BRF1.1	319.8 (40.9)	14.1 (26.8)	3.52 (19.5)	12.28 (26.7)	1.2 (44.4)	76 (29.8)
CVF1.7	317.4 (42.0)	17.2 (22.2)	29.22 (28.1)	14.8 (16.0)	1.17 (33.1)	98 (21.6)
CV11	305.7 (68.2)	8.7 (20.7)	31.7 (18.1)	15.4 (22.8)	0.56 (19.7)	92 (25.2)
BR1	279 (39.0)	16.6 (18.1)	3.84 (17.3)	11.1 (23.7)	1.5 (28.2)	57 (21.5)
BR2	266.9 (33.4)	22.2 (30.9)	3.18 (13.2)	8.47 (32.7)	2.7 (37.4)	58 (19.4)
CV2	263.6 (56.1)	11.2 (28.3)	34.23 (18.8)	14.4 (22.0)	0.78 (21.2)	91 (23.4)
BR3	226.4 (39.6)	18.2 (12.9)	3.13 (26.8)	7.89 (29.4)	2.3 (30.5)	49 (27.8)
BR4	217.7 (58.3)	18.2 (18.2)	2.93 (29.8)	9.49 (31.6)	1.9 (29.4)	54 (26.3)
BRF1.2	212.8 (36.3)	12.8 (12.2)	3.14 (15.0)	7.78 (23.1)	1.9 (16.5)	46 (24.1)
BR5	188.3 (51.8)	16.6 (23.4)	2.87 (24.3)	7.7 (28.3)	2.2 (24.2)	46 (24.1)
CV3	186.6 (41.3)	8.4 (17.5)	28.6 (16.7)	13.6 (15.1)	0.61 (18.2)	85 (24.8)
BR6	164.0 (49.0)	16.5 (17.9)	3.34 (32.4)	8.25 (29.5)	2 (52.3)	46 (32.8)
BR7	143.9 (42.2)	16 (29.0)	2.69 (22.7)	7.82 (29.0)	2.1 (22.6)	48 (26.7)
BR8	109.5 (30.8)	15.5 (9.5)	2.64 (20.2)	7.88 (25.8)	2 (23.4)	41 (34.2)
BR9	63.1 (41.7)	16.9 (23.5)	2.76 (18.9)	4.74 (22.3)	3.6 (15.5)	27 (15.2)
BU1	33.30 (28.3)	27.60 (15.5)	16.20 (20.2)	3.10 (17.9)	0.19 (21.2)	14.00 (11.7)
BU2	28.7 (1.6)	19.45 (1.5)	19.25 (3.3)	4.05 (0.2)	0.21 (0.1)	15.00 (0.9)
BY1	27.7 (3.7)	20.35 (1.0)	22.50 (4.5)	3.25 (0.7)	0.14 (0.1)	13.50 (2.1)
BM	26.6 (5.9)	16.76 (4.6)	9.61 (2.5)	2.69 (0.4)	0.29 (0.1)	11.25 (2.6)
BU3	22.4 (0.4)	23.45 (4.0)	19.60 (1.5)	2.30 (0.3)	0.12 (0.1)	9.50 (0.7)
BU4	21.1 (0.8)	19.20 (2.2)	18.85 (3.6)	2.15 (0.1)	0.12 (0.1)	13.50 (2.1)
BY2	20.6 (1.3)	20.75 (0.6)	18.45 (1.6)	2.80 (0.4)	0.15 (0.1)	11.50 (0.7)
BV	19.7 (0.6)	14.80 (0.4)	18.95 (1.2)	2.40 (0.2)	0.13 (0.1)	10.50 (0.7)

Broccoli hybrids F1 resemble cauliflower inflorescence and, for this reason, showed a lower CS ratio than for broccoli landraces which are characterized by higher values of IH (Table 43). The cauliflower genotype showed intermediate IS values varying from 0.5 to 1.17 for CVF1.2 and CVF1.7, respectively. The CWRs group showed the lowest IS value, which varied from 0.1 for BU3, BU4, and BV to 0.3 for BM (Table 43). Concerning IA character, it exhibited the highest values for the cauliflower genotypes showing the average value of 102.9◦, varying from 85◦ to 117◦ for CV3 and CV9, respectively (Table 1). Broccoli genotypes are characterized by a reduced amplitude compared to the cauliflower group, and it varied from 279◦ to 76◦ for BR9 and BRF1.1, respectively (Table 43). The CWRs group showed the lowest IA values due to their different

simple inflorescence architecture that is slenderer than cauliflower and broccoli genotypes, and it varied from 9.5◦ to 15◦ for BU3 and BU2, respectively (Table 43).

Pearson's correlation showed a significant correlation among IW and the descriptors ID1, ID2 and IA. On the other hand, the IS descriptor is derived from the ratio between ID1 and IH and is negatively related to ID2 (Table 44). Concerning IA, it was positively correlated to ID1, IW and ID2, respectively (Table 44)

Table 44. Pearson correlation among traits.

Genotype	IW	IH	ID2	ID1	IS	IA
IW	1					
IH	0.024	1				
ID2	**0.680****	-0.035	1			
ID1	**0.880****	-0.066	**0.724****	1		
IS	-0.117	-0.068	**-0.638****	-0.107	1	
IA	**0.847****	-0.033	**0.706****	**0.980****	-0.086	1

The correlation among the molecular markers and the inflorescence descriptors showed a high significant correlation with the allelic variation 155 bp of AP1 (P1), which was correlated negatively with IH and positively with IW, ID1, ID2 and IA (Table 45). The allelic variant of 157 bp of BoTHTL1 was positively correlated with IH and negatively with IW, IA, ID1 and IS, respectively, in decrescent order (Table 45). The allele of 165 bp found for P2 was positively correlated with ID1, ID2 and IA, while it was negatively correlated with IW. The allelic variation of 184 bp detected for the marker BoAB1 (P3) was negatively correlated with IS, ID1, WI and IA, and positively with IH (Table 45).

Table 45. Correlation among all the allelic variants detected by the molecular markers used and the analysed traits to individuate the most associated alleles to the examined traits.

Allelic variant	Rank of IW	Rank of IH	Rank of ID2	Rank of ID1	Rank of IS	Rank of IA
P1_155	**0.622****	-0.471**	0.521**	0.622**	0.032	0.677**
P1_156	-0.101	0.156	0.219	-0.097	0.202	-0.135
P1_164	-0.375	0.072	-0.082	-0.334	-0.283	-0.306
P2_153	-0.288	0.189	0.219	0.308	0.00	-0.264
P2_157_2	-0.338*	0.405**	-0.088	-0.372*	-0.376*	-0.372*
P2_162	-0.152	-0.029	-0.418**	-0.266	0.196	-0.175
P2_165	-0.461*	-0.220	**0.583****	**0.594****	-0.014	0.538**
P2_168	0.160	0.021	0.226	0.205	0.050	0.204
P3_180	0.010	0.033	0.069	0.046	-0.003	0.095
P3_184	-0.455**	0.440**	0.123	-0.455**	-0.477**	-0.433*
P3_186	-0.233	0.296	-0.214	-0.257	0.062	-0.187
P3_190	0.257	-0.440*	0.268	0.303	0.192	0.226
P3_192	0.418*	-0.324	0.222	0.424**	0.156	0.436**
P3_194	0.140	-0.015	-0.068	0.068	0.146	0.174
P4_282	-0.139	0.199	-0.097	-0.184	-0.168	-0.232
P4_288	0.460**	-0.333*	0.308	**0.522****	0.172	**0.568****
P4_291	-0.462**	0.381*	-0.462**	-0.477**	0.148	0.485**
P5_294	-0.343*	0.410**	0.078	-0.376*	-0.391*	0.384*
P5_304	0.306	0.050	0.089	0.217	0.165	0.330*
P5_308	0.384*	-0.474**	0.132	0.449**	0.478**	0.380*

The allelic variant of 288 bp of the BoPLD1 marker (P4) was positively correlated with IA, ID1 and IW, and negatively with IH, whereas on the other hand, the allelic variant of 291 bp was positively correlated to IA and IH, and negatively correlated to ID1, IW and ID2, respectively, in decrescent order (Table 45). The PBCGSSRBo39 (P5) allele of 294 bp was positively correlated with IH and IA, and negatively correlated with IW, ID1 and IS. Finally, the allelic variant of 308 bp of the marker P5 was positively correlated with IS, ID1, IW and IA, respectively, in decrescent order, and negatively correlated with IH (Table 45).

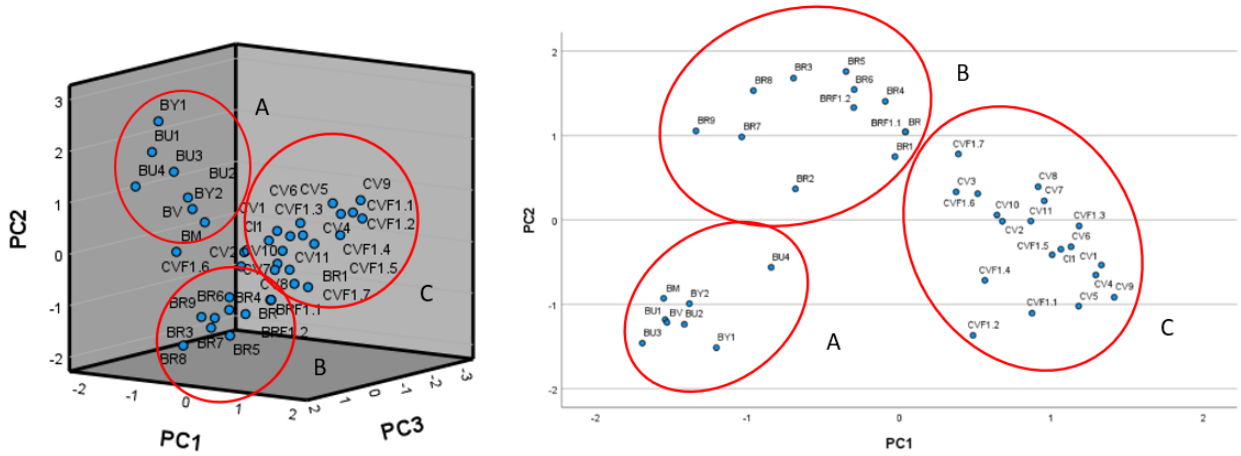
On the basis of the correlation observed among the inflorescence descriptors and the alleles detected for the five primers utilized we directed our attention to the alleles most correlated with at least four correlations with the six inflorescence descriptors utilized. The most correlated alleles chosen were the following: P1_155, P2_153, P2_157, P2_162, P2_165, P2_168, P3_184, P3_186, P3_190, P3_192, P4_288, P4_291, P5_294, P5_304 and P5_308 (Table 46).

Table 46. Principal component of the rank of all the examined traits and for all the correlated allelic variants.

	PC1	**PC2**	**PC3**
IW (g)	**0.900**	0.093	-0.155
IH (cm)	-0.564	0.108	0.132
ID2 (cm)	0.670	0.653	-0.045
ID1 (cm)	**0.938**	0.135	-0.049
IS	0.200	-0.858	0.147
IA (°)	**0.919**	0.131	-0.213
P1_155	0.671	0.197	-0.112
P2_153	-0.350	-0.035	0.067
P2_160	-0.482	0.422	0.074
P2_162	-0.183	-0.396	-0.411
P2_165	0.579	0.283	0.462
P3_184	-0.570	0.503	0.089
P3_186	-0.353	-0.196	-0.144
P3_188	-0.269	-0.041	-0.041
P3_190	0.323	-0.085	0.693
P3_192	0.509	-0.150	-0.287
P4_288	0.673	-0.066	0.098
P4_291	-0.643	-0.162	0.031
P5_294	-0.503	0.523	0.027
P5_304	0.226	-0.046	-0.765
P5_308	0.526	-0.361	0.571
Variance (%)	*32.60*	*11.57*	*9.68*

Utilizing the data from the above cited allelic variants for the five primers utilized and for the six inflorescence descriptors, we established the related PCA, for which the main component (PC1) is positively correlated with ID1, IA, IW, P4_288, P1_155 and ID2, respectively, in decrescent order, whereas it was negatively correlated with P4_291, IH and P3_184 and represented 32.60 % of the total variance (Table 46, Figure 20). With regard to the second principal component (PC2), it was positively correlated with P5_294 and negatively correlated with IS, and it represented 11.57% of the total variance (Table 46, Figure 20). Concerning the third component (PC3), it was positively correlated with P3_190 and negatively correlated with P5_304, and it represented 9.68% of the total variance (Table 46, Figure 20).

Based on the correlation and the PCA observed and to better discriminate the six inflorescence morphotypes studied, we chose among the 20 alleles detected 5 of them correlated with at least 4 of the 6 inflorescence descriptors utilized. The most correlated alleles were P1_155, P2_165, P3_184, P4_288 and P5_308 (Table 46). Each allelic variants of the markers P1, P2 and P4 of 155, 165 and 288bp, was correlated to all the examined traits allowing the genotypes distribution in different clusters, each of them represented by a different morphotype (Figure 20). The broccoli landrace BR9 and the cauliflower F1 hybrid CVF1.2 were isolated from the morphotype cluster for their distinctive such as the slender and the compact, huge inflorescence for BR9 and CVF1.2, respectively.



a b

Figure 22. PCA plots (a and b) performed for all the correlated alleles and traits detected (a) and for the five much more correlated (b), respectively.

The PCA analysis performed on the highest correlated alleles with the inflorescence descriptors showed the PC1 positively correlated that ID1, IA, IW, P1_155, ID2, P4_288, P2_165 and P2_192, and negatively correlated with P4_291, P3_184 and IH, representing 49.80% of the total variance (Table 47, Figure 22b). Concerning the PC2, it was positively correlated to IS and negatively correlated to ID2, and it represented 15.29% of the total variance (Table 47, Figure 22b).

Table 47. PCs matrix related to the bio-morphometric analysis and the selected allelic variants.

	PC1	**PC2**
IW (g)	**0.910**	-0.058
IH (cm)	-0.554	-0.119
ID2 (cm)	**0.741**	-0.595
ID1 (cm)	**0.952**	-0.060
IS	0.101	**0.888**
IA (°)	**0.941**	-0.100
P1_155	**0.752**	-0.078
P2_165	**0.631**	-0.031
P3_192	0.506	0.258
P4_288	**0.644**	0.156
P5_308	0.524	0.641
Variance (%)	*49.08*	*15.29*

The PCA plot established by the 15 chosen alleles showed the genotypes studied distributed in three main groups (Figure 21a). The first group (A) is represented by the CWRs characterized by high value of IH and low values of IW and IA (Figure 21a). The second group (B) is represented by the broccoli genotypes distinguishable by high IS values and by the intermediate values of IH, IW, ID1, ID2 and IA (Figure 21a). Group C, instead, is represented by cauliflower genotypes followed by the broccoli F1 hybrids showing the highest values for IW, IH, ID2, ID1 and IA and the lowest for IS (Figure 21a). The PCA plot performed utilizing the most correlated allele for each primer, confirmed the three groups observed earlier but distinguished them better (Figure 21a). Group A is represented by all the B. oleracea complex species (n = 9), group B by the broccoli landraces and hybrids F1, and group C by the cauliflower landraces and hybrids F1, validating the efficiency of the five alleles and of the SSRs utilized to distinguish among B. oleracea crops and complex species (n = 9) (Figure 21b). The PCA obtained utilizing the three highest correlated allelic variances is shown in Figure 21. In fact, the allelic variances P1_155, P2_165 and P4_288, which show the highest correlation with the examined bio-morphometric traits allowing the genotypes distribution in different clusters, are each represented by the different inflorescence morphotypes studied (Figure 23).

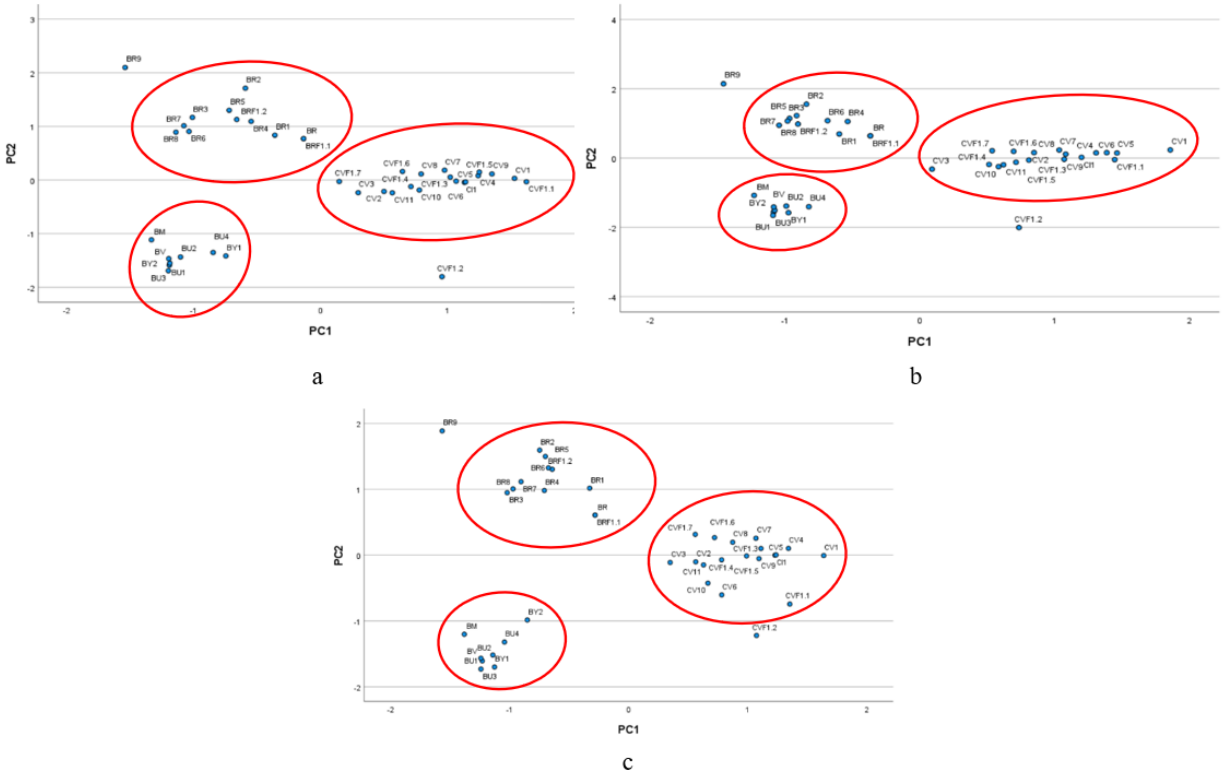


Figure 23. PCA plots for the three best alleles selected among the different molecular markers used, which were: the allelic variant of P1 of 155 bp (a), the one of P2 of 165 bp (b), and the one of P4 of 288bp (c), respectively.

Discussion

B. oleracea species includes many important vegetable crops exhibiting high morphological diversity among them and their cultivars. In our work, the main inflorescence mor- phometric traits (IW, IH, ID1, ID2,

IS and IA) allow us to distinguish among the *B. oleracea* inflorescence morphotypes, in accordance with Branca *et al*. (2018) and Treccarichi *et al*. (2021). The plant materials were selected from the *B. oleracea* core and the Brassica wild relatives species (n = 9) collection of the Di3A of the University of Catania to individuate the morphometric and genetic diversity of the inflorescence just before the anthesis stage. Broccoli landraces showed low values of IW due to how they were traditionally consumed, which was focused on the consumption of the small elongated primary inflorescence having small tender and sweet leaves (Timpanaro *et al*., 2012; Branca *et al*., 2012). As confirmed by the bio-morphometric and molecular analysis performed in the present work and by several additional authors, the Sicilian broccoli and cauliflower landraces are well differentiated from each other and from the F1 hybrids (Gomes *et al*., 2001). In general, broccoli F1 hybrids resemble the cauliflower inflorescence architecture that is clearly differentiated by its huge hypertrophic inflorescence and wide angle of curvature. As reported by several authors, in fact, the allelic distribution of *BoCAL* and *BoAP1* also have contributed to the diversification process of the Calabrese broccoli and of the cauliflower purple type, which is typical of the northeast side of Sicily (Smith and King, 2000; Maggioni *et al*., 2018). *B. oleracea* wild relatives (n = 9), furthermore, have differential traits from the *B. oleracea* crops that can be improved for their resistance to biotic and abiotic stresses and to improve organoleptic and nutraceutical properties for enhancing the bioactive compound amount and profile by assessing and exploiting their genetic diversity (Branca *et al*., 2012).

The *B. oleracea* complex species (n = 9) utilized in our work are diploid species and coexist along the Sicilian and the genetic flux among them and with different *B. oleracea* crops and landraces was ascertained (Branca *et al*., 2012). MADS box genes are differentially conserved in the Brassica genome, and their differential expression on the different *B. oleracea* crops and organs are responsible for the flower induction and for the inflorescence development. The functional characterization of the following genes was performed by Sheng *et al*. (2019), highlighting their different expression patterns and the molecular regulation of the flower development. In our previous work, we already detected for each SSR locus different numbers of alleles among the accessions and the inflorescence morphotypes studied; BoAP1 (P1) showed 12 alleles, BoTHL1 (P2) 8 alleles, BoABI1 (P3) 9 alleles, BoPLD1 (P4) 6 alleles, and PBCGSSRBo39 (SP5) 39 11 alleles, in accordance with Branca *et al*. (2018) and Treccarichi *et al*. (2021). Several of the following alleles, were unconsciously selected and maintained by the growers selected for the size of the hypertrophic inflorescence and probably they were also introgressed by the genetic flux among the *B. oleracea* wild relatives (n = 9) and the first domesticated kales and sprouting broccoli landraces (Maggioni *et al*., 2013). The correlation among the allelic variants and the inflorescence bio-morphometric traits showed that they increase in terms of value when *BoPLD1* (P4) locus tends to heterozygosity. In reality we have observed the P4_288 allele which is homozygous or heterozygous for broccoli and cauliflower whereas for all the B. oleracea complex specie s (n = 9), except for one of the two *B. incana* studied (BY2), it is absent (Figure 23) (Treccarichi *et al*., 2021). In the work of Tonguç and Griffith (2004), the molecular markers P1, P2, P3 and P4 were characterized and identified as candidate markers to assess genetic similarity in broccoli, cabbage and cauliflower, and they showed the polymorphism information content (PIC) value of 0.70, 0.60, 0.58 and 0.45 for P3, P4, P2 and P1,

respectively. For the BoAP1 (P1) the allele P1_155 is generally heterozygous for broccoli and cauliflower, whereas for all the B. oleracea complex species (n = 9), except for one population each of B. incana (BY1) and *B. rupestris* (BU4) studied, it is absent (Figure 23). Regarding BoTHL1 (P2) the allele P2_165 generally expresses a heterozygous condition for broccoli and cauliflower, and it was absent for all *B. oleracea* complex species (n = 9), except for one *B. rupestris* studied (BU4), is absent (Figure 23). For the BoAB1 (P3) the allelic variants P3_184 is always absent for broccoli and cauliflower, whereas for *B. oleracea* complex species (n = 9), it was homozygous for two populations of *B. rupestris* (BU1, BU4) and for two populations of *B. incana* (BY1, BY2) (Figure 21). With regard to P5, it was developed and characterized by Burgess *et al*. (2006) in silico by genome shotgun sequences and showed the highest PIC which was 0.83. In fact, we detected the allele P5_308 which was generally homozygous in cauliflower and broccoli landraces and absent for all the B. oleracea complex species (n = 9), except for one of the four *B. rupestris* (BU4), which in previous studies seems to be an escape population, is absent. The high number of allelic variants individuated in our previous study confirmed that the following molecular markers, can be exploited for the construction of a genetic map with the different annotation related to the polymorphic loci and for the identification of diploid and amphiploid Brassica taxa. The following molecular markers also allowed us to perform a hierarchical clustering dendrogram distinguishing both broccoli and cauliflower landraces and F1 hybrids, and their crosses, respectively, in each different phylogenetic clade (Branca *et al*., 2018). Noteworthy, for all the primers selected, the broccoli landrace BR9 and the cauliflower F1 hybrid CVF1.2 were isolated from the morphotype cluster for their distinctive features, such as the slender and the compact, huge inflorescence for BR9 and CVF1.2, respectively (Figure 21). Herein, we are providing more information about the MADS box domain allelic distribution and diversity focusing on the ones strictly related to the inflorescence traits. The data discussed will be utilized shortly for validating them by the GBS dataset in progress in the frame of the genotyping activities of the EU H2020 BRESOV project. On the other hand, the alleles individuated can already be a solid base for using them for selecting progenies by MAS for hypertrophic inflorescence and size for organic breeding of broccoli and cauliflower and for establishing new organic heterogenous materials requested by the EU Directive 848/2018.

EXPERIMENTAL TRIAL B

We investigated the presence of molecular markers associated with the tolerance or resistance to two important tomato viruses, Tomato spotted wilt virus (TSWV) and Tomato mosaic virus (ToMV), in a set of tomato accessions. Our results indicate that none of the examined accessions carried the *Sw-5* gene associated with TSWV resistance (Figure 24). However, we found that accession 6 (PO166) was heterozygous for the *Tm2* gene, which is associated with ToMV resistance and therefore, showed tolerance to the virus (Figure 25). These findings suggest that while the examined accessions may lack the *Sw-5* gene, *Tm2* could be a potential target for breeding programs aimed at increasing tomato virus resistance.

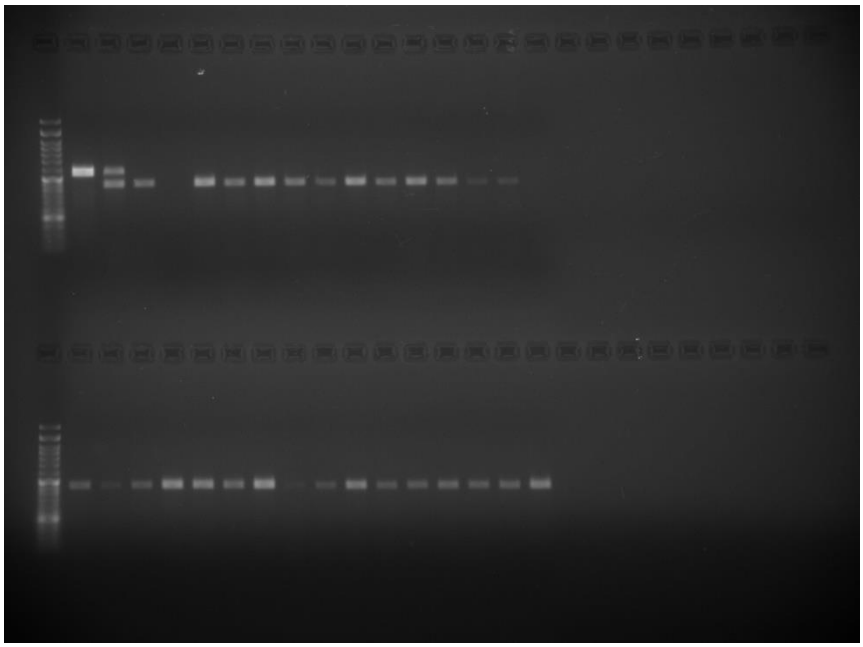


Figure 24. Evaluation of the genotypes resistant to the TSWV. All the accessions showed no resistance. As we can observe, after the 100 bp ladder we can see the dominant homozygous, the heterozygous, the recessive homozygous and the negative control.

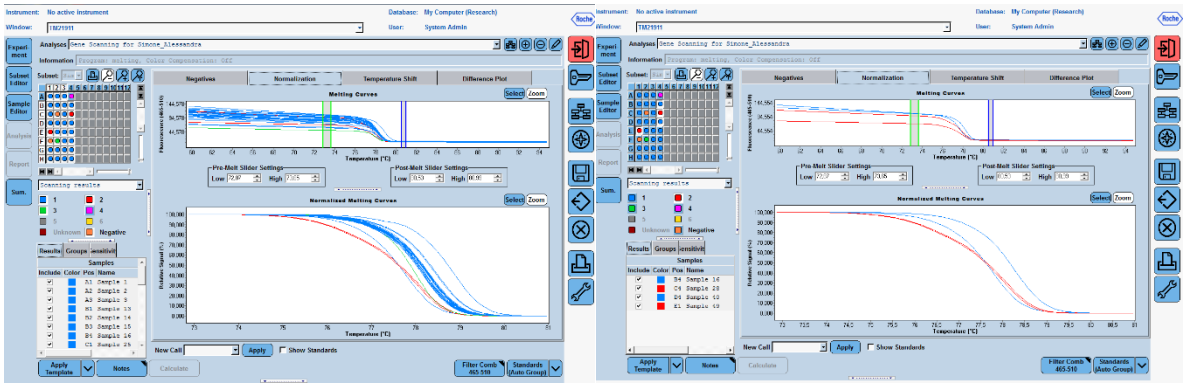


Figure 25. Variation of the melting temperature for all the samples (a), and for the sample 6 (b), for which, basing on the different temperature registered, it showed the heterozygosity of the genes *Tm2*.

Concerning the resistance against pathogen *Fusarium oxysporum* f sp *lycopersici*, all the genotypes showed no presence of the resistant genetic pattern due their homozygosity at *I2* locus. Conversely, genotyping performed with *Ve* gene against *Verticillium* diseases, displayed the presence of in the genotypes 7, 11, 13, 23 and 27. The previously mentioned genotypes were homozygous resistant at the *Ve locus*, except for the genotype 23 which was heterozygous resistant. Additionally, all the genotypes displayed susceptibility to the TYLC because they were homozygous susceptible at *Ty5* locus. Finally, only the genotype 5, was heterozygous resistant for at the *Mi* locus.

2.5.4. Conclusion

EXPERIMENTAL TRIAL A

Genotyping techniques based on molecular markers can be useful for improving knowledge about putative genes controlled by quantitative loci regulating several complex traits such as the inflorescence size. Based on the achieved results, the allelic variants P1_155, P2_165 and P4_288 of the markers BoAP1, BoTHL1 and BoPLD1, respectively, were the most associated with the increase of inflorescence size, and they also facilitate genotype distribution into several clusters by Principal Component Analysis (PCA), represented by each different inflorescence morphotype studied. These three selected alleles could be utilized as molecular markers for organic breeding programs by molecular assisted selection (MAS), and they could be helpful to individuate progenies with hypertrophic inflorescence after crossing broccoli lines and cauliflower with B. oleracea wild relatives (n = 9) for transferring useful forgotten alleles, during the domestication process, for increasing biotic and abiotic stress resistance and for organoleptic, nutritional and nutraceutical traits. Of course, the matrix utilized will soon be compared with the new GBS dataset that will permit us to finely validate our present work highlighting the several mutations responsible of the hypertrophic inflorescence of *B. oleracea*. The molecular markers individuated which could be used for the fast selection of a new resilient, efficient and sustainable cultivar exploiting the wild ancestor of *Brassica oleracea* crops.

EXPERIMENTAL TRIAL B

Molecular markers play a pivotal role in revolutionizing our ability to detect virus-resistance genes, enhancing crop breeding efficiency, and ultimately contributing to global food security. By pinpointing these crucial genetic traits with precision, molecular markers empower researchers and breeders to develop resilient crops more swiftly, mitigating the devastating impact of plant viruses on agriculture worldwide. Their importance in safeguarding our food supply cannot be overstated. In our conducted experiment, the presence of the *Tm2*^{2} resistant gene was solely identified in one specific accession (PO166), likely a result of deliberate trait selection. Conversely, other genetic materials, having undergone selection within Italian local gardens, exhibited an absence of resistance genes within their DNA profiles. As concern the resistance against Verticillium diseases, we found the resistance genes in the genotypes 7 (PO211), 11 (PO5), 13 (PS05), 23 (PS35), and 27 (PS51). The transfer of the disease resistant alleles *Tm2*^{2} and *Ve* in the other accessions, could represent a pivotal basis for the development of tomato genetic lines suitable for organic farming.

3. Conclusion and perspectives

The primary objective of the three distinct research lines undertaken in this PhD thesis was to identify genotypes suited for organic farming. In the first research line, a significant discovery was made as the various species within the *B. oleracea* L. complex (n=9) and *S. lycopersicum* cultivars were effectively grouped into distinct clusters, each representing unique morphotypes. These distinctions were based on both phenotypic and genetic traits, providing a robust foundation for future breeding efforts.

Moving to the second research line, a key outcome was the identification of valuable phenotypic and genetic resources capable of withstanding diverse stresses. Within this context, the utilization of Brassica CRWs cultivars emerged as a promising avenue to enhance commercial varieties through traditional breeding methods. Additionally, for tomatoes, the adoption of improved rootstocks developed via biotechnological approaches presents a pivotal perspective for advancing organic agriculture.

Finally, in the third research line, noteworthy achievements included the development and exploration of molecular markers that facilitate the selection of hypertrophic inflorescence traits in B. oleracea. Furthermore, a tomato accession with genetic SNPs associated with ToMV resistance was identified.

These collective findings, accomplished over the course of the past three years, serve as a robust foundation for future breeding endeavours and hold significant promise for the advancement of organic farming practices.

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Using Simple Sequence Repeats in 9 *Brassica* Complex Species to Assess Hypertrophic Curd Induction

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Abstract: Five Simple Sequence Repeats (SSRs) were used to assess the relationship between inflorescence characteristics and their allelic variation in 53 *Brassica oleracea* and *Brassica* wild relatives (n = 9). Curd morphometric traits, such as weight (CW), height (CH), diameter (CD1), shape (CS) inflorescence curvature angle (CA), and its curd stem diameter (CD2), were measured. The aim of the work was to analyze the relationships among the allelic patterns of the SSRs primers utilized, and their status of homo or heterozygosity registered at each locus, as well as the inflorescence morphometric traits in order to individuate genomic regions stimulating the hypertrophy of this reproductive organ. The relationships found explain the diversity among *B. oleracea* complex species (n = 9) for the inflorescence size and structure, allowing important time reduction during the breeding process by crossing wild species, transferring useful resistance, and organoleptic and nutraceutical traits. The five SSRs loci were BoABI1, BoAP1, BoPLD1, BoTHL1, and PBCGSSRBo39. According to the allelic variation ascertained, we evaluated the heterozygosity index (H) for each SSR above cited. The results showed a significant interaction between the H index of the BoPLD1 gene and the inflorescence characteristics, summarized by the First Principal Component (PC1) (p-value = 0.0244); we ascertained a negative correlation between the H index and inflorescence characteristics, namely CW, CH, CD1, CD2, CA. The homozygosity BoPLD1 alelles, indicated by the H index, affect the inflorescence characteristics and broccoli and cauliflower yields.

Keywords: *Brassica* complex species; MADH–box genes; SSRs assay; heterozygosity index; allelic variance; curd morphometric traits

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1. Introduction

Brassica crops include several interesting species which are strictly related to crop wild relatives (CWRs) during their domestication process [1]. The Mediterranean region represents one of the main domestication and diversification centers of *Brassica* genus, in particular in Sicily where the cytodeme is represented by several wild relatives such as *Brassica macrocarpa* Guss., *B. villosa* Biv., B. *rupestris*, and B. *incana* [2].

The brassica genus includes three diploids (2n) (AA, BB, CC) and three tetraploids (4n) (AABB, AACC, BBCC) main species as described in the U's triangle model [3]. The *B. oleracea* complex species (n = 9) belongs to genome C (n = 9) and it represents the primary gene pool of the *Brassica* genus. This genus shows high genetic variability due to the genetic self-incompatibility characterizing the landraces and their CWRs and to several domestication processes [4]. Genetic diversity of *B. oleracea* is shown by the several varieties obtained by different domestication processes in a number of geographic areas which include broccoli, cauliflower, cabbage, kale, kohlrabi, savoy cabbage, and Brussel sprouts.

Brassica wild relatives could be a source of cytoplasmic male sterility (androsterility) for the development of hybrid seed of *Brassica* crops and they can provide genes for

resistance to different diseases and pests and for these traits they can be used in breeding programs [5].

Cauliflower and broccoli are characterized by the floral induction of hypertrophic inflorescence [6]. Broccoli crop, as reported by Viani, originated from wild cabbage while cauliflower head derived from the improving process of broccoli addressed to the reduction of branches length, flower bud size, and the absence of their pigmentation.

Flower development genes were studied by Bowman et al. who found several genes involved such as *apetala 1* and *cauliflower* in *Arabidopsis* [7]. These genes are closely related to members of the MADH-box genes family and a mutant copy of them is present in *B. oleracea* genome. Irish and Sussex characterized several floral phenotypes produced by the recessive homeotic *apetala 1* (*ap1*) mutation in *Arabidopsis* and the homozygote for this mutation showed weak inflorescence affecting floral primordia formation [8].

Smith and King proposed a simple genetic model based on segregation of recessive alleles for BoAP1 and BoCAL candidate genes which showed differences in stage of arrest between cauliflower and Calabrese broccoli [9]. According to Smith and King's allelic distribution genetic model, the domestication process reduced the allelic diversity by promoting loci affecting the arrest of floral development which determined the inflorescence hypertrophy and then the domestication for cauliflower's curd phenotype; the Sicilian Purple was indicated as an important intermediate of this domestication process [10].

BoABI1, BoAP1, BoPLD1, and BoTHL1were designed to amplify genomic DNA region by Tonguc and Griffiths [11]. They were used to assess genetic similarity between several *B. oleracea* cultivars, belonging to three varietal groups (cabbage, cauliflower, and broccoli) while PBCGSSRBo39 was designed by Burgess et al. [12] to provide a useful molecular marker for crop improvement which was derived from shotgun sequencing programs.

Simple sequence repeats markers can be a useful tool to find genetic relationships among genotypes and related species provided from different countries. They can be used also as chloroplastic SSRs (cpSSRs) to avoid multiple gene copy number problems in polyploidy species [13].

In this study, the inflorescence morphometric traits of several accessions of broccoli and cauliflower landraces and commercial varieties, and *Brassica* relatives were measured [14], and additionally the five SSRs above cited were utilized to analyze the allelic variation among the accessions used and to associate them with inflorescence characteristics.

2. Materials and Methods

Plant material was represented by fifty-three accessions belonging to the Department of Agriculture, Food and Environment (Di3A) of the University of Catania-UNICT (Table 1). Seeds were sown in the first week of July in cellular trays placed under greenhouse conditions. The seedlings were transplanted after 5 weeks on the experimental farm of the University of Catania, (37°27′ N, 15°40′ E, 10 m a.s.l.) in single rows, with 1.0 m between the rows and 0.5 m between the plants along the rows, at crop density of 2 plants/m^2. The experimental design was composed of four replicates (10 plants each) placed in randomized blocks as described by Branca et al. [15]; plants were grown in open fields.

For the accessions, inflorescence morphological data were registered following the International Board for Plant Genetic Resources (IBPGR) [16] descriptors related to the curd. Inflorescence morpho-biometric traits such as weight (CW), height (CH), diameter (CD1), shape (CS), angle of curvature (CA), and inflorescence stem thickness (CD2) were measured and calculated at the laboratory of Biotechnology of Vegetable and Flower Crops of the Di3A UNICT department. The inflorescence before anthesis was cut five centimeters before the first branch of inflorescence and for it the CW was registered by analytical balance, the CH and CD1 were calculated using a meter rule while CD2 was calculated using a caliber. The inflorescence shape (CS) parameter can be used to distinguish broccoli and cauliflowers from the CWRs and is derived from the ratio between CH and CD1. Curvature angle CA was registered with a goniometer by calculating the angle limited to between the central vertical inflorescence axes and the tangent to the extreme part of it.

Table 1. List of *B. oleracea* complex species (*n* = 9) accessions utilized.

Accession Code	Laboratory Code	Origin	Species
UNICT 3876	CV 171 Menhir F1	ISI sementi	CV
UNICT 3190	BR 15 S 1 A	Modica (RG)	CV
UNICT 4137	CV 99 S2 B	Adrano (CT)	CV
UNICT 4145	BR 13 S3 AC	Modica (RG)	CV
UNICT 3878	CV 173 Freedom	3878 Royal Sluis	CV
UNICT 4138	CV 76 S2	Acireale (CT)	CV
UNICT 3652	CV 159	Catania	CV
UNICT 3900	BR 13 A X CV98/21	DISPA 4	CV
UNICT 3902	CV 33 S1	Royal Sluis	CV
UNICT 3895	CV 98/2 X CV 136 EG	DISPA 2	CV
UNICT 3880	CV 175 White Flash	Sakata	CV
UNICT 3879	CV 174 Graffiti	ISI sementi	CV
UNICT 3089	CV 75 S3AC	Acireale (CT)	CV
UNICT 3906	CV 24 S4	Biancavilla (CT)	CV
UNICT 3892	CV 98/2 X BR 13 S3	DISPA 3	CV
UNICT 579	BR 41	Modica (RG)	CV
UNICT 3578	BR 165 Marathon	Esasem	BR
UNICT 3893	CV 136 EG X CV98/2	DISPA 1	CV
UNICT 3671	CV 72 S2	Catania (CT)	CV
UNICT 583	BR 46	Vittoria (RG)	BR
UNICT 658	BR 45 S1	Acireale (CT)	BR
UNICT 3669	BR 17 S2	Ragusa (RG)	CV
UNICT 658	BR 129	Roccella Valdemone (ME)	BR
UNICT 657	BR 128	Roccella Valdemone (ME)	BR
UNICT 651	BR 122 Packman	Petoseed	BR
UNICT 655	BR 126	Adrano (CT)	BR
UNICT 3674	CV 19 S2 A	Piazza Armerina (EN)	CV
UNICT 637	BR 106	Cefalù (PA)	BR
UNICT 3675	BR 94 S1	Francavilla (ME)	BR
UNICT 3668	BR 115 S1	Troina (EN)	BR
UNICT 574	BR 36	Biancavilla (CT)	BR
UNICT 342	*Brassica macrocarpa 1*	Favignana (TP)	BM
UNICT 733	*Brassica rupestris 1*	San Vito Lo Capo (TP)	BU
UNICT 342	*Brassica macrocarpa 2*	Favignana (TP)	BM
UNICT 342	*Brassica macrocarpa 3*	Favignana (TP)	BM
UNICT 3512	*Brassica incana 1*	Agnone Bagni (SR)	BY
UNICT 3270	*Brassica rupestris 2*	Bivongi (RC)	BU
UNICT 3270	*Brassica rupestris 3*	Bivongi (RC)	BU
UNICT 342	*Brassica macrocarpa 4*	Favignana (TP)	BM
UNICT 3512	*Brassica incana 2*	Agnone Bagni (SR)	BY
UNICT 342	*Brassica macrocarpa 5*	Favignana (TP)	BM
UNICT 732	*Brassica rupestris 4*	Roccella Valdemone (ME)	BU
UNICT 732	*Brassica rupestris 2*	Roccella Valdemone (ME)	BU
UNICT 342	*Brassica macrocarpa 6*	Favignana (TP)	BM
UNICT 736	*Brassica rupestris 5*	Ragusa Ibla (RG)	BU
UNICT 342	*Brassica macrocarpa 7*	Favignana (TP)	BM
UNICT 4158	*Brassica incana 3*	Sortino (SR)	BY
UNICT 736	*Brassica rupestris 6*	Ragusa Ibla (RG)	BU
UNICT 3040	*Brassica villosa 1*	Marianopoli (CL)	BV
UNICT 736	*Brassica rupestris 7*	Ragusa Ibla (RG)	BU
UNICT 342	*Brassica macrocarpa 8*	Favignana (TP)	BM
UNICT 4158	*Brassica incana 4*	Sortino (SR)	BY
UNICT 3040	*Brassica villosa 2*	Marianopoli (CL)	BV

Legend: CV—Cauliflower; BR—Broccoli; BY—*B. incana*; BM—*B. macrocarpa*; BU—*B. rupestris*; BV—*B. villosa.*

For morphological data, the mean values of the analyzed parameters of every accession were used to prepare a numerical matrix.

Genomic DNA was extracted from seedlings upon reaching the 6–8 leaved stage in young leaves tissues as reported by Tonguç and Griffiths utilizing the kit GenEluteTM Plant Genomic DNA Miniprep (Sigma Aldrich Inc.).

Extracted DNA was measured using a spectrophotometer Shimadzu at wavelengths of 260 and 280 nm, quantified by visual comparison on ethidium bromide-stained agarose gels. The final DNA concentration followed the protocol established by Branca et al. (2018) which includes 200 ng of template DNA.

The primers flanking SSR sequences (Table 2) were obtained in accordance with Tonguc and Griffiths (2004) for BoTHL1, BoAP1, BoPLD1, and BoABI1; concerning the PBCGSSRBO39 primers sequence, this was retrieved by Burgess et al. The position of the primers was checked using Assembly: GCA_0006955251.1 within Ensembl.

Table 2. List of primers utilized with their sequences and chromosome position.

GenBank	Primers Name	SSR Motif	Primer Sequence (Forward, Reverse)	Chromosome
AF113918	BoPLD1	$(CT)_7(AT)_{7-1}$	GACCACCGACTCCGATCTC AGACAAGCAAAATGCAAGGAA	C5
AF180355	BoABI1	$(TC)_{16}$	TATCAGGGTTTCCTGGGTTG GTGAACAAGAAGAAAAGAGAGCC	C1
AF273844	BoTHL1	$(CTT)_7$	GCCAAGGAGGAAATCGAAG AAGTGTCAATAAGGCAACAAGG	C9
U67451	BoAP1	$(AT)_{9-1}$	GGAGGAACGACCTTGATT GCCAAAATATACTATGCGTCT	C6
BH479680	PBCGSSRBo39	$[GGTCG]_4$	AACGCATCCATCCTCACTTC TAAACCAGCTCGTTCGGTTC	C7

Five SSRs primers used were chosen following Branca et al., selecting them from ten primers, performed by Branca et al. for phylogenetic analysis and to assess the genetic similarity between several *B. oleracea* cultivars and wild Brassica species, belonging to two varietal groups (cauliflower and broccoli) as well as to estimate genetic divergence using F_{ST} statistic; broccoli cultivars clustered with cauliflower cultivars as predicted and wild species showed major genetic differences [13].

The basic local alignment search tool (BLAST) was performed to check amplicon size and to compare results with amplificated sequences registered in an online database which was represented by BLAST (version 1.17) and Ensembl. The Uniprot database (release 2021, version 3) was used to study encoding regions close to the gene of interest.

The SSRs studied are located in different regions of the plant genome: BoABI1 is located in chromosome 1 region: 1,229,915,511-12,992,170 within the gene Bo1g041870 coding the ABI1 protein. The second SSR BoTHL1 is located on chromosome: 17,254,558: 17,255,176 within the Bo9g058820 gene, a homolog of thioredoxin 3 in Arabidopsis thaliana. The microsatellite PBCGSSRBo39 is located inside the Bo7g105720 gene on chromosome 7, BoAP1 is located inside chromosome 6: 33,883,667-33,887,357 inside the Bo6g108600 gene, one of MADS-box gene family members (Ap1Like).

BoPLD1 marker is located in the fifth chromosome in *B. oleracea* from 46,037,340 bp to 46,037,606 bp.

After DNA purification, PCR-based amplification was performed in 20 µL of final volume. The reaction mixture was composed of 200 ng of DNA template, 200 µM of each dNTP 3.75 mM MgCl2, 1X Taq DNA polymerase buffer, and 2 mM Primer according to Branca et al. (2018). DNA amplification was conducted in a Perkin Elmer 9700 thermocycler (ABI, Foster City, CA, USA) with the following parameters: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 50 °C for 1 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 7 min. At the end of reaction, amplicons were stored at 4 °C. PCR products were loaded into 4% agarose gels (UNILAB Life Science, Taipei, Taiwan) and the electrophoresis run at a

voltage of 100 V for 5–6 h in 1 X TBE buffer [15]. Capillary electrophoresis was performed using ABI PRISM 3130 Genetic 191 Analyser (Applied Biosystems, Waltham, MA, USA) as described by Branca et al. (2013) and Branca et al. Fragment sizes were determined by the GeneMapper 3.7 software (Applied Biosystems, Waltham, MA, USA). The allele peaks were checked by performing capillary electrophoresis and also checked using GeneMapper software. Each allele peak was manually rechecked by the operator.

3. Data Analysis

Allelic detection occurred in coding allelic status on the basis of their molecular weights using numeric scores: 2 (homozygosity), 1 (heterozygosity), and 0 (absence of any allele).

Allelic frequency data were elaborated to calculate heterozygosity index (H) which indicates the frequencies of heterozygosity in a population; an H value close to 1 suggests a large degree of heterozygosity within the populations while an H value close to 0 suggests homozygosity. Statistical analysis was performed to evaluate the correlation between the heterozygosity index for each locus and the inflorescence morpho-biometric traits as CW, CD1, CD2, and PC1.

For statistical analysis, the main inflorescence morpho-biometric characteristics, with exception of CS were used to calculate the Principal Component Analysis (PCA) to obtain a single parameter to summarize the inflorescence characteristics. CS was discarded due to the origin of this parameter which is derived from CH and CD1. PCA was performed using RStudio software 3.6.3 and a linear regression model was used to obtain information about the relationships between the heterozygosity index of each locus and the inflorescence bio-morphometric traits. PCA data were scaled to have unit values.

4. Results

Inflorescence morphometric traits CW, CH, CD1, CD2, and angle of curvature CA were registered for the Di3A accessions establishing a morphological database (Table 3).

The inflorescence morphometric variance was detected using data on plant bio-morphometric parameters recorded on Sicilian broccoli and cauliflower landraces and their F1 hybrids which show a large diversity among the genotypes.

The curd weight showed higher values for cauliflower landraces and F1 hybrids, than the CWRs analyzed, which registered lower values. Among cauliflower accessions, CV 171 Menhir F1 recorded the highest value (10,958 g); landrace curds weighed less than those collected from F1 hybrids and that explain the worldwide diffusion of these genotypes due to their yield. Curd diameter (CD1) was related to CW and, as registered, showed lower values in CWRs accessions while cultivated accessions with higher CD1 values, such as CV 99 S2 B recorded the largest curd diameter, respectively 21.1 cm (Table 3).

Broccoli types showed an elongated inflorescence as shown by CS values while cauliflowers showed more compact and flattened curd such as compared to the CWRs. Broccoli landraces showed the highest CS values compared to commercial cultivars.

The curvature angle (CA) also showed the large phenotypical variability among broccoli and cauliflowers landraces and hybrids F1, and their CWRs. CA distinguish well cauliflowers from broccoli and CWRs; cauliflowers were characterized by the highest CA value. Broccoli accessions showed lower CA values than cauliflowers. CWRs were characterized by the absence of the hypertrophic inflorescence developed from the apical meristem and they showed the lowest CA values; *B. macrocarpa* accession Favignana 1 showed the lowest CA value (7°).

Phenotypical variability was explained using a correlation model for each bio-morpho-metric descriptor; PC1 showed 46.75% of the total variance among the accessions (Table 4) and it is significantly correlated to CW, CD1, CD2 and CA. PC2 overlaps with CH which is one of the major traits affecting inflorescence morphology and therefore it was not used for this analysis.

Table 3. Inflorescence morphometric characteristic in descending order, from the heaviest to the lightest. The parameters measured were curd weight (CW), height (H), curvature angle (CA), curd and stem diameters (CD1 and CD2) and principal component 1 (PC1).

Laboratory Code	CW (g)	CH (cm)	CD2 (cm)	CD1 (cm)	CS (cm)	CA (°)	PC1
CV 171 Menhir F1	1095.8 (21.1)	11.1 (8.4)	42.32 (8.5)	18 (8.7)	0.62 (9.6)	110 (21.9)	3.794
BR 15 S 1 A	965.7 (37.4)	15.4 (14.6)	39.82 (16.4)	20.7 (17.4)	0.74 (16.6)	105 (19.4)	3.588
CV 99 S2 B	666.6 (42.5)	15.2 (13.2)	34.09 (19.6)	21.1 (15.0)	0.72 (12.1)	112 (20.4)	2.925
BR 13 S3 AC	628.8 (33.7)	16.8 (16.6)	38.13 (18.5)	19.7 (14.6)	0.85 (14.6)	101 (22.5)	2.742
CV 173 Freedom	605 (33.8)	89 (16.7)	30.99 (10.3)	16.9 (11.8)	0.53 (12.1)	113 (13.3)	2.171
CV 76 S2	567.3 (38.2)	14.5 (15.6)	36.96 (19.8)	19.5 (13.1)	0.74 (17.2)	113 (13.5)	2.722
CV 159	564.9 (37.0)	14.5 (20.7)	34.55 (12.6)	20 (15.1)	0.72 (18.7)	104 (16.7)	2.561
BR 13 A X CV98/21	554.5 (56.7)	18.8 (20.4)	30.84 (26.9)	19.5 (19.3)	0.96 (29.8)	107 (17.7)	2.397
CV 33 S1	541.5 (54.7)	13.7 (24.4)	32.25 (21.9)	18.9 (29.6)	0.72 (18.3)	112 (22.3)	2.452
CV 98/2 X CV 136 EG	503.9 (35.4)	16.8 (28.4)	32.36 (18.1)	16.5 (17.9)	1.02 (34.4)	100 (27.4)	2.039
CV 175 White Flash	467.09 (41.1)	7.46 (20.9)	29.97 (13.3)	14.6 (15.7)	0.51 (11.1)	101 (15.6)	1.777
CV 174 Graffiti	461.8 (47.1)	10.8 (16.1)	32.98 (13.9)	17.5 (16.4)	0.62 (17.4)	110 (19.3)	2.198
CV 75 S3AC	453.5 (49.7)	11 (17.2)	35.54 (17.9)	18.1 (27.3)	0.61(22.5)	117 (15.8)	2.404
CV 24 S4	443 (55.9)	12.7 (23.0)	36.41 (24.2)	16.7 (23.8)	0.76 (32.4)	91 (26.5)	1.977
CV 98/2 X BR 13 S3	438.8 (84.4)	17.6 (24.4)	28.81 (28.1)	16.8 (29.4)	1.05 (34.2)	93 (17.7)	1.723
BR 41	378.3 (46.2)	10.2 (21.0)	36.8 (16.9)	17.2 (19.5)	0.59 (17.8)	113 (17.5)	2.184
BR 165 Marathon	319.8 (40.9)	14.1 (26.8)	3.52 (19.5)	12.28 (26.7)	1.2 (44.4)	76 (29.8)	0.061
CV 136 EG X CV98/2	317.4 (42.0)	17.2 (22.2)	29.22 (28.1)	14.8 (16.0)	1.17 (33.1)	98 (21.6)	1.410
CV 72 S2	305.7 (68.2)	8.7 (20.7)	31.7 (18.1)	15.4 (22.8)	0.56 (19.7)	92 (25.2)	1.470
BR 46	279 (39.0)	16.6 (18.1)	3.84 (17.3)	11.1 (23.7)	1.5 (28.2)	57 (21.5)	−0.342
BR 45 S1	266.9 (33.4)	22.2 (30.9)	3.18 (13.2)	8.47 (32.7)	2.7 (37.4)	58 (19.4)	−0.595
BR 17 S2	263.6 (56.1)	11.2 (28.3)	34.23 (18.8)	14.4 (22.0)	0.78 (21.2)	91 (23.4)	1.379
BR 129	226.4 (39.6)	18.2 (12.9)	3.13 (26.8)	7.89 (29.4)	2.3 (30.5)	49 (27.8)	−0.821
BR 128	217.7 (58.3)	18.2 (18.2)	2.93 (29.8)	9.49 (31.6)	1.9 (29.4)	54 (26.3)	−0.659
BR 122 Packman	212.8 (36.3)	12.8 (12.2)	3.14 (15.0)	7.78 (23.1)	1.9 (16.5)	46 (24.1)	−0.877
BR 126	188.3 (51.8)	16.6 (23.4)	2.87 (24.3)	7.7 (28.3)	2.2 (24.2)	46 (24.1)	−0.951
CV 19 S2 A	186.6 (41.3)	8.4 (17.5)	28.6 (16.7)	13.6 (15.1)	0.61 (18.2)	85 (24.8)	0.905
BR 106	164 (49.0)	16.5 (17.9)	3.34 (32.4)	8.25 (29.5)	2 (52.3)	46 (32.8)	−0.940
BR 94 S1	143.9 (42.2)	16 (29.0)	2.69 (22.7)	7.82 (29.0)	2.1 (22.6)	48 (26.7)	−1.008
BR 115 S1	109.5 (30.8)	15.5 (9.5)	2.64 (20.2)	7.88 (25.8)	2 (23.4)	41 (34.2)	−1.158
BR 36	63.1 (41.7)	16.9 (23.5)	2.76 (18.9)	4.74 (22.3)	3.6 (15.5)	27 (15.2)	−1.664
Brassica macrocarpa 5	36.7 (21.1)	8.2 (12.1)	14.5 (16.3)	3.4 (23.1)	0.23 (27.9)	12 (10.2)	−1.572
Brassica rupestris	33.3 (28.3)	27.6 (15.5)	16.2 (20.2)	3.1 (17.9)	0.19 (21.2)	14 (11.7)	−1.574
Brassica macrocarpa 3	31.2 (19.8)	18.6 (21.2)	10.8 (23.6)	2.4 (16.2)	0.22 (19.8)	15 (12.6)	−1.781
Brassica macrocarpa 1	30.9 (23.2)	15.4 (18.4)	7.3 (20.7)	3.1 (19.2)	0.42 (38.4)	9 (7.9)	−1.915
Brassica incana 1	30.3 (21.9)	21.1 (19.2)	25.7 (26.3)	3.8 (21.7)	0.15 (26.5)	12 (11.7)	−1.201
Brassica rupestris 3	29.8 (19.8)	20.5 (12.2)	16.9 (20.5)	4.2 (22.2)	0.25 (21.6)	13 (7.3)	−1.463
Brassica rupestris 2	27.5 (17.5)	18.4 (9.1)	21.6 (23.4)	3.9 (25.4)	0.18 (17.8)	17 (10.2)	−1.271
Brassica macrocarpa 8	27.2 (18.4)	13.2 (21.2)	8.5 (19.5)	2.9 (19.1)	0.34 (18.9)	14 (11.9)	−1.827
Brassica incana 3	25.1 (21.8)	19.6 (24.6)	19.3 (31.3)	2.7 (17.1)	0.14 (27.1)	15 (9.8)	−1.477
Brassica macrocarpa 7	24 (21.2)	21.5 (27.2)	11.4 (21.2)	2.5 (19.5)	0.22 (21.0)	10 (8.1)	−1.837
Brassica rupestris 6	22.7 (20.1)	26.3 (20.4)	20.7 (28.2)	2.1 (25.3)	0.1 (19.2)	9 (7.2)	−1.573
Brassica rupestris 7	22.1 (18.9)	20.6 (26.1)	18.5 (18.4)	2.5 (19.2)	0.14 (18.8)	10 (8.3)	−1.591
Brassica macrocarpa 2	21.7 (18.4)	15.8 (21.2)	8.2 (19.2)	3 (18.8)	0.37 (18.9)	12 (7.7)	−1.873
Brassica rupestris 4	21.6 (16.2)	19.8 (9.1)	7.3 (16.3)	2.1 (16.9)	0.29 (15.9)	11 (8.2)	−1.998
Brassica macrocarpa 6	21.6 (20.3)	17.6 (13.6)	21.4 (19.7)	2.1 (17.4)	0.1 (16.5)	15 (9.0)	−1.451
Brassica incana 2	21.5 (15.2)	20.3 (21.1)	19.6 (19.1)	3.1 (21.1)	0.16 (19.8)	12 (9.2)	−1.482
Brassica rupestris 5	20.5 (19.0)	20.8 (16.9)	16.3 (20.1)	2.2 (22.2)	0.13 (20.5)	12 (6.3)	−1.670
Brassica villosa 1	20.1 (18.2)	15.1 (12.1)	19.8 (19.2)	2.6 (18.4)	0.13 (19.3)	11 (9.2)	−1.514
Brassica rupestris 1	19.8 (16.1)	21.6 (19.5)	8.9 (16.2)	2.1 (14.2)	0.24 (16.0)	7 (6.1)	−2.001
Brassica macrocarpa 4	19.8 (17.2)	23.2 (20.3)	19.6 (23.3)	2.6 (21.8)	0.13 (23.2)	11 (8.9)	−1.545
Brassica incana 4	19.7 (9.1)	21.2 (23.2)	17.3 (21.2)	2.5 (18.8)	0.14 (20.2)	11 (8.0)	−1.627
Brassica villosa 2	19.2 (18.4)	14.5 (9.1)	18.1 (15.2)	2.2 (19.2)	0.12 (19.1)	10 (6.8)	−1.616

Legend: number in brackets indicates standard deviation.

Table 4. Correlation coefficients of single descriptors with the three main principal components (PCs).

	PC1	PC2	PC3
CW	0.508	0.06	0.229
CH	−0.032	0.998	0.010
CD1	0.438	0.022	−0.898
CD2	0.527	−0.022	0.251
CA	0.521	0.004	0.279
% variance	46.75	25.21	16.34

Morphometric traits were subsequently elaborated and correlated to the genetic data by statistical analysis.

Each SSR locus exhibited a different number of alleles among the accessions studied: BoTHL1 showed eight alleles, PBCGSSRBo39 eleven alleles, BoPLD1 six alleles, BoAP1 showed twelve alleles, and BoABI1 nine alleles. Allelic data were processed to measure genetic diversity for each locus within the different *Brassica* accessions examined and to calculate the H index (Table 5).

Table 5. Multiple regression on several loci heterozygosity indices of four plant growth parameters: CW—Curd weight, CD1—Curd inflorescence diameter; CD2—Curd Stem thickness and their First Principal Component (PC1).

	Estimate	Std. Error	*p*-Value
CW on H indices			
BoTHL1	95.48	128.87	0.4643
PBCGSSRBo39	170.24	130.62	0.2021
BoPLD1	−248.18	137.86	0.0888
BoAP1	142.67	99.97	0.1635
BoABI1	−178.45	117.18	0.1379
CD1 on H indices			
BoTHL1	4.5004	3.0624	0.1518
PBCGSSRBo39	−0.6356	3.1040	0.8391
BoPLD1	−6.9635	3.2759	0.0416
BoAP1	2.7333	2.3756	0.2587
BoABI1	−3.3929	2.7845	0.2322
CD2 on H indices			
BoTHL1	8.413	7.049	0.2417
PBCGSSRBo39	1.682	7.145	0.8154
BoPLD1	−19.056	7.541	0.0168
BoAP1	1.124	5.468	0.8386
BoABI1	−12.926	6.410	0.0525
PC1 on H indices			
BoTHL1	1.1348	0.8870	0.2102
PBCGSSRBo39	0.3260	0.8990	0.7193
BoPLD1	−2.2453	0.9488	0.0244
BoAP1	0.6661	0.6881	0.3405
BoABI1	−1.2391	0.065	0.1346

The correlation between CW and the five locus H index did not show significant *p*-value, although the BoPLD1 one was weakly significant (smaller than 0.10). On the other hand, significant correlations were observed among BoPLD1 H index and CD1, CD2, and PC1 (Table 5). The negative sign of the estimate coefficient confirms the association between the heterozygosity index and BoPLD1; when the H index increases the size of the inflorescence and the thickness of the stem decrease. The analysis also showed no significant correlation between the H index of the other loci (BoAP1, BoTHL1, BoAB1, PBCGSSRBo39) and inflorescence characteristics.

5. Discussion

The Di3A core collection describes the evolution of the domestication process from the *Brassica* wild species (n = 9) to the broccoli and cauliflower crops by comparing the main morphometric traits of the inflorescence and the allele diversity of the molecular primers utilized during human selection.

The domestication process is explained by the use of five SSR primers which show a wide range of alleles among the growing and wild species belonging to the *B. oleracea* complex species (n = 9). Some alleles, useful for increasing the inflorescence size, were unconsciously subject of selection by the growers in order to fix the hypertrophic inflorescence of broccoli and cauliflower. In addition, the broccoli and cauliflower domestication processes have been affected by the genetic flux among the *Brassica* wild relatives (n = 9), and the first domesticated sprouting broccoli was permitted to enlarge the inflorescence size gradually and define its shape of the hybrids F1 of broccoli and cauliflower [17]. In Branca et al. Fst was calculated in order to measure the genetic distance among accessions; the genetic diversity shown by the five SSRs primers utilized in relation to the *B. oleracea* complex species (n = 9) accessions, permitted us to classify them in relation to their domestication process (CWRs, landraces of cauliflowers and broccoli and their hybrids F1. MADS–box genes family includes several transcriptional factors involved in the growth and development of the inflorescence after its reproductive induction, flowering time, fruit development, and ripening [18]. During the last decades, several genomic studies were reported to explain the role played by some homeotic genes involved in the development of the hypertropic inflorescence, called head for broccoli and curd for cauliflower. Several genes such as *apetala* 1 (AP1) were reported to be involved in the inflorescence structure controlling its meristematic development. The transcript of AP1 gene (RefSeq ID: XP_013590290.1) was described by Sheng et al., 2019 as showing high levels of expression in different tissues and in particular in the curd and the flower [19]. These genes are related to the development of the reproductive organs and they belong to the MADH-box genes family [20]. *BoCal* is one of the related genes involved in curd formation; the mutant alleles seem to stop flower development and a simple genetic model has been proposed [9].

BoPLD1 marker is located in the fifth chromosome of *B. oleracea* from 46,037,340 bp to 46,037,606 bp in an untranscribed region (accession: LR031877), near the region encoding Phospolipase D (UniProtKB-A0A3P6FGA7). This catalytic enzyme is encoded from BOLC5T33808H gene and is involved in glycerolphospholipids hydrolysis at the terminal phosphodiesteric bond.

Taking into consideration the stem diameter (CD2) and the diameter of the inflorescence (CD1), the correlation between their values and the H index is significant only for the BoPLD1 locus while it is weakly significant with respect to the weight of the inflorescence (Table 5). The analysis of the negative correlation coefficient between these two inflorescence morphometric traits (CD1 and CD2) permits us to deduce that the more the H index associated with BoPLD1 increases, and the related locus tends to show higher heterozygosity, the more such a parameter affects the inflorescence size. This could be correlated with the observation that in the CWRs there is greater heterozygosity than in the hybrids F1 but the size of the inflorescence, its stem, and its weight decrease for the former. The sequence hosting the microsatellite placed in the initial portion of the gene *Bo5g126670,* just before the first exon, could not exclude the presence of a repeat affecting the transcription of the gene itself.

The data acquired consent to delineate the next steps of this study sequencing the polymorphisms present in the upstream region of the *Bo5g126670* gene that could be involved in the inflorescence hypertrophy of the *B. oleracea complex species* (n = 9). These variations could be used for marker-assisted selection (MAS) and for individuating in advance, during the breeding program utilized CWRs—the individuals who express hypertrophic inflorescence are an object of interest for further field evaluation.

6. Conclusions

BoPLD1 marker heterozygosity (H index) shows significant interaction with several inflorescence morpho-biometric characteristics and when BoPLD1 alleles tend to homozygosity an increase of inflorescence and curd size are observed. These results permit us to continue to investigate by sequencing these primers to individuate the SNPs useful for distinguishing the broccoli types with hypertrophic inflorescence during the organic breeding programs. The crossing plans among the broccoli breeding lines and the *Brassica* wild relatives, aiming to transfer forgotten alleles during the domestication process, will be useful for increasing the resistance against biotic and abiotic stresses, and for nutritional, organoleptic and nutraceutical traits. The molecular marker will reduce the cost of evaluation field transplanting with only the selected individuals expressing the broccoli inflorescence phenotype, reducing the number of individuals to grow and to analyze.

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Molecular Markers for Detecting Inflorescence Size of *Brassica oleracea* L. Crops and *B. oleracea* Complex Species (n = 9) Useful for Breeding of Broccoli (*B. oleracea* var. *italica*) and Cauliflower (*B. oleracea* var. *botrytis*)

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Abstract: The gene flow from *Brassica oleracea* L. wild relatives to *B. oleracea* vegetable crops have occurred and continue to occur ordinarily in several Mediterranean countries, such as Sicily, representing an important hot spot of diversity for some of them, such as broccoli, cauliflower and kale. For detecting and for exploiting the forgotten alleles lost during the domestication processes of the *B. oleracea* crops, attention has been pointed to the individuation of specific markers for individuating genotypes characterized by hypertrophic inflorescence traits by the marker assisted selection (MAS) during the first plant growing phases after the crosses between broccoli (*B. oleracea* var. *italica*)/cauliflower (*B. oleracea* var. *botrytis*) with *B. oleracea* wild relatives (n = 9), reducing the cultivation and evaluation costs. The desired traits often found in several *B. oleracea* wild relatives are mainly addressed to improve the plant resistance to biotic and abiotic stresses and to increase the organoleptic, nutritive and nutraceutical traits of the products. One of the targeted traits for broccoli and cauliflower breeding is represented by the inflorescences size as is documented by the domestication processes of these two crops. Based on the previous results achieved, the numerical matrix, obtained utilizing five simple sequence repeats (SSRs), was analyzed to assess the relationship among the main inflorescence characteristics and the allelic variation of the SSRs loci analyzed (BoABI1, BoAP1, BoPLD1, BoTHL1 and PBCGSSRBo39), both for the *Brassica oleracea* and *B. oleracea* wild relatives (n = 9) accessions set. The main inflorescence morphometric characteristics, such as weight, height, diameter, shape, inflorescence curvature angle and its stem diameter, were registered before the flower anthesis. We analyzed the correlations among the allelic variation of the SSRs primers utilized and the inflorescence morphometric characteristics to individuate genomic regions stimulating the hypertrophy of the reproductive organ. The relationships found explain the diversity among *B. oleracea* crops and the *B. oleracea* complex species (n = 9) for the inflorescence size and structure. The individuated markers allow important time reduction during the breeding programs after crossing wild species for transferring useful biotic and abiotic resistances and organoleptic and nutraceutical traits to the *B. oleracea* crops by MAS.

Keywords: *Brassica* complex species (n = 9); MADH–box genes; SSRs assay; heterozygosity index; allelic variance; inflorescence morphometric traits

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1. Introduction

Molecular markers provide a simple, rapid and non-destructive method for selection by genotyping, which can be utilized at any plant stages for significantly reducing the time, cost and other resources required for breeding programs to develop varieties [1]. Marker-Assisted Selection (MAS) is a technique for identifying and localizing genes associated with

the key plant traits in its genome. The goal of plant breeding is to create new varieties that combine several traits defined by the breeder to meet the needs of farmers and consumers. It is also of great interest in programs for the introgression of a gene of interest into an elite variety by the backcross plan. MAS is an effective tool in plant breeding, especially when the target phenotypic traits are laborious or expensive to measure and which can be supported by the molecular markers [2].

Brassica is a genus of the dicotyledonous plants belonging to the *Brassicaceae* family, native to Eurasia and the Mediterranean basin, and it includes about forty species [3]. They are generally annual or biennial herbaceous plants, in some case perennial, with cross-shaped flowers characteristic of *Cruciferae* (now *Brassicaceae*). Cultivated species have a very diversified plant morphology, depending on whether they have been domesticated for their leaves, petioles, buds, flowers, roots or seeds. They are grown as vegetables, condiments, oilseeds or medicinal plants. Four species are mainly cultivated with an important role in the human diet: *Brassica oleracea* (various coles), *Brassica nigra* (black mustard), *Brassica napus* (rapeseed, rutabaga) and *Brassica rapa* (turnip, rape, Chinese cabbage) [4]. Among the *Brassica oleracea* crops, cauliflower (*B. oleracea* L. var. *botrytis* L.) and broccoli (*B. oleracea* L. var. *italica*) are the only two crops grown for their hypertrophic reproductive organs, whereas all the others have constantly modified vegetative organs that represent the products [5]. The domestication process of broccoli and cauliflower probably began since Roman times, however, the similar morphological structure of the edible organs of both crops has repeatedly caused confusion in finding unique descriptions for each of them. The apparent similarity of broccoli and cauliflower inflorescence and the similar hypertrophy of the inflorescence may have influenced the scientific and common names, which are interchangeable in some cases [5–7]. *B. oleracea* vegetable products provide a high level of bioactive compounds conferring high antioxidant activity related to the richness of glucosinolates, isothiocyanates and polyphenols contained in the edible portion of the plant [8,9].

Recent DNA analysis using molecular techniques supports a high degree of similarity between Sicilian wild *Brassica* species (n = 9) and *B. oleracea* crops [10–13]. More recently, additional similarities have been observed among *B. oleracea* crops and the *Brassica oleracea* complex species (n = 9) widespread in the Mediterranean basin than have been observed for the Atlantic wild *Brassica* ones [14–19].

Molecular markers have proven to be important tools for assessing genetic variation and relationships in plant species above all for organic breeding and for establishing the organic heterogenic materials as described by the EU Directive 848/2018 [20]. Some molecular markers are represented by simple sequence repeats (SSRs), alternately known as microsatellite markers, which have been successfully used for evaluating the genetic variability and for distinguishing among them the nearly related *Brassica* genotypes [21–23], because of their codominance and ability to reveal a high number of alleles for locus, resulting in a high degree of reproducibility and variability [24].

The MADS-box genes are involved in plants for the control of all major aspects of their development, such as the differentiation between male and female gametophytes, the development of embryos and seeds, roots, flowers and fruits, and the determination of flowering time [25]. Bowman et al. [26] studied flower development genes, and they found several genes involved, like *apetala 1* and *cauliflower*, also observed in *Arabidopsis thaliana*. These genes are closely related to members of the MADS-box gene family, and a mutant copy of these is present in the *B. oleracea* genome C. Irish and Sussex [27] characterized by a lot of floral morphotypes produced by the homeotic recessive *apetala 1* (*ap1*) mutation in *Arabidopsis* and the homozygote for this mutation which demonstrated low inflorescence affecting the formation of floral buds.

Exercising the simple sequence repeat (SSR) marker BoAP1 advanced a number of alleles which were found in the wild *B. oleracea* complex species (n = 9) rather than in cabbage and cauliflower. *BoAp1-a* locus located in a single genomic region on the chromosome 6 of *B. oleracea* with the additional ones (BoCAL, BoLFY, BoAP1-c, BoREM1) of the

MADS-box genes family [28]. Smith and King [29] proposed a genetic model grounded on the segregation of the recessive alleles for BoCAL and BoAP1 candidate genes that showed differences during the plant stage of flower development arrest between broccoli and cauliflower. According to Smith and King's allelic distribution genetic model, the domestication strategy reduced the allelic diversity by promoting *loci* affecting the arrest of floral development that determined the first inflorescence hypertrophy of broccoli and then, by further selection, the cauliflower's curd inflorescence morphotype; the Sicilian Purple cauliflower was indicated as an important intermediate of this domestication pathway. The four primers BoAP1, BoABI1, BoPLD1 and BoTHL1 were designed by Tonguç and Griffiths [30] to investigate the genomic DNA for evaluating the genetic similarity among several *Brassica oleracea* cultivars belonging to three varietal groups (broccoli, cauliflower and cabbage). One additional primer PBCGSSRBo39 was designed by Burgess et al. [31] to demonstrate a useful molecular marker for crop improvement that was derived from shotgun sequencing methods.

These five SSR primers (BoAP1, BoABI1, BoPLD1, BoTHL1 and PBCGSSRBo39) were chosen by Branca et al. [32] by opting for them from among others primers, for phylogenetic analysis and for evaluating the genetic similarity among different *B. oleracea* accessions (cauliflower and broccoli) and *B. oleracea* complex species (n = 9), as well as to estimate genetic divergence using the FST statistical parameter, where broccoli cultivars grouped with cauliflower cultivars as expected and wild species showed major genetic differences. Sheng et al. [33] characterized and mapped 91 MADS-box transcription factors able to discern from type I (Mα, Mβ, Mγ) and type II (MIKCC, MIKC*) genetic groups as a consequence of phylogenetic and gene structure analysis: 59 genes were randomly distributed on 9 chromosomes, and 23 were located in 19 scaffolds, while 9 of them were not located due to the lack of information on the NCBI database (Sheng et al., 2019). Treccarichi et al. [20] used the set of markers used by Branca et al. [34] to calculate the genetic diversity among nine accessions of *B. oleracea* crops and *B. oleracea* complex species (n = 9) and to evaluate the hypertrophic induction of the curd. The SSRs assay can also be exploited in population genetics to discover allelic variants related to interesting traits, and it could also be a topic for the breeders that can apply it to inherit them in the F2 population [35].

In the present work, the above cited five SSR primers based on the sequences of several MADS-box genes were used to analyze the allelic variation of different Sicilian landraces and hybrids F1 of cauliflower and broccoli, and of some *B. oleracea complex* species (n = 9), for associating them with the inflorescence morphometric traits that have been measured for each accession. The following manuscript aims to identify the most interesting allelic variants to use as organic breeding tool for broccoli MAS.

2. Results

2.1. Bio-Morphometric Analysis

Based on the bio-morphometric characteristics of the inflorescence analyzed were inflorescence weight (IW), height (IH), diameter (ID1), stem thickness (ID2), shape (IS) and angle of curvature (IA). With regard to IW, it varied among genotype from 1095.8 to 16.65 g, for CVF1.1 and BV, respectively (Table 1). IW showed the highest values in cauliflower F1 hybrids and landraces, followed by broccoli heirlooms and landraces. CWRs showed the lowest value of IW varying from 33.3 to 16.7 g, for BU1 and BV, respectively. With regard to IH, the CWRs group, represented by the accessions BM, BU1, BU2, BU3, BU4, BV, BY1, BY2 showed the highest IH values followed by the lowest IW, ID2, ID1 and IA due to the characteristics of their inflorescence architecture, which is large and thin, with large flower buds, low inflorescence density and low bolting resistance. IH varied for the CWRs group from 14.8 to 27.6 cm, while in cauliflower and broccoli groups, it varied from 7.5 to 22.2 cm for CVF1.4 and BR2, respectively. Concerning ID2, we observed the highest values for cauliflower morphotype varying from 28.6 to 39.8 mm for CV3 and CV1, respectively, while broccoli genotypes exhibited an average value of 3.1 cm varying from 2.6 to 3.8 mm for BR8 and BR1, respectively. The crop wild relatives group showed an average ID2 value

of 17.9 mm varying from 9.6 to 22.5 mm for BM and BY1, respectively (Table 1). Concerning ID1 it showed the highest value for the cauliflower group varying from 13.6 to 21.1 cm for CV3 and CV4, respectively, and it varied for the broccoli group from 4.7 to 12.3 cm for BR9 and BRF1.1, respectively (Table 1). IS showed the highest values for broccoli accessions varying from 1.2 to 3.6 for BRF1.1 and BR9, respectively (Table 1).

Table 1. Inflorescence morphometric traits analyzed.

Accession	IW	IH	ID2	ID1	IS	IA
CVF1.1	1095.8 (21.1) *	11.1 (8.4)	42.3 (8.5)	18.0 (8.7)	0.6 (9.6)	110.0 (21.9)
CV1	965.7 (37.4)	15.4 (14.6)	39.8 (16.4)	20.7 (17.4)	0.7 (16.6)	105.0 (19.4)
CV4	666.6 (42.5)	15.2 (13.2)	34.1 (19.6)	21.1 (15.0)	0.7 (12.1)	112.0 (20.4)
CI1	628.8 (33.7)	16.8 (16.6)	38.1 (18.5)	19.7 (14.6)	0.9 (14.6)	101.0 (22.5)
CVF1.2	605.0 (33.8)	8.9 (16.7)	31.0(10.3)	16.9 (11.8)	0.5 (12.1)	113.0 (13.3)
CV5	567.3 (38.2)	14.5 (15.6)	37.0 (19.8)	19.5 (13.1)	0.7 (17.29)	113.0 (13.5)
CV6	564.9 (37.0)	14.5 (20.7)	34.6 (12.6)	20 (15.1)	0.7 (18.7)	104.0 (16.7)
CV7	554.5 (56.7)	18.8 (20.4)	30.8 (26.9)	19.5 (19.3)	0.9 (29.8)	107.0 (17.7)
CVF1.3	541.5 (54.7)	13.7 (24.4)	32.3 (21.9)	18.9 (29.6)	0.7 (18.3)	112.0 (22.3)
CV8	503.9 (35.4)	16.8 (28.4)	32.4 (18.1)	16.5 (17.9)	1.0 (34.4)	100.0 (27.4)
CVF1.4	467.1 (41.1)	7.5 (20.9)	30.0 (13.3)	14.6 (15.7)	0.5 (11.1)	101.0 (15.6)
CVF1.5	461.8 (47.1)	10.8 (16.1)	33.0 (13.9)	17.5 (16.4)	0.6 (17.4)	110.0 (19.3)
CV9	453.5 (49.7)	11 (17.2)	35.6 (17.9)	18.1 (27.3)	0.6 (22.5)	117.0 (15.8)
CV10	443 (55.9)	12.7 (23.0)	36.4 (24.2)	16.7 (23.8)	0.8 (32.4)	91.0 (26.5)
CVF1.6	438.8 (84.4)	17.6 (24.4)	28.8 (28.1)	16.8 (29.4)	1.1 (34.2)	93.0 (17.7)
CI2	378.3 (46.2)	10.2 (21.0)	36.8 (16.9)	17.2 (19.5)	0.6 (17.8)	113.0 (17.5)
BRF1.1	319.8 (40.9)	14.1 (26.8)	3.5 (19.5)	12.3 (26.7)	1.2 (44.4)	76.0 (29.8)
CVF1.7	317.4 (42.0)	17.2 (22.2)	29.2 (28.1)	14.8 (16.0)	1.2 (33.1)	98.0 (21.6)
CV11	305.7 (68.2)	8.7 (20.7)	31.7 (18.1)	15.4 (22.8)	0.6 (19.7)	92.0 (25.2)
BR1	279 (39.0)	16.6 (18.1)	3.8 (17.3)	11.1 (23.7)	1.5 (28.2)	57.0 (21.5)
BR2	266.9 (33.4)	22.2 (30.9)	3.2 (13.2)	8.5 (32.7)	2.7 (37.4)	58.0 (19.4)
CV2	263.6 (56.1)	11.2 (28.3)	34.2 (18.8)	14.4 (22.0)	0.8 (21.2)	91.0 (23.4)
BR3	226.4 (39.6)	18.2 (12.9)	3.1 (26.8)	7.9 (29.4)	2.3 (30.5)	49.0 (27.8)
BR4	217.7 (58.3)	18.2 (18.2)	2.9 (29.8)	9.5 (31.6)	1.9 (29.4)	54.0 (26.3)
BRF1.2	212.8 (36.3)	12.8 (12.2)	3.1 (15.0)	7. 8 (23.1)	1.9 (16.5)	46.0 (24.1)
BR5	188.3 (51.8)	16.6 (23.4)	2.9 (24.3)	7.7 (28.3)	2.2 (24.2)	46.0 (24.1)
CV3	186.6 (41.3)	8.4 (17.5)	28.6 (16.7)	13.6 (15.1)	0.6 (18.2)	85.0 (24.8)
BR6	164.0 (49.0)	16.5 (17.9)	3.3 (32.4)	8.3 (29.5)	2.0 (52.3)	46.0 (32.8)
BR7	143.9 (42.2)	16.0(29.0)	2.7 (22.7)	7.8 (29.0)	2.1 (22.6)	48.0 (26.7)
BR8	109.5 (30.8)	15.5 (9.5)	2.6 (20.2)	7.9 (25.8)	2.0 (23.4)	41.0 (34.2)
BR9	63.1 (41.7)	16.9 (23.5)	2.7 (18.9)	4.7 (22.3)	3.6 (15.5)	27.0 (15.2)
BU1	33.3 (28.3)	27.6 (15.5)	16.2 (20.2)	3.1 (17.9)	0.2 (21.2)	14.0 (11.7)
BU2	28.7 (1.6)	19.5(1.5)	19.3 (3.3)	4.1 (0.2)	0.21 (0.1)	15.0 (0.9)
BY1	27.7 (3.7)	20.4 (1.0)	22.5 (4.5)	3.3(0.7)	0.1 (0.1)	13.5 (2.1)
BM	26.6 (5.9)	16.7 (4.6)	9.6 (2.5)	2.7 (0.4)	0.3 (0.1)	11.3 (2.6)
BU3	22.4 (0.4)	23.5 (4.0)	19.6 (1.5)	2.0 (0.3)	0.1 (0.1)	9.5 (0.7)
BU4	21.1 (0.8)	19.2 (2.2)	18.9(3.6)	2.2(0.1)	0.1 (0.1)	13.5 (2.1)
BY2	20.6 (1.3)	20.8 (0.6)	18.5 (1.6)	2.8 (0.4)	0.2 (0.1)	11.5 (0.7)
BV	19.7 (0.6)	14.8 (0.4)	19.0 (1.2)	2.4 (0.2)	0.1 (0.1)	10.5 (0.7)

numbers in brackets () * indicate the standard deviation.

Broccoli hybrids F1 resemble cauliflower inflorescence and, for this reason, showed a lower CS ratio than for broccoli landraces which are characterized by higher values of IH (Table 1). The cauliflower genotype showed intermediate IS values varying from 0.5 to 1.17 for CVF1.2 and CVF1.7, respectively. The CWRs group showed the lowest IS value, which varied from 0.1 for BU3, BU4, and BV to 0.3 for BM (Table 1). Concerning IA character, it exhibited the highest values for the cauliflower genotypes showing the average value of 102.9°, varying from 85° to 117° for CV3 and CV9, respectively (Table 1). Broccoli genotypes are characterized by a reduced amplitude compared to the cauliflower group, and it varied from 279° to 76° for BR9 and BRF1.1, respectively (Table 1). The CWRs group showed the lowest IA values due to their different simple inflorescence architecture that is slenderer than cauliflower and broccoli genotypes, and it varied from 9.5° to 15° for BU3 and BU2, respectively (Table 1).

2.2. Identification of the Best Molecular Marker by the Association between Their Allelic Variants and the Bio-Morphometric Traits

Pearson's correlation showed a significant correlation among IW and the descriptors ID1, ID2 and IA. On the other hand, the IS descriptor is derived from the ratio between ID1 and IH and is negatively related to ID2 (Table 2). Concerning IA, it was positively correlated to ID1, IW and ID2, respectively (Table 2).

Table 2. Pearson's correlation among traits.

Genotype	IW	IH	ID2	ID1	IS	IA
IW	1					
IH	0.024	1				
ID2	0.680 **	−0.035	1			
ID1	0.880 **	−0.066	0.724 **	1		
IS	−0.117	−0.068	−0.638 **	−0.107	1	
IA	0.847 **	−0.033	0.706 **	0.980 **	−0.086	1

** indicates that the correlation is significant at $p < 0.01$.

By analysing the correlation among the matrix data of the considered alleles detected and the inflorescence morpho-biometric traits, we individuated the ones that correlated the highest.

The correlation among the molecular markers and the inflorescence descriptors showed a high significant correlation with the allelic variation 155 bp of AP1 (P1), which was correlated negatively with IH and positively with IW, ID1, ID2 and IA (Table 3). With regards to the P2 (BoTHL1), the allelic variant of 157 bp molecular weight was positively correlated with IH and negatively with IW, IA, ID1 and IS, respectively, in decrescent order (Table 3). The allele of 165 bp found for P2 was positively correlated with ID1, ID2 and IA, while it was negatively correlated with IW. The allelic variation of 184 bp detected for the marker BoAB1 (P3) was significantly negatively correlated with IS, ID1, WI and IA, and positively with IH (Table 3). The allelic variant of 288 bp of the BoPLD1 marker (P4) was positively correlated with IA, ID1 and IW, and negatively with IH, whereas on the other hand, the allelic variant of 291 bp was positively correlated to IA and IH, and negatively correlated to ID1, IW and ID2, respectively, in decrescent order (Table 3). The PBCGSSRBo39 (P5) allele of 294 bp was positively correlated with IH and IA, and negatively correlated with IW, ID1 and IS. Finally, the allelic variant of 308 bp of the marker P5 was positively correlated with IS, ID1, IW and IA, respectively, in decrescent order, and negatively correlated with IH (Table 3).

Table 3. Correlation among all the allelic variants detected by the molecular markers used and the analyzed traits to individuate the most associated alleles of the examined traits.

Allelic Variant	IW	IH	ID2	ID1	IS	IA
P1_155	0.622 **	−0.471 **	0.521 **	0.622 **	0.032	0.677 **
P1_156	−0.101	0.156	0.219	−0.097	0.202	−0.135
P1_164	−0.375	0.072	−0.082	−0.334	−0.283	−0.306
P2_153	−0.288	0.189	0.219	0.308	0.00	−0.264
P2_157	−0.338 *	0.405 **	−0.088	−0.372 *	−0.376 *	−0.372 *
P2_162	−0.152	−0.029	−0.418 **	−0.266	0.196	−0.175
P2_165	−0.461 *	−0.220	0.583 **	0.594 **	−0.014	0.538 **
P2_168	0.160	0.021	0.226	0.205	0.050	0.204
P3_180	0.010	0.033	0.069	0.046	−0.003	0.095
P3_184	−0.455 **	0.440 **	0.123	−0.455 **	−0.477 **	−0.433 *
P3_186	−0.233	0.296	−0.214	−0.257	0.062	−0.187
P3_190	0.257	−0.440 *	0.268	0.303	0.192	0.226
P3_192	0.418 *	−0.324	0.222	0.424 **	0.156	0.436 **
P3_194	0.140	−0.015	−0.068	0.068	0.146	0.174
P4_282	−0.139	0.199	−0.097	−0.184	−0.168	−0.232

Table 3. *Cont.*

Allelic Variant	IW	IH	ID2	ID1	IS	IA
P4_288	0.460 **	−0.333 *	0.308	0.522 **	0.172	0.568 **
P4_291	−0.462 **	0.381 *	−0.462 **	−0.477 **	0.148	0.485 **
P5_294	−0.343 *	0.410 **	0.078	−0.376 *	−0.391 *	0.384 *
P5_304	0.306	0.050	0.089	0.217	0.165	0.330 *
P5_308	0.384 *	−0.474 **	0.132	0.449 **	0.478 **	0.380 *

* and ** indicate that the correlation is significant at $p < 0.05$ and $p < 0.01$, respectively.

On the basis of the correlation observed among the inflorescence descriptors and the alleles detected for the five primers utilized we directed our attention to the alleles most correlated with at least four correlations with the six inflorescence descriptors utilized. The most correlated alleles chosen were the following: P1_155, P2_153, P2_157, P2_162, P2_165, P2_168, P3_184, P3_186, P3_190, P3_192, P4_288, P4_291, P5_294, P5_304 and P5_308 (Tables 3 and 4).

Table 4. Principal component of the rank of all the examined traits and for the most correlated allelic variants detected.

	PC1	PC2	PC3
IW	0.900	0.093	−0.155
IH	−0.564	0.108	0.132
ID2	0.670	0.653	−0.045
ID1	0.938	0.135	−0.049
IS	0.200	−0.858	0.147
IA	0.919	0.131	−0.213
P1_155	0.671	0.197	−0.112
P2_153	−0.350	−0.035	0.067
P2_157	−0.482	0.422	0.074
P2_162	−0.183	−0.396	−0.411
P2_165	0.579	0.283	0.462
P2_168	−0.269	−0.041	−0.041
P3_184	−0.570	0.503	0.089
P3_186	−0.353	−0.196	−0.144
P3_190	0.323	−0.085	0.693
P3_192	0.509	−0.150	−0.287
P4_288	0.673	−0.066	0.098
P4_291	−0.643	−0.162	0.031
P5_294	−0.503	0.523	0.027
P5_304	0.226	−0.046	−0.765
P5_308	0.526	−0.361	0.571
Variance (%)	32.60	11.57	9.68

Utilizing the data from the above cited allelic variants for the five primers utilized and for the six inflorescence descriptors, we established the related PCA, for which the main component (PC1) is positively correlated with ID1, IA, IW, P4_288, P1_155 and ID2, respectively, in decrescent order, whereas it was negatively correlated with P4_291, IH and P3_184 and represented 32.60 % of the total variance (Table 4, Figure 1a). With regard to the second principal component (PC2), it was positively correlated with P5_294 and negatively correlated with IS, and it represented 11.57% of the total variance (Table 4, Figure 1a). Concerning the third component (PC3), it was positively correlated with P3_190 and negatively correlated with P5_304, and it represented 9.68% of the total variance (Table 4, Figure 1a).

Based on the correlation and the PCA observed and to better discriminate the six inflorescence morphotypes studied, we chose among the 20 alleles detected 5 of them correlated with at least 4 of the 6 inflorescence descriptors utilized. The most correlated alleles chosen were P1_155, P2_165, P3_184, P4_288 and P5_308 (Tables 3 and 4).

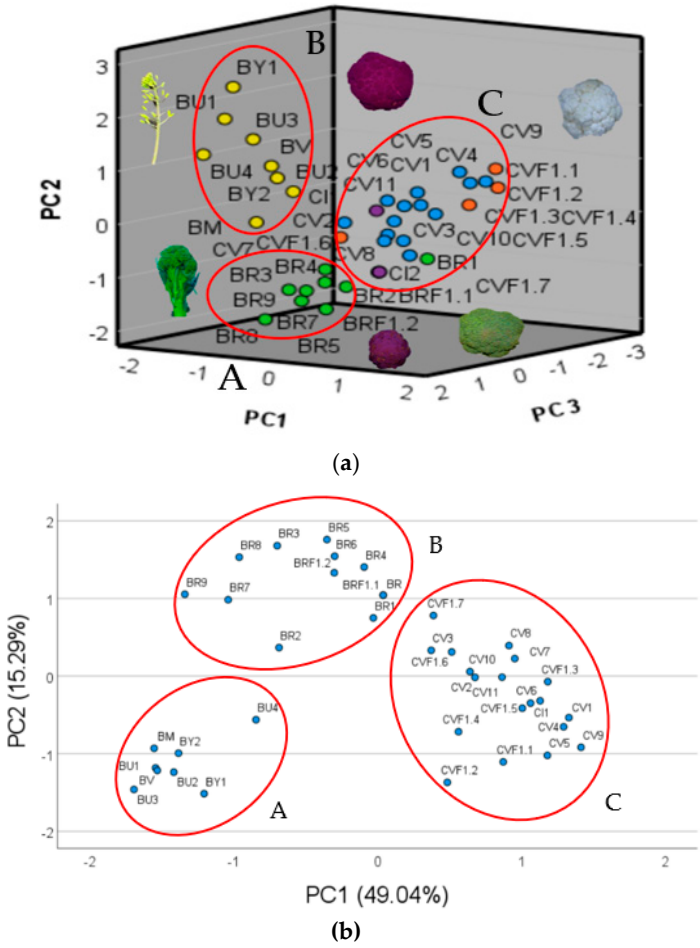

Figure 1. PCA plot performed utilizing 20 alleles selected for the primers utilized and all the inflorescence descriptors (**a**) and PCA plot performed utilizing the 5 more highly correlated alleles with the inflorescence traits (**b**), respectively.

The PCA analysis performed on the highest correlated alleles with the inflorescence descriptors showed the PC1 positively correlated that ID1, IA, IW, P1_155, ID2, P4_288, P2_165 and P2_192, and negatively correlated with P4_291, P3_184 and IH, representing 49.80% of the total variance (Table 5, Figure 1b). Concerning the PC2, it was positively correlated to IS and negatively correlated to ID2, and it represented 15.29% of the total variance (Table 5, Figure 1b).

Table 5. The PCs matrix related to the bio-morphometric analysis and the selected allelic variants.

	PC1	PC2
IW	0.910	−0.058
IH	−0.554	−0.119
ID2	0.741	−0.595
ID1	0.952	−0.060
IS	0.101	0.888
IA	0.941	−0.100
P1_155	0.752	−0.078
P2_165	0.631	−0.031
P3_192	0.506	0.258
P4_288	0.644	0.156
P5_308	0.524	0.641
Variance (%)	*49.08*	*15.29*

The PCA plot established by the 15 chosen alleles showed the genotypes studied distributed in three main groups (Figure 1a). The first group (A) is represented by the CWRs characterized by high value of IH and low values of IW and IA (Figure 1a). The second group (B) is represented by the broccoli genotypes distinguishable by high IS values and by the intermediate values of IH, IW, ID1, ID2 and IA (Figure 1a). Group C, instead, is represented by cauliflower genotypes followed by the broccoli F1 hybrids showing the highest values for IW, IH, ID2, ID1 and IA and the lowest for IS (Figure 1a). The PCA plot performed utilizing the most correlated allele for each primer, confirmed the three groups observed earlier but distinguished them better (Figure 1a). Group A is represented by all the *B. oleracea* complex species (n = 9), group B by the broccoli landraces and hybrids F1, and group C by the cauliflower landraces and hybrids F1, validating the efficiency of the five alleles and of the SSRs utilized to distinguish among *B. oleracea* crops and complex species (n = 9) (Figure 1b).

The PCA obtained utilizing the three highest correlated allelic variances is shown in Figure 2. In fact, the allelic variances P1_155, P2_165 and P4_288, which show the highest correlation with the examined bio-morphometric traits allowing the genotypes distribution in different clusters, are each represented by the different inflorescence morphotypes studied (Figure 2).

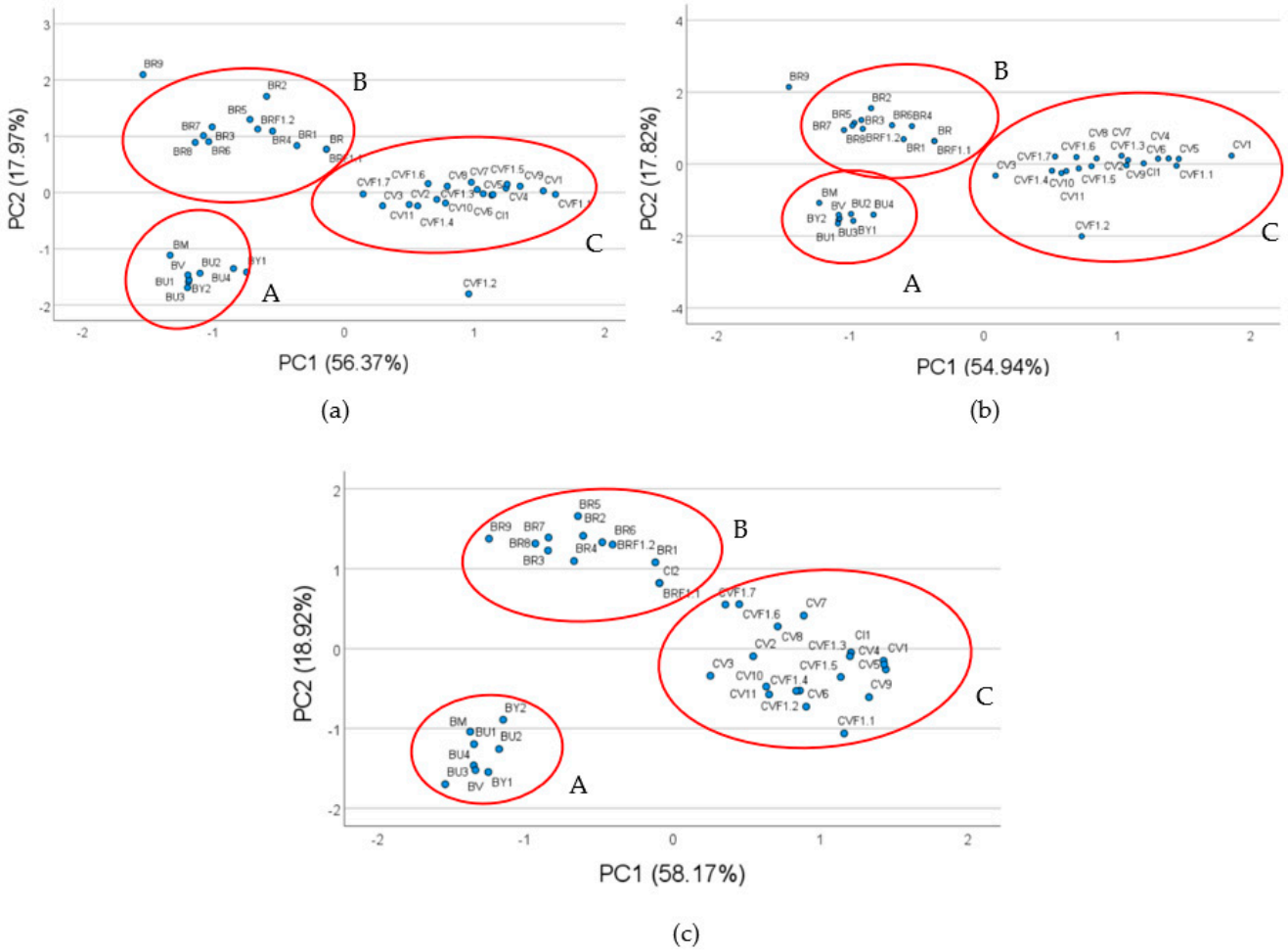

Figure 2. PCA plots performed utilizing three of the most correlated alleles with the inflorescence traits: allele of 155 bp for P1 (**a**), allele of 165 bp for P2 (**b**), allele of 288 bp for P4 (**c**), respectively.

3. Discussion

B. oleracea species includes many important vegetable crops exhibiting high morphological diversity among them and their cultivars. In our work, the main inflorescence mor-

phometric traits (IW, IH, ID1, ID2, IS and IA) allow us to distinguish among the *B. oleracea* inflorescence morphotypes, in accordance with Branca et al. [32] and Treccarichi et al. [20]. The plant materials were selected from the *B. oleracea* core and the *Brassica* wild relatives species (n = 9) collection of the Di3A of the University of Catania to individuate the morphometric and genetic diversity of the inflorescence just before the anthesis stage. Broccoli landraces showed low values of IW due to how they were traditionally consumed, which was focused on the consumption of the small elongated primary inflorescence having small tender and sweet leaves [32,36]. As confirmed by the bio-morphometric and molecular analysis performed in the present work and by several additional authors, the Sicilian broccoli and cauliflower landraces are well differentiated from each other and from the F1 hybrids [37]. In general, broccoli F1 hybrids resemble the cauliflower inflorescence architecture that is clearly differentiated by its huge hypertrophic inflorescence and wide angle of curvature. As reported by several authors, in fact, the allelic distribution of BoCAL and BoAP1 also have contributed to the diversification process of the Calabrese broccoli and of the cauliflower purple type, which is typical of the northeast side of Sicily [16,29].

B. oleracea wild relatives (n = 9), furthermore, have differential traits from the *B. oleracea* crops that can be improved for their resistance to biotic and abiotic stresses and to improve organoleptic and nutraceutical properties for enhancing the bioactive compound amount and profile by assessing and exploiting their genetic diversity [38,39]. The *B. oleracea* complex species (n = 9) utilized in our work are diploid species and coexist along the Sicilian and the genetic flux among them and with different *B. oleracea* crops and landraces was ascertained [38].

MADS box genes are differentially conserved in the *Brassica* genome, and their differential expression on the different *B. oleracea* crops and organs are responsible for the flower induction and for the inflorescence development. The functional characterization of the following genes was performed by Sheng et al. [33], highlighting their different expression patterns and the molecular regulation of the flower development.

In our previous work, we already detected for each SSR locus different numbers of alleles among the accessions and the inflorescence morphotypes studied; BoAP1 (P1) showed 12 alleles, BoTHL1 (P2) 8 alleles, BoABI1 (P3) 9 alleles, BoPLD1 (P4) 6 alleles, and PBCGSS-RBo39 (SP5) 39 11 alleles, in accordance with Branca et al. [32] and Treccarichi et al. [20]. Several of the following alleles, were unconsciously selected and maintained by the growers selected for the size of the hypertrophic inflorescence and probably they were also introgressed by the genetic flux among the *B. oleracea* wild relatives (n = 9) and the first domesticated kales and sprouting broccoli landraces [13]. The correlation among the allelic variants and the inflorescence bio-morphometric traits showed that they increase in terms of value when BoPLD1 (P4) *locus* tends to heterozygosity. In reality we have observed the P4_288 allele which is homozygous or heterozygous for broccoli and cauliflower whereas for all the *B. oleracea* complex species (n = 9), except for one of the two *B. incana* studied (BY2), it is absent (Figure 1) [20].

In the work of Tonguç and Griffith [30], the molecular markers P1, P2, P3 and P4 were characterized and identified as candidate markers to assess genetic similarity in broccoli, cabbage and cauliflower, and they showed the polymorphism information content (PIC) value of 0.70, 0.60, 0.58 and 0.45 for P3, P4, P2 and P1, respectively. For the BoAP1 (P1) the allele P1_155 is generally heterozygous for broccoli and cauliflower, whereas for all the *B. oleracea* complex species (n = 9), except for one population each of *B. incana* (BY1) and *B. rupestris* (BU4) studied, it is absent (Figure 1). Regarding BoTHL1 (P2) the allele P2_165 generally expresses a heterozygous condition for broccoli and cauliflower, and it was absent for all *B. oleracea* complex species (n = 9), except for one *B. rupestris* studied (BU4), is absent (Figure 1). For the BoAB1 (P3) the allelic variants P3_184 is always absent for broccoli and cauliflower, whereas for *B. oleracea* complex species (n = 9), it was homozygous for two populations of *B. rupestris* (BU1, BU4) and for two populations of *B. icanca* (BY1, BY2) (Figure 1).

With regard to P5, it was developed and characterized by Burgess et al. [31] in silico by genome shotgun sequences and showed the highest PIC which was 0.83. In fact, we detected the allele P5_308 which was generally homozygous in cauliflower and broccoli landraces and absent for all the *B. oleracea* complex species (n = 9), except for one of the four *B. rupestris* (BU4), which in previous studies seems to be an escape population, is absent.

The high number of allelic variants individuated in our previous study confirmed that the following molecular markers, can be exploited for the construction of a genetic map with the different annotation related to the polymorphic loci and for the identification of diploid and amphiploid *Brassica* taxa. The following molecular markers also allowed us to perform a hierarchical clustering dendrogram distinguishing both broccoli and cauliflower landraces and F1 hybrids, and their crosses, respectively, in each different phylogenetic clade [32].

Noteworthy, for all the primers selected, the broccoli landrace BR9 and the cauliflower F1 hybrid CVF1.2 were isolated from the morphotype cluster for their distinctive features, such as the slender and the compact, huge inflorescence for BR9 and CVF1.2, respectively (Figure 2). Herein, we are providing more information about the MADS box domain allelic distribution and diversity focusing on the ones strictly related to the inflorescence traits. The data discussed will be utilized shortly for validating them by the GBS dataset in progress in the frame of the genotyping activities of the EU H2020 BRESOV project.

On the other hand, the alleles individuated can already be a solid base for using them for selecting progenies by MAS for hypertrophic inflorescence and size for organic breeding of broccoli and cauliflower and for establishing new organic heterogenous materials requested by the EU Directive 848/2018.

4. Materials and Methods

4.1. Plant Material

Plant material includes 31 accessions of Sicilian landraces of broccoli (*B. oleracea* var. *italica*) and cauliflower (*B. oleracea* var. *botrytis*) and 8 *B. oleracea* complex species population (crop wild relatives—CWRs) belonging to the *Brassica* active collection of the Department of Agriculture, Food and Environment (Di3A) of the University of Catania (UNICT), as shown in Table 6 and Figure 3. The plants were transplanted in an open field by block randomized experimental design, as described by Branca et al. [32]. Plants were characterized by their agronomical traits related to the inflorescence production, following the International Board for Plant Genetic Resources (IBPGR) descriptors. Examined traits include (IW), height (IH), diameter (ID1), shape (IS), angle of curvature (IA) and inflorescence stem thickness (ID2) and were analyzed by the laboratory of Biotechnology of Vegetable and Flower Crops of the Di3A department of the University of Catania (UNICT).

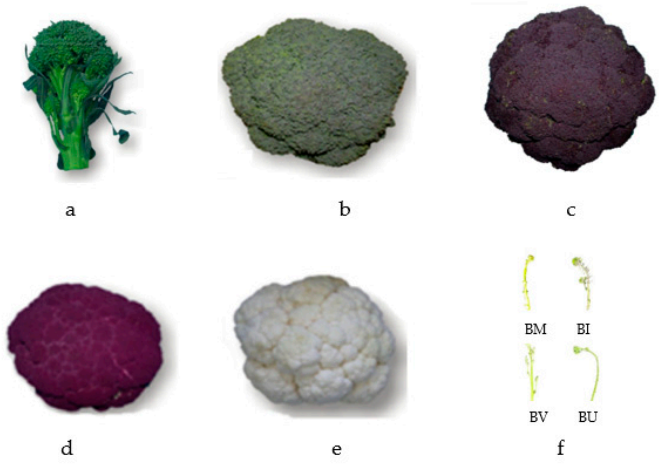

Figure 3. Inflorescence induction in relation of the different morphotypes tested which are, in the following order, (**a**): BR5, (**b**): BRF1.1, (**c**): BR41, (**d**): CV10, (**e**): CVF1.1, (**f**): BM1, BI1, BV and BU1, respectively.

Table 6. List of *B. oleracea* complex species (*n* = 9) utilized in the experiment, with cauliflowers and broccoli F1 and landraces, respectively, and crop wild relatives.

Accession Code	Laboratory Code	Origin	Species
UNICT 583	BR 46	Vittoria	BR1
UNICT 658	BR 45 S1	Acireale	BR2
UNICT 658	BR 129	Roccella Valdemone	BR3
UNICT 657	BR 128	Roccella Valdemone	BR4
UNICT 655	BR 126	Adrano	BR5
UNICT 637	BR 106	Cefalù	BR6
UNICT 3675	BR 94 S1	Francavilla	BR7
UNICT 3668	BR 115 S1	Troina	BR8
UNICT 574	BR 36	Biancavilla	BR9
UNICT 3578	BR 165 Marathon	Esasem	BRF1.1
UNICT 651	BR 122 Packman	Petoseed	BRF1.2
UNICT 4145	BR 13 S3 AC	Modica	CI1
UNICT 579	BR 41	Modica	CI2
UNICT 3190	BR 15 S 1 A	Modica	CV1
UNICT 3669	BR 17 S2	Ragusa	CV2
UNICT 3674	CV 19 S2 A	Piazza Armerina	CV3
UNICT 4137	CV 99 S2 B	Adrano	CV4
UNICT 4138	CV 76 S2	Acireale	CV5
UNICT 3652	CV 159	Catania	CV6
UNICT 3900	BR 13 A X CV98/21	Di3A	CV7
UNICT 3895	CV 98/2 X CV 136 EG	Di3A	CV8
UNICT 3089	CV 75 S3AC	Acireale	CV9
UNICT 3906	CV 24 S4	Biancavilla	CV10
UNICT 3671	CV 72 S2	Catania	CV11
UNICT 3876	CV 171 Menhir F1	ISI sementi	CVF1.1
UNICT 3878	CV 173 Freedom	3878 Royal Sluis	CVF1.2
UNICT 3902	CV 33 S1	Royal Sluis	CVF1.3
UNICT 3880	CV 175 White Flash	Sakata	CVF1.4
UNICT 3879	CV 174 Graffiti	ISI sementi	CVF1.5
UNICT 3892	CV 98/2 X BR 13 S3	DISPA 3	CVF1.6
UNICT 3893	CV 136 EG X CV98/2	DISPA 1	CVF1.7
UNICT 342	*Brassica macrocarpa 1*	Favignana	BM
UNICT 733	*Brassica rupestris 1*	San Vito Lo Capo	BU1
UNICT 3270	*Brassica rupestris 2*	Bivongi	BU2
UNICT 732	*Brassica rupestris 3*	Roccella Valdemone	BU3
UNICT 736	*Brassica rupestris 4*	Ragusa Ibla	BU4
UNICT 3040	*Brassica villosa 1*	Marianopoli	BV
UNICT 3512	*Brassica incana 1*	Agnone Bagni	BY1
UNICT 4158	*Brassica incana 2*	Sortino	BY2

Legend: CV—Cauliflower; CI—Ciurietti landrace; BR—Broccoli; BY—*B. incana*; BM—*B. macrocarpa*; BU—*B. rupestris*; BV—*B. villosa*.

IW was calculated using an analytical scale, while the IH (cm) and ID1 (cm) traits were calculated using a meter rule, and ID2 (mm) was noted using a calibre. The IS descriptor represents the ratio between IH and ID1, while curvature angle IA (°) is the angle limited by the central vertical axes and the tangent one at the extreme part of the inflorescence, and it was calculated using goniometer.

4.2. DNA Extraction and PCR

DNA extraction was performed using the kit GenEluteTM Plant Genomic DNA Miniprep (Sigma Aldrich Inc., St. Louis, MI, USA) and 200 ng μL^{-1} were used for PCR reaction, as reported by Branca et al. [32]. PCRs were done using the primers list reported in Table 7, obtaining the flanking SSRs sequences by Tonguç and Griffiths [30] for BoTHL1, BoAP1, BoPLD1, and BoABI1 and by Burgess et al. [31]. SSRs genome allocation were checked using the basic local alignment search tool (BLAST) (version 1.17) and Ensembl database (release 2021, version 3) and Uniprot database (release 2021, version 3) was

used to study encoding regions close to the gene of interest. DNA amplification was performed in a Perkin Elmer 9700 thermocycler (ABI, Foster City, CA, USA) as reported by Branca et al. [40]. Capillary electrophoresis was carried out by ABI PRISM 3130 Genetic 191 Analyser (Applied Biosystems, Waltham, MA, USA) as described by Branca et al. [32,37] and GeneMapper 3.7 software (Applied Biosystems, Waltham, MA, USA) was used to note the fragments size manually checking each alleles peak.

Table 7. List of primers utilized with their sequences and chromosome (C) position.

Name	SSR Motif	Primer Sequence (Forward, Reverse)	C	Position (from–to); (bp)	Code
BoAP1	$(AT)_{9\text{-}1}$	GGAGGAACGACCTTGATT GCCAAAATATACTATGCGTCT	C6	33,883,667–33,887,357	P1
BoTHL1	$(CTT)_7$	GCCAAGGAGGAAATCGAAG AAGTGTCAATAAGGCAACAAGG	C9	17,254,558–17,255,176	P2
BoABI1	$(TC)_{16}$	TATCAGGGTTTCCTGGGTTG GTGAACAAGAAGAAAAGAGAGCC	C1	1,229,915,511–12,992,170	P3
BoPLD1	$(CT)_7(AT)_{7\text{-}1}$	GACCACCGACTCCGATCTC AGACAAGCAAAATGCAAGGAA	C5	46037340–46,037,606	P4
PBCGSSRBo39	$[GGTCG]_4$	AACGCATCCATCCTCACTTC TAAACCAGCTCGTTCGGTTC	C7	50595248–50595537	P5

4.3. Data Analysis

The Allelic data set was codified by numeric scores, distinguished from 0 (absence of any allele), 1 (heterozygosity), 2 (homozygosity). The matrix generated from the following annotations was used for the sub-mentioned statistical analysis and is available in the H2020 BRESOV repository on the Zenodo database and is also present in the Supplementary data in Table S1. The Statistical analysis was performed using the SPSS software version 27. Data were transformed using the percentage rank of the analyzed matrix and normalized using the angular coefficient (DEGRES(ASIN(RACINE(x/100))). Pearson's correlation was performed to identify the allelic variants involved in the size of inflorescence. The alleles that showed the highest correlation with the morphometric traits were selected. Moreover, the principal component analysis (PCA), as a powerful tool for clustering and dimension reduction, was also performed to discriminate the accessions studied and explain the variability among genotypes by the main components reducing the size of data by the factorial analysis regression method.

5. Conclusions

Genotyping techniques based on molecular markers can be useful for improving knowledge about putative genes controlled by quantitative loci regulating several complex traits such as the inflorescence size. Based on the achieved results, the allelic variants P1_155, P2_165 and P4_288 of the markers BoAP1, BoTHL1 and BoPLD1, respectively, were the most associated with the increase of inflorescence size, and they also facilitate genotype distribution into several clusters by Principal Component Analysis (PCA), represented by each different inflorescence morphotype studied. These three selected alleles could be utilized as molecular markers for organic breeding programs by molecular assisted selection (MAS), and they could be helpful to individuate progenies with hypertrophic inflorescence after crossing broccoli lines and cauliflower with *B. oleracea* wild relatives (n = 9) for transferring useful forgotten alleles, during the domestication process, for increasing biotic and abiotic stress resistance and for organoleptic, nutritional and nutraceutical traits. Of course, the matrix utilized will soon be compared with the new GBS dataset that will permit us to finely validate our present work highlighting the several mutations responsible of the hypertrophic inflorescence of *B. oleracea*. The molecular markers individuated which could be used for the fast selection of a new resilient, efficient and sustainable cultivar exploiting the wild ancestor of *Brassica oleracea* crops.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/plants12020407/s1. Supplementary data Table S1 are represented by the SSRs matrix provided by the association of the different molecular patterns of the detected alleles, to the bio morphometric traits, registered in Table 3. The following additional data permit one to distinguish among the different accessions and the different allelic variants, the condition of homozygosity (2), heterozygosity (1) or its absence (0).

Author Contributions: Conceptualization, F.B.; methodology, F.B., H.B.A. and S.T.; software, H.B.A. and S.T.; validation, F.B., S.T., M.A. and H.B.A.; formal analysis, S.T.; investigation, S.T., F.B., A.T., H.B.A., M.A. and R.C.; resources, F.B. and A.T.; data curation, S.T. and H.B.A.; writing—original draft preparation, S.T. and H.B.A.; writing—review and editing, S.T., F.B. and A.T.; visualization, F.B. and A.T.; supervision, F.B.; project administration, F.B.; funding acquisition, F.B. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The matrix data presented in this study are deposited on Kibana and Zenodo database repositories, and it will be available after the embargo period of one year foreseen in the BRESOV Consortium Agreement.

Conflicts of Interest: The authors declare no conflict of interest.

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Exploitation of a Grafting Technique for Improving the Water Use Efficiency of Eggplant (*Solanum melongena* L.) Grown in a Cold Greenhouse in Mediterranean Climatic Conditions

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Abstract: Grafting techniques have been intricately associated with the optimization of water use efficiency (WUE). In this study, various eggplant (*Solanum melongena* L.) rootstock–scion combinations were compared under three irrigation regimes (IR): 50% deficit in water volume (IR50), a doubling of irrigation volume (IR200), and normal watering (IR100). The cultivar Black Bell (Bb) was employed as a scion, while the rootstock adopted included the F1 hybrids Energy (En) and Beaufort (Be) and one accession of *S. torvum* (To). The trial encompassed the evaluation of no- and self-grafted plants. Plants grown in a cold greenhouse in Sicily were assessed for their morphological parameters, as well as their fruit production and quality. The leaf analysis encompassed the evaluation of chromatic parameters and water potential. Significant variation was observed for plant height, exhibiting the lowest values in self-grafted combinations. The leaf water potential varied significantly in relation to the rootstock–scion combination employed and to the irrigation regime. Fruit quality traits displayed significant variations for chromatic parameters L* and a*, as well as for the fruit's longitudinal and transversal diameters and the soluble solid content. The number of fruits and fruit production per plant varied significantly in relation to the rootstock–scion combination; the highest fruit production was recorded for Black Bell grafted onto *S. torvum* grown by IR50. The fruit weight displayed a significant interaction between the experimental factors under study. Notably, for the WUE calculated in relation to fruit production, a significant interaction between the experimental factors studied was ascertained. The highest WUE was registered for IR50, specifically for To/Bb. This research aims to develop a comprehensive water-efficient organic farming protocol for sustainable agriculture.

Keywords: water uptake; rootstocks; scions; vegetable; organic farming; aubergine

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1. Introduction

The grafting technique for vegetable crops is a highly effective method for controlling pests and diseases and for promoting plant growth and development. This technique renders the fusion of two distinct plant genotypes at the grafting point possible, with the aim of enhancing the scion attributes of rusticity and vigor [1] This ancient practice involves the fusion of the tissues of two different plant varieties to create a single, stronger organism that can control pests and diseases better, thereby improving crop yield and produce quality [1–3]. Grafting is a widely adopted global practice for the management of soil-borne pests and diseases, particularly those that can substantially impact the cultivation of *Solanaceae* and *Cucurbitaceae* plant families [4–6].

This technique is intricately linked to water use efficiency (WUE), which is a measure that elucidates how effectively a plant utilizes water resources for producing biomass and product yield [7,8]. WUE represents the amount of water required to generate the obtained

yield. It can also be evidenced by the ratio of biomass obtained under ordinary irrigation regimes, considering the amount of water consumed through plant evapotranspiration during plant growth and development processes, compared to the deficit irrigation ones. Furthermore, WUE quantifies how efficiently plants convert water into useful outputs in terms of growth and crop production [9,10]. It is a crucial parameter in agriculture and ecology because it reflects a plant's ability to thrive in water-limited environments, which have an impact on photosynthetic activity [11–13]. The exploitation of the WUE trait is particularly valuable in regions where water resources are limited, or in the face of changing climate conditions where droughts are more frequent [14–16]. Drought stress can impact not only the quantity of crop yield but also its quality, potentially altering the production of metabolites [16–18]. The improvement of the crop WUE is a key objective in agricultural research as it can lead to more sustainable and resilient agricultural practices. It can reduce water waste and increase crop productivity in water-stressed areas. Consequently, both grafting techniques and strategies aimed at enhancing WUE hold significant promise for adoption in organic farming conditions, contributing to more efficient and environmentally friendly agricultural practices [19–23]. Within this context, organic farming needs the development of suitable genetic materials and cultivars capable of thriving under organic farming. This often requires the use of biotechnological tools [24,25].

While the primary purpose of grafting is often to combine desirable traits, such as disease resistance or improved fruit amount and quality, its connection to water use efficiency cannot be understood. One of the most critical aspects of grafting in relation to WUE is the choice of rootstock. The rootstock, often chosen for its adaptability to specific environmental conditions, plays a pivotal role in regulating water uptake and distribution within the grafted plant [26,27]. Different rootstocks can exhibit varied levels of drought tolerance, water absorption efficiency, and resistance to waterborne diseases. By carefully selecting a rootstock that suits the local climate and soil conditions, growers can optimize water utilization. Moreover, grafting can enhance a plant's WUE by reducing the overall water demand of the plant [28–30]. When grafting is successful, the scion can benefit from the well-established root system of the rootstock. This means that the scion may require less water to sustain itself since it can draw upon the rootstock's water reserves. As a result, grafted plants can thrive in conditions where non-grafted counterparts might struggle due to limited water availability [31,32].

In the present study, the tested eggplant genotypes, encompassing different combinations of rootstocks and scions (including self and non-grafted plants), were evaluated under three levels of irrigation management. The different irrigation regimes consisted of a 50% deficit in water volume (IR50), a doubling of irrigation volume (IR200), and normal watering (IR100). Hence, a primary objective of this study was to assess how plant growth and development are influenced by altering the water volume, specifically by using either half or double the normal irrigation amount. This evaluation took into account various combinations of rootstocks known for their ability to access water from deeper soil layers. Another objective of this study was to identify the most effective combinations under conditions of water deficiency, with the aim of improving water use efficiency (WUE). This study aimed to develop novel agricultural protocols specifically designed for organic farming. Bio-morphometric analysis of the plants was carried out, including the analysis of fruits' quality traits. Moreover, leaf chromatic parameters and water potential were evaluated to determine how leaf tissue responded in accordance with the plant's hydration status under the three distinct irrigation regimes studied.

2. Materials and Methods

2.1. Plant Material

The trial was conducted in a cold greenhouse located in Marina di Ragusa (RG, Sicily, Italy). This area was chosen for its representativeness of Sicilian greenhouse cultivation, primarily due to the presence of rot-knot nematodes. The geographical coordinates of the greenhouse were 36°47′15.5″ N, 14°33′18.6″ E.

A split-plot experimental design was employed with four replicates for each combination of the two experimental factors under consideration. The first experimental factor concerned the various genotypes (GEs), which were represented by the distinct rootstock–scion combinations, and the second experimental factor encompassed the irrigation regime (IR), involving the adoption of three distinct watering levels. As concerns the evaluated GE, the eggplant cultivar Black Bell (Bb) from the seed company PetoSeed was employed as the scion in each combination. The rootstock utilized included the interspecific tomato hybrid F1 Beaufort (Be), the intraspecific tomato hybrid F1 Energy (En), and one accession of the wild species *Solanum torvum* belonging to the GenBank of vegetables of the Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A) of the Catania University. In addition to the previously mentioned combinations, the auto-grafted and the non-grafted plants of the cv Black Bell were also evaluated (Bb/Bb and Bb, respectively). Consequently, all the rootstock–scion combinations tested were To/Bb, Be/Bb, En/Bb, the self-grafted Bb/Bb, and the non-grafted Bb.

Sowing was conducted in cellular trays, and the plantlets were transplanted during the second decade of October 2021. All grafting combinations were executed using the oblique cutting method in a specialized nursery. The transplanting occurred within a cold greenhouse that had previously hosted tomato (*Solanum lycopersicum*) cultivation in the growing season before the trial. The plants were cultivated at the crop density of 2 plants m^{-2} (0.5 m $\times$ 1.0 m). Plants were characterized using the morphological traits reported in Table 1, with their respective codes and units of measure.

Table 1. List of descriptors used with their respective codes and units of measure.

Code	Trait
PH	Plant height (cm)
PBDR	Plant basal diameter of the rootstock (mm)
PBDS	Plant basal diameter of the scion (mm)
PDGP	Plant diameter at the grafting point (mm)
LL*	Leaf CIE chromatic parameter L*
La*	Leaf CIE chromatic parameter a*
Lb*	Leaf CIE chromatic parameter b*
Fl1, 2, 3, 4	Days of anthesis from transplant (days)
FUL*	Fruit chromatic parameter L*
FUa*	Fruit chromatic parameter a*
FUb*	Fruit chromatic parameter b*
FLD	Fruit longitudinal diameter (cm)
FTD	Fruit transversal diameter (cm)
FDM	Fruit dry matter (%)
FSSC	Fruit soluble solid content (°Brix)
FP	Fruits per plant (n)
few	Fruit weight (g)
FPP	Fruits production per plant (g)
WP	Leaf water potential (-MPa)

During the growing cycle, plants were characterized for their bio morphometric parameters at 21, 42, and 84 days after transplanting (DAT). The traits evaluated during the growing cycle were plant height (PH), the basal diameter of the rootstock and of the scion (PBDR and PBDS, respectively), and the plant diameter at the grafting point (PDGP). These traits were also evaluated at the end of the growing cycle, which was in July 2021. Furthermore, during the growing cycle, the number of days required after transplanting for the first, second, third, and fourth flowers to open was also recorded. During the trial, the leaf chromatic CIEL*a*b* parameters were registered using the colorimeter (Chroma meter CR-200, MINOLTA, Osaka, Japan). In the leaf chromatic analysis performed, L* represented lightness, a* indicated the red/green coordinate, and b* signified the yellow/blue coordinate. The average air temperature throughout the growth cycle ranged from 11.5 °C

in the second decade of December 2021 to 30.5 °C at the end of June 2022. The temperatures inside were recorded using the USB data logger (Testo, 174-T, Sparta, NJ, USA).

2.2. Differential Irrigation Set-Up

The second experimental factor was the irrigation regime (IR), which consisted of a 50% deficit in water volume (IR50), a doubling of irrigation volume (IR200), and normal watering (IR100). The total amounts of water per plant provided during the growing period were 46.40 L plant^{-1} for IR50, 92.80 L plant^{-1} for IR100, and 185.81 L plant^{-1} for IR200 (Table 2). Different irrigation volumes were applied in different phenological stages according to the Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale index. The definition of the optimal requirement of water per plant was estimated according to previous works [33,34]. Particularly, the irrigation volume employed for the control IR100 was calculated following the model of "FAO irrigation and drainage paper number 56" proposed by Allen et al. [35]. This model, which is based on the Penman–Monteith formula [36], considered crop coefficients specific to eggplant cultivation in a cold greenhouse in Sicily, along with global solar radiation. To minimize soil evaporation, plastic mulching was adopted. To ensure the scheduled water supply to the plants, a dripping irrigation system managed by timers was employed. Notably, each irrigation thesis was spatially separated from the others by a border raw.

Table 2. Variation of the irrigation volume (L plant^{-1}) in relation to the different phenological stages (according to the BBCH scale for *Solanaceae*) and to the different irrigation regimes applied (IR50, IR100, and IR200).

DAT	Phenological Stage	IR100	IR50	IR200
17–73	from fourth leaves on main shoot to the fourth flower open	8.10	4.05	16.35
74–112	from the fourth flower open to the 10% of the fruit ripened	4.33	2.16	8.74
113–166	from the 10% of the fruit ripened to the 80% of fruit ripened	24.42	12.21	48.84
167–229	from 80% of the fruit ripened to the harvested product	55.94	27.97	111.89
TOTAL		**92.80**	**46.40**	**185.81**

2.3. Fruit Analysis

Fruit quality traits were assessed, including chromatic parameters (FUL*, FUa*, and FUb*, respectively), longitudinal (FLD) and transversal diameters (FTD), dry matter content (FDM), and soluble solid content (FSSC) (Table 1). Chromatic CIEL*a*b* parameters of the fruits were measured using a colorimeter (Chroma meter CR-200, MINOLTA, Japan). Among the fruit chromatic parameters analyzed, L* indicated lightness, a* was the red/green coordinate, and b* was the yellow/blue coordinate. The soluble solid content was determined using a digital refractometer (DBX-55A, ATAGO, Italy, Milan). The fruit production components registered were the number of fruits per plant (FP), the weight of individual fruit (FEW), and the total fruit production per plant (FPP) (Table 1).

2.4. Water Use Efficiency (WUE)

WUE was calculated for the fruit production per plant (FPP) in relation to the volume of water used per plant. The formula used was in accordance with a previous study [37].

$$WUE = FPP \cdot IW^{-1}$$

where FPP was the fruit production per plant and IW was the water volume, expressed in m^3, employed for the plant. WUE was expressed in kg m^{-3}.

2.5. Leaf Water Potential

Leaf water potential (WP) values, measured in -MPa, were recorded on three specific days corresponding to distinct phenological phases, according to the BBCH index. The

first assessment took place on January 26 during the phenological phase of the third visible flower bud, with temperatures ranging from 9 to 10 °C and relative humidity levels between 80% and 90%. The second assessment occurred during the fruit development phase, specifically at the first fruit cluster stage, with temperatures ranging from 27 to 32 °C and humidity levels of 40%. Finally, the third assessment was conducted during the phenological phase of fruit ripening, with temperatures ranging from 24.5 to 26 °C. The three assessments were chronologically labeled in the manuscript as A1c, A2h, and A3i, representing the assessments conducted in cold, hot, and intermediate temperature conditions, respectively. WP measurements were performed using the Scholander pressure chamber (PMS Instrument Company, PMS-600, Albany, OR, USA).

2.6. Data Analysis

The data were subjected to analysis of variance (ANOVA) using the Newman–Keuls method, and this analysis was conducted using CoStat software version 6.4 (CoHort software, Birmingham, UK). The experimental design included the first factor consisting of four repetitions for each rootstock–scion combination (GE) and the second factor representing the three irrigation levels (IR). Tukey's multiple comparisons test was also performed by CoStat software to assess significant differences in relation to the experimental factor studied. Subsequently, the means for each repetition were utilized to calculate Pearson's correlations among all the examined traits, as well as to perform the principal components analysis (PCA) with the extraction of the three main components. Pearson's correlation and the PCA were carried out using IBM SPSS version 27 software (IBM, Armonk, NY, USA).

3. Results

3.1. Plant Characterization during the Growing Cycle

Significant interactions between the genotype (GE) and the days after transplanting (DAT) were observed for the plant height (PH) recorded during the growing cycle. Similarly, there were significant interactions between the irrigation regime (IR) and the DAT for the PH (Table 3). In relation to the interaction GE × DAT, the PH ranged from 21.04 mm to 60.58 mm for Bb/Bb grown at 21 DAT and Be/Bb growth at 84 DAT, respectively. Conversely, in relation to the interaction between the irrigation regime (IR) and the DAT, the PH value spanned from 21.35 cm for plants grown in IR200 at 21 DAT to 57.86 when grown in IR50 at 84 DAT (Table 3). As a result, the findings revealed notably higher PH values in plants cultivated under IR50 and IR100 conditions compared to those under IR200 conditions. This suggests a more efficient utilization of irrigation water by plants subjected to normal watering or half the amount.

Regarding the plant basal diameter of the rootstock (PBDR), a significant interaction of GE × DAT was observed. Within this context, PBDR values ranged from 4.43 mm for Bb/Bb at 21 DAT to 18.08 mm for Be/Bb at 84 DAT (Table 3). Concerning the plant basal diameter of the scion (PBDS), it exhibited significant fluctuations among the different GEs, ranging from 6.28 mm for Bb/Bb to 9.74 mm for Bb. Furthermore, a significant variation of the PBD value in relation to the DAT was ascertained, spanning from 5.12 mm at 21 DAT to 11.15 mm at 84 DAT (Table 3).

As concerns the plant diameter at the grafting point (PDGP), significant interactions of GE × IR and GE × DAT were observed. PDGP values ranged from 6.79 mm for Bb/Bb grown under IR200 to 17.14 mm for Be/Bb grown under IR50. On the other hand, the PDGP varied from 5.32 mm to 20.97 mm for Bb/Bb at 21 DAT and Be/Bb at 84 DAT, respectively (Table 3). Overall, PDGP values exhibited significantly higher values in plants grown in IR50 and IR100 than those cultivated by IR200. These results unequivocally indicate a better management of water by plants grown with normal watering (IR100) or half the amount (IR50).

Table 3. Variation of the plant growth parameter in relation to the different rootstock–scion combinations, to the different irrigation regimes (IR100, IR50, and IR200), and to the days after transplant (DAT), which were 21, 42, and 84. The analyzed traits were plant height (PH), the basal diameter of the rootstock (PBDR), the basal diameter of the scion (PBDS), and the diameter at the grafting point (PDGP).

		PH						**PBDR**						**PBDS**						**PDGP**					
	DAT	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**x̄**	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**x̄**	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**x̄**	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**x̄**
IR100	**21**	21.71	25.27	21.97	21.71	24.60	23.05	5.42	6.26	7.60	4.40	0.00	4.74	5.38	4.58	5.29	4.22	6.59	5.21	6.26	9.45	10.13	5.58	0.00	6.28
	42	34.43	41.55	42.19	28.38	31.81	35.67	7.65	9.15	11.65	6.20	0.00	6.93	7.79	6.62	7.86	5.92	10.44	7.73	9.16	13.99	14.50	7.58	0.00	9.05
	84	58.25	63.44	61.65	44.74	52.78	56.17	12.82	15.95	17.69	9.12	0.00	11.12	12.69	10.14	12.06	8.92	14.28	11.62	13.52	21.32	21.71	11.00	0.00	13.51
x̄		38.13	43.42	41.94	31.61	36.40	38.30 a	8.63	10.45	12.31	6.57	0.00	7.60 a	8.62	7.11	8.40	6.35	10.44	8.19 a	9.65	14.92	15.45	8.05	0.00	9.61 b
IR50	**21**	21.48	25.19	22.33	21.69	22.48	22.63	5.91	6.63	7.82	4.81	0.00	5.04	6.02	4.43	5.42	4.55	6.90	5.46	7.20	9.91	10.48	5.60	0.00	6.64
	42	35.01	42.63	42.74	28.93	39.61	37.79	7.80	9.40	11.87	6.39	0.00	7.09	8.21	6.95	7.83	6.26	11.59	8.17	9.03	14.63	15.76	7.96	0.00	9.48
	84	56.76	62.53	64.41	49.25	56.35	57.86	12.61	15.82	19.94	10.18	0.00	11.71	12.99	10.52	13.52	10.29	13.08	12.08	14.25	22.07	25.18	11.13	0.00	14.53
x̄		37.75	43.45	43.16	33.29	39.48	39.43 a	8.77	10.62	13.21	7.13	0.00	7.95 a	9.07	7.30	8.92	7.03	10.52	8.57 a	10.16	15.54	17.14	8.23	0.00	10.22 a
IR200	**21**	20.45	23.65	21.61	19.71	21.34	21.35	4.65	5.70	7.14	4.07	0.00	4.31	4.85	4.26	5.08	3.76	5.53	4.69	5.70	8.57	9.56	4.78	0.00	5.72
	42	30.06	34.45	38.91	25.54	29.75	31.74	7.16	8.02	11.03	5.61	0.00	6.36	7.05	5.89	7.45	5.37	8.90	6.93	8.60	12.32	14.03	6.89	0.00	8.37
	84	47.19	50.47	55.66	37.28	43.51	46.82	11.97	16.44	16.59	7.13	0.00	10.42	11.22	9.14	10.92	7.20	10.32	9.76	12.64	19.53	22.25	8.69	0.00	12.62
x̄		32.57	36.19	38.73	27.51	31.53	33.30 b	7.93	10.05	11.59	5.60	0.00	7.03 a	7.71	6.43	7.82	5.44	8.25	7.13 a	8.98	13.47	15.28	6.79	0.00	8.90 c

Means per genotype

	PH						**PBDR**						**PBDS**						**PDGP**					
DAT	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**x̄**	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**x̄**	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**x̄**	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**x̄**
21	21.21	24.71	21.97	21.04	22.81	22.35 c	5.33	6.20	7.52	4.43	0.00	4.70 c	5.41	4.42	5.26	4.18	6.34	5.12 c	6.39	9.31	10.05	5.32	0.00	6.08 c
42	33.17	39.54	41.28	27.62	33.72	35.07 b	7.54	8.86	11.52	6.07	0.00	6.80 b	7.68	6.49	7.71	5.85	10.31	7.61 b	8.93	13.65	14.76	7.47	0.00	8.85 b
84	54.07	58.81	60.58	43.76	50.88	53.62 a	12.47	16.07	18.08	8.81	0.00	11.09 a	12.30	9.93	12.17	8.80	12.56	11.15 a	13.47	20.97	23.05	10.27	0.00	13.38 a
x̄	36.15 b	41.02 a	41.28 a	30.81 c	35.80 b	37.01	8.45 bc	10.38 ab	12.37 a	6.44 c	0.00 d	7.53	8.46 a	6.95 a	8.38 a	6.28 a	9.74 a	7.96	9.60 c	14.64 b	15.95 a	7.69 d	0.00 e	8.90

Significancy of the differences by ANOVA Newman-Keuls method

	PH	**PBDR**	**PBDS**	**PDGP**
GE	***	***	*	***
IR	***	**n.s.**	**n.s.**	***
DAT	***	***	*	***
GE × IR	**n.s.**	**n.s.**	**n.s.**	*
GE × DAT	***	***	**n.s.**	***
IR × DAT	**	**n.s.**	**n.s.**	**n.s.**
GE × IR × DAT	**n.s.**	**n.s.**	**n.s.**	**n.s.**

***, **, and * indicate p-values $\leq$ 0.001, 0.01, and 0.05, respectively. Letters indicate significant differences according to the Tukey test ($p < 0.05$). n.s. represents not significant.

3.2. Plant Characterization at the End of the Growing Cycle

At the end of the growing cycle, a significant interaction between genotype (GE) and irrigation regime (IR) was observed for plant height (PH). In this context, PH values ranged from 118.03 cm for Bb/Bb grown under IR100 to 180.13 cm for Be/Bb grown under IR50 (Table 4). Regarding the plant basal diameter of the rootstock (PBDR), it exhibited significant variation among the evaluated genotypes, ranging from 12.42 mm for Bb/Bb to 29.27 mm for Be/Bb (Table 4). Similarly, the plant basal diameter of the scion (PBDS) also varied significantly among the different genotypes, with values ranging from 11.93 mm for Bb/Bb to 15.92 mm for To/Bb (Table 4). Furthermore, the plant diameter at the grafting point (PDGP) displayed significant variation among the genotypes, varying from 13.62 mm for Bb/Bb to 34.03 mm for Be/Bb (Table 4). Remarkably, at the end of the growth cycle, the disparities observed among the various IRs used were bridged. Specifically, similar values were recorded for PBDR, PBDS, and PDGP when comparing IR50 and IR200 with the control, IR100. The most substantial variations of the diameters recorded were observed in relation to the different rootstock–scion combinations among all the analyzed morphological traits (Figure 1).

Figure 1. Variation of the plant basal diameter of the rootstock and the scion and at the grafting point in relation to the different eggplant rootstock–scion combinations employed. (**a**) Black Bell grafted onto *S. torvum* (To/Bb); (**b**) Black Bell grafted onto Energy F1 rootstock (En/Bb); (**c**) Black Bell grafted onto Beaufort F1 rootstock (Be/Bb); (**d**) Black Bell self-grafted (Bb/Bb); (**e**) Black Bell non-grafted (Bb). Of note is the grafting imbalance observed for the combinations En/Bb and Be/Bb.

3.3. Leaf Chromatic Parameters

The leaf chromatic parameter related to lightness (LL*) exhibited significant variation among the different rootstock–scion combinations (GE), ranging from 36.34 for To/Bb to 38.53 for Bb (Table 5). Additionally, the chromatic parameter La* displayed significant variation among the GE, with values ranging from −11.20 for To/Bb to −13.58 for Bb. Finally, the chromatic parameter Lb* showed significant variations both among the GE and among the different irrigation regimes (IR). Regarding the GE, Lb* values ranged from 14.91 for To/Bb to 21.04 for Bb. In terms of the IR, Lb* values varied from 17.47 for IR100 to 19.48 for IR200 (Table 5).

3.4. Days for the Flowers Opening

The days for the anthesis of the first flower (Fl1) exhibited significant variation among the different rootstock–scion combinations tested (GE). Specifically, Fl1 values ranged from 50.33 DAT to 79.67 DAT for Bb and Bb/Bb, respectively (Table 6). Additionally, there were notable differences in the days for the anthesis of the second flower (Fl2) across the various genotypes (Table 6). Fl2 values spanned from 70.13 DAT to 101.61 DAT for Bb and Bb/Bb, respectively. Conversely, the days for the anthesis of the third flower (Fl3) showed significant variation influenced by both the genotype and the irrigation regime (IR). In terms of genotype, Fl3 ranged from 79.54 DAT to 104.41 DAT for Bb and Bb/Bb, respectively (Table 6). In contrast, concerning the irrigation regime (IR), Fl3 values fluctuated between 87.47 DAT and 98.26 DAT for IR50 and IR200, respectively.

Table 4. Variation of the plant morphometric traits, analyzed at the end of the trial, in relation to the different rootstock–scion combinations and to the irrigation regimes (IR100, IR50, and IR200).

	IR100						IR50						IR200					
	To/Bb	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**x̄**	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**x̄**	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**x̄**
PH	171.90	136.87	178.89	118.03	120.58	145.25 a	157.38	143.13	180.13	120.79	132.50	146.78 a	162.46	144.61	143.05	136.88	139.00	145.20 a
PBDR	16.66	26.44	30.27	12.46	12.90	19.75 a	16.22	26.85	29.62	12.43	13.02	19.63 a	15.40	27.37	27.91	12.37	13.13	19.24 a
PBDS	16.90	11.21	15.02	13.03	12.90	13.81 a	15.84	11.91	15.38	11.99	13.02	13.63 a	15.04	12.68	15.31	11.79	13.13	13.59 a
PDGP	18.29	31.22	33.85	13.72	0.00	19.42 a	18.30	30.84	34.44	13.72	0.00	19.46 a	18.23	30.80	33.79	13.42	0.00	19.25 a

Means per genotype

	To/Bb	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**x̄**
PH	163.91 a	141.53 b	167.35 a	125.23 c	130.69 c	145.74
PBDR	16.09 b	26.89 a	29.27 a	12.42 c	13.02 c	19.54
PBDS	15.92 a	11.93 b	15.23 a	12.27 b	13.02 b	13.68
PDGP	18.27 b	30.95 a	34.03 a	13.62 c	0.00 d	19.37

Significancy of the differences by ANOVA Newman-Keuls method

	PH	**PBDR**	**PBDS**	**PDGP**
GE	***	***	***	***
IR	**n.s.**	**n.s.**	**n.s.**	**n.s.**
GE × IR	***	**n.s.**	**n.s.**	**n.s.**

*** indicate p-values ≤ 0.001. Letters indicate significant differences according to the Tukey test ($p < 0.05$). n.s. represents not significant.

Table 5. Variation of the leaf chromatic CIE parameters in relation to the different irrigation regimes (IR100, IR50, and IR200).

	IR100						IR50						IR200					
	To/Bb	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**x̄**	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**x̄**	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**x̄**
LL*	37.94	38.57	37.67	38.26	39.88	38.46 a	35.23	37.24	39.71	37.09	37.91	37.44 a	35.84	39.22	36.23	37.16	37.79	37.25 a
La*	−11.23	−13.45	−11.16	−12.67	−12.86	−12.27 a	−10.53	−13.55	−11.44	−12.72	−13.81	−12.41 a	−11.82	−12.72	−13.18	−11.86	−13.48	−12.61 a
Lb*	15.36	19.37	14.94	18.05	19.62	17.47 ab	14.09	19.38	16.43	19.31	20.75	17.99 ab	15.27	22.77	18.46	18.17	22.74	19.48 a

Means per genotype

	To/Bb	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**Mean**
LL*	36.34 b	38.34 a	37.87 a	37.50 a	38.53 a	37.72
La*	−11.20 b	−13.24 a	−11.93 ab	−12.42 ab	−13.38 a	−12.43
Lb*	14.91 c	20.51 a	16.61 bc	18.51 b	21.04 a	18.31

Significancy of the differences by ANOVA Newman−Keuls method

	LL*	**La***	**Lb***
GE	*	**	***
IR	**n.s.**	**n.s.**	*
GE × IR	**n.s.**	**n.s.**	**n.s.**

***, **, and * indicate p-values ≤ 0.001, 0.01, and 0.05, respectively. Letters indicate significant differences according to the Tukey test ($p < 0.05$). n.s. represents not significant.

Table 6. Variation of days of anthesis expressed in days after transplant (DAT) in relation to the first, second, third, and fourth flowers open (Fl1, Fl2, Fl3, and Fl4, respectively).

	IR100						IR50						IR200					
	To/Bb	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**$\bar{x}$**	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**$\bar{x}$**	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**$\bar{x}$**
Fl1	65.17	62.13	51.05	74.78	53.00	61.23 a	58.58	66.50	50.71	78.04	45.63	59.89 a	56.17	70.13	52.94	86.18	52.38	63.56 a
Fl2	88.25	92.75	84.67	98.31	73.33	87.46 a	84.54	95.58	77.92	100.17	62.50	84.14 a	82.54	99.42	85.52	106.36	74.54	89.68 a
Fl3	99.08	98.04	96.20	103.73	81.63	95.74 a	94.79	93.17	78.00	102.46	70.29	87.74 b	98.46	102.88	96.23	107.03	86.72	98.26 a
Fl4	111.71	114.96	109.45	117.37	92.33	109.16 a	115.46	115.46	99.42	110.17	83.92	104.88 a	101.82	112.29	105.76	117.68	100.10	107.53 a

Means per genotype

	To/Bb	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**$\bar{x}$**
Fl1	59.97 bc	66.25 b	51.57 c	79.67 a	50.33 c	61.56
Fl2	85.11 bc	95.92 ab	82.70 c	101.61 a	70.13 d	87.09
Fl3	97.44 ab	98.03 ab	90.14 b	104.41 a	79.54 c	93.91
Fl4	109.66 ab	114.24 a	104.88 b	115.07 a	92.12 c	107.19

Significancy of the differences by ANOVA Newman-Keuls method

	Fl1	**Fl2**	**Fl3**	**Fl4**
GE	***	***	***	***
IR	**n.s.**	**n.s.**	***	**n.s.**
GE × IR	**n.s.**	**n.s.**	**n.s.**	*

*** and * indicate the *p*-values $\leq$ 0.001 and 0.05, respectively. Letters indicate significant differences according to the Tukey test ($p < 0.05$). n.s. represents not significant.

Finally, regarding the days for the anthesis of the fourth flower (Fl4), a significant interaction between the GE and the IR was observed. The values for Fl4 varied from 83.92 for Bb when grown under IR50 conditions to 117.68 for Bb/Bb when grown under IR200 conditions (Table 6). Overall, there was a significant reduction in the time for anthesis for Fl4 only in plants grown under IR50 conditions, indicating that the reduced water supply represented by IR50 accelerated the flowering process. Notably, the time for anthesis exhibited significant variations based on the specific rootstock–scion combinations for all the recorded flowering traits.

3.5. Fruit Quality Parameters and Production

Regarding the fruit chromatic parameter L* (FUL*), a significant variation was observed among the GE and the IR. FUL* values exhibited significant differences among the genotypes, ranging from 26.42 for En/Bb to 27.15 for Bb (Table 7). Similarly, FUL* values varied across different IRs, ranging from 26.47 for IR50 to 27.05 for IR200 (Table 7). The fruit chromatic parameter a* (FUa*) also displayed significant variations among the GE and the IR. In the different rootstock–scion combinations, FUa* ranged from 4.08 for Be/Bb to 5.46 for Bb. Conversely, concerning the IR, FUa* spanned from 4.28 for IR200 to 5.05 for IR50 (Table 7). In contrast, no significant variation was observed in the fruit chromatic parameter b* (FUb*) among the tested genotypes and the three irrigation regimes.

Moving to the fruit longitudinal diameter (FLD), its value exhibited significant variation across the GE and the IR. FLD values differed among the genotypes, ranging from 16.12 cm for Bb to 19.93 cm for En/Bb. In terms of the irrigation regime (IR), FLD ranged from 17.14 cm for IR50 to 18.67 cm for IR100 (Table 7). As concerns the fruit transversal diameter (FTD), it showed a significant variation among the GE and the IR. In relation to the GE, there were registered values from 7.90 cm to 9.42 cm for Bb and En/Bb. Contrarily, in relation to the IR, the FTD varied from 8.51 cm to 9.11 cm, respectively. For the fruit dry matter (FDM), a significant interaction between the GE and the IR was observed. Within this context, FDM values ranged from 6.54% to 7.47% for Be/Bb and Bb/Bb, both grown under IR50 (Table 7). Regarding the fruit soluble solid content (FSSC), its value significantly varied concerning both the GE and the IR. Among the genotypes, the FSSC ranged from 4.71 °Brix to 5.42 °Brix for Be/Bb and Bb, respectively.

Moreover, the FSSC varied with respect to the IR, ranging from 4.97 °Brix when grown under IR100 to 5.35 °Brix for IR100 (Table 7).

Concerning the fruit yield components, the number of fruits per plant (FP) exhibited significant variation due to both the genotype (GE) and the irrigation regime (IR). Among the genotypes, the FP ranged from 7.01 to 10.26 fruits per plant for Bb/Bb and To/Bb, respectively. In contrast, regarding the IR, the FP varied from 7.87 to 9.53 fruits per plant for IR200 and IR50, respectively (Table 8).

For the fruit weight (FWE), a significant interaction between the genotype and the irrigation regime was determined. Consequently, FWE values ranged from 296.71 g to 450.52 g for Bb and To/Bb, both grown under IR50 (Table 8). In fact, the enhanced optimization of irrigation water was evident for this parameter, specifically among those related to fruit yield components. More precisely, FWE exhibited the highest efficiency in converting water into fruit biomass under IR50 conditions.

Finally, the fruit production per plant (FPP) displayed significant variation associated with both the different rootstock–scion combinations (GE) and the three irrigation regimes (IR). Specifically, the FPP varied from 2286.31 g to 4407.61 g for Bb/Bb and To/Bb, respectively. Concerning the irrigation regime, the FPP ranged from 2877.53 g under IR200 to 3593.53 g under IR100 (Table 8).

Table 7. Variation of the fruit quality traits in relation to the different rootstock–scion combinations and to the irrigation regimes (IR50, IR100, and IR200). The analyzed traits were the chromatic CIEL*a*b* parameters (FUL*, FUa*, and FUb*, respectively), the fruit lateral and transversal diameter (FLD and FTD), the fruit dry matter (FDM), and the soluble solid content (FSSC).

	IR100						IR50						IR200					
	To/Bb	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**$\bar{x}$**	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**$\bar{x}$**	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**$\bar{x}$**
FUL*	26.29	26.38	26.48	26.61	27.35	26.62 b	26.35	26.45	26.28	26.48	26.82	26.47 b	26.93	26.43	26.69	27.93	27.29	27.05 a
FUa*	5.70	4.34	4.34	4.96	5.92	5.05 a	4.54	4.05	4.05	4.78	5.09	4.50 ab	4.08	4.45	3.85	3.64	5.37	4.28 b
FUb*	−0.50	−0.46	−0.33	−0.45	−0.38	−0.43 a	−0.48	−0.49	−0.41	−0.50	−0.46	−0.47 a	−0.45 a	−0.50	−0.39	−0.24	−0.30	−0.38 a
FLD	19.52	20.34	18.62	16.98	17.09	18.51 a	20.13	20.18	18.93	17.68	16.46	18.67 a	19.40	19.27	16.03	16.18	14.82	17.14 b
FTD	9.18	9.45	9.29	9.29	8.11	9.06 a	8.98	9.51	8.94	9.82	8.29	9.11 a	9.38	9.29	8.29	8.28	7.31	8.51 b
FDM	6.72	6.73	6.54	7.47	6.94	6.88 a	6.87	6.77	6.59	7.33	6.95	6.90 a	6.85	6.75	6.88	6.80	6.85	6.82 a
FSSC	5.26	5.19	4.75	5.72	5.83	5.35 a	4.98	5.03	4.61	4.89	5.35	4.97 b	4.68	4.89	4.76	5.05	5.09	4.89 b

Means per genotype

Trait	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**$\bar{x}$**
FUL*	26.52 b	26.42 b	26.48 b	27.00 ab	27.15 a	26.72
FUa*	4.77 ab	4.28 b	4.08 b	4.46 b	5.46 a	4.61
FUb*	−0.47 a	−0.48 a	−0.38 a	−0.40 a	−0.38 a	−0.42
FLD	19.68 a	19.93 a	17.86 b	16.94 bc	16.12 c	18.11
FTD	9.18 a	9.42 a	8.84 a	9.13 a	7.90 b	8.89
FDM	6.81 b	6.75 b	6.67 b	7.20 a	6.91 ab	6.87
FSSC	4.97 bc	5.04 abc	4.71 c	5.22 ab	5.42 a	5.07

Significancy of the differences by ANOVA Newman−Keuls method

	FUL*	**FUa***	**FUb***	**FLD**	**FTD**	**FDM**	**FSSC**
GE	**	***	**n.s.**	***	***	***	***
IR	**	**	**n.s.**	**	**	**n.s.**	***
GE × IR	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	*	**n.s.**

***, **, and * indicate p-values $\leq$ 0.001, 0.01, and 0.05, respectively. Letters indicate significant differences according to the Tukey test ($p < 0.05$). n.s. represents not significant.

Table 8. Variation of the yield components in relation to the different rootstock–scion combinations and to the different irrigation regimes (IR50, IR100, and IR200). The analyzed parameters were the number of fruits per plant (FP), the fruit weight (FWE), and the fruit production per plant (FPP), both expressed in g plant^{-1}.

	IR100						IR50						IR200					
	To/Bb	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**$\bar{x}$**	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**$\bar{x}$**	**To/Bb**	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**$\bar{x}$**
FP	10.70	10.66	9.38	7.47	9.46	9.53	10.46	9.41	9.04	7.40	7.71	8.80	9.63	7.68	8.08	6.17	7.79	7.87
FWE	417.25	384.30	432.21	313.01	320.65	373.48	450.52	426.10	439.71	338.11	296.71	390.23	422.00	384.14	365.19	323.30	304.16	359.76
FPP	4458.49	4098.06	4029.39	2344.22	3037.47	3593.53 a	4694.87	4027.11	3961.86	2500.62	2275.45	3491.98 a	4069.48	2962.44	2950.52	2014.09	2391.10	2877.53 b

Means per genotype

	To/Bb	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	**$\bar{x}$**
FP	10.26 a	9.25 ab	8.83 ab	7.01 c	8.32 bc	8.73
FWE	429.92 a	398.18 b	412.37 ab	324.81 c	307.17 c	374.49
FPP	4407.61 a	3695.87 b	3647.26 b	2286.31 c	2568.01 c	3321.01

Significancy of the differences by ANOVA Newman-Keuls method

	FP	**FWE**	**FPP**
GE	***	***	***
IR	**	**	***
GE × IR	**n.s.**	**	**n.s.**

*** and ** indicate p-values $\leq$ 0.001 and 0.01. Letters indicate significant differences according to the Tukey test ($p < 0.05$). n.s. represents not significant.

3.6. Water Use Efficiency for the Fruit Production

The water use efficiency (WUE) ranged in IR100 from 25.26 kg m^{-3} to 48.04 kg m^{-3} for Bb/Bb and To/Bb, respectively (Table 9). On the other hand, in IR50, the WUE spanned from kg m^{-3} to 49.04 kg m^{-3} to 101.18 kg m^{-3} for Bb and To/Bb, respectively. Finally, as concerns IR200, the WUE varied from 10.84 kg m^{-3} to 21.90 kg m^{-3} (Table 9).

Table 9. Water use efficiency (WUE), expressed in kg m^{-3}, calculated for fruit production (FPP). It was calculated through the ratio between the fruit production per plant (kg) and the liter of irrigation water (IW), expressed in m^3.

	To/Bb	En/Bb	Be/Bb	Bb/Bb	Bb	$\bar{x}$
IR100	48.04	44.16	43.42	25.26	32.73	38.72 b
IR50	101.18	86.79	85.38	53.89	49.04	75.26 a
IR200	21.90	15.94	15.88	10.84	12.87	15.49 c
Means per genotype						
	To/Bb	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	$\bar{x}$
WUE	57.04 a	48.96 b	48.23 b	30.00 c	31.55 c	43.15
Significancy of the differences by ANOVA Newman-Keuls method						
				WUE		
GE				***		
IR				***		
GE × IR				***		

*** indicates p-value ≤ 0.001. Letters indicate significant differences according to the Tukey test ($p < 0.05$).

3.7. Water Potential of the Leaves

The leaf water potential (WP) exhibited significant variation among the three irrigation regimes (IR) employed. Additionally, a significant interaction between the genotype (GE) and the temperature at the assessment moment (AT) was observed. In terms of the IR, WP values ranged from −4.49 MPa for IR100 to −5.80 MPa for IR50 (Table 10). Furthermore, as a result of the GE × AT interaction, the WP varied from −0.98 MPa for En/Bb at A3i to −14.02 MPa for Bb/Bb at A2h (Table 10).

3.8. Pearson's Correlation among Traits

As a result of the Pearson's correlation analysis, the most correlated traits were plant height (PH), fruit weight, and production (FWE and FPP, respectively). Specifically, for the PH, a robust positive correlation with 12 traits was detected, which were FWE, FPP, PBDS, La*, FP, PDGP, PBDR, and FLD (Table 11). On the other hand, the PH was negatively correlated with FDM, FSSC, Lb*, and WP. In addition, the fruit production per plant (FPP) showed a strong correlation with 13 traits (Table 10). Specifically, it exhibited a positive correlation with FP, FEW, FLD, PH, PBDS, PDGP, PBDR, FTD, and La*. Conversely, the FPP exhibited a negative correlation with FUL*, Lb*, FDM, and FUb*. Finally, the FWE was correlated with a total of 14 traits. Within this context, it displayed a positive correlation with FPP, PH, FLD, PDGP, FP, PBDR, La*, PBDS, and FTD. Conversely, the FWE was negatively correlated with Lb*, FUL*, FDM, WP, and FSSC (Table 11).

3.9. PCA Analysis

The first component extracted (PC1) was positively correlated with PH, PBDR, FWE, FPP, PDGP, FLD, FP, and FTD. On the other hand, it showed a negative correlation with FDM, FSSC, Lb*, and FUL* (Table 12). Moreover, the second component extracted (PC2) was positively correlated with WP and FUa*, while it was negatively correlated with FUb*. As concerns the third component (PC3), it exhibited a strong positive correlation with La* and PBDS and a negative correlation with LL* (Table 12).

Table 10. Variation of the water potential (WP) expressed in -MPa in relation to the different rootstock–scion combinations, to the different irrigation regimes (IR100, IR50, and IR200), and to the temperature during the assessment (AT). The WP was registered at cold, hot, and intermediate temperatures (A1c, A2c, and A3i, respectively).

	AT	To/Bb	En/Bb	Be/Bb	Bb/Bb	Bb	$\bar{x}$
IR100	**A1c**	3.90 ± (0.55)	2.19 ± (0.46)	2.24 ± (0.84)	3.95 ± (0.90)	4.09 ± (0.33)	3.27 ± (0.97)
	A2h	10.20 ± (2.75)	7.38 ± (2.56)	4.80 ± (2.17)	14.58 ± (3.35)	15.03 ± (2.49)	10.40 ± (4.45)
	A3i	2.68 ± (0.92)	1.60 ± (0.82)	1.05 ± (0.26)	2.10 ± (0.18)	3.50 ± (2.42)	2.19 ± (0.95)
$\bar{x}$		5.59 ± (4.04) c	3.72 ± (3.18) d	2.70 ± (1.92) e	6.88 ± (6.74) b	7.63 ± (6.65) a	5.29 ± (4.46) b
IR50	**A1c**	4.59 ± (1.00)	2.65 ± (1.12)	2.86 ± (1.07)	4.53 ± (1.18)	5.10 ± (0.67)	3.95 ± (1.11)
	A2h	8.73 ± (4.25)	13.68 ± (2.00)	7.30 ± (4.44)	13.43 ± (6.92)	12.13 ± (6.54)	11.05 ± (2.88)
	A3i	5.5 ± (1.36)	4.5 ± (2.45)	1.25 ± (0.21)	4.68 ± (1.96)	5.53 ± (4.05)	4.29 ± (1.76)
$\bar{x}$		6.27 ± (2.18) b	6.94 ± (5.91) ab	3.80 ± (3.13) c	7.55 ± (5.10) a	7.59 ± (3.94) a	6.43 ± (4.00) a
IR200	**A1c**	3.76 ± (0.54)	1.75 ± (0.35)	1.64 ± (0.35)	4.10 ± (0.58)	4.00 ± (0.48)	3.05 ± (1.24)
	A2h	11.93 ± (1.15)	11.00 ± (2.28)	7.43 ± (4.25)	14.08 ± (1.25)	10.95 ± (3.43)	11.08 ± (2.40)
	A3i	3.23 ± (1.07)	1.95 ± (1.23)	0.63 ± (0.22)	2.8 ± (1.12)	2.78 ± (1.11)	2.28 ± (1.03)
$\bar{x}$		6.30 ± (4.78) b	4.9 ± (5.28) d	3.23 ± (3.67) e	7.23 ± (6.59) a	5.91 ± (4.41) c	5.47 ± (4.87) b
Means per genotype							
		To/Bb	**En/Bb**	**Be/Bb**	**Bb/Bb**	**Bb**	$\bar{x}$
A1c		4.08 ± (0.44)	2.25 ± (0.45)	2.20 ± (0.62)	4.19 ± (0.30)	4.40 ± (0.61)	3.53 ± (1.02) b
A2h		10.28 ± (1.60)	6.51 ± (3.16)	10.68 ± (1.48)	14.02 ± (0.58)	12.70 ± (2.10)	10.75 ± (2.57) a
A3i		3.8 ± (1.50)	0.98 ± (1.58)	2.68 ± (0.32)	3.19 ± (1.33)	3.93 ± (1.43)	3.06 ± (1.13) b
$\bar{x}$		6.05 ± (3.66) a	3.24 ± (2.90) c	5.18 ± (4.76) b	7.13 ± (5.98) a	7.01 ± (4.93) a	5.78 ± (4.31)
Significancy of the differences by ANOVA Newman Keuls method							
	GE	**IR**	**AT**	**GE × IR**	**GE × AT**	**IR × AT**	**GE × IR × AT**
WP	***	*	***	**n.s.**	***	**n.s.**	**n.s.**

Numbers in brackets represent the standard deviation. *** and * indicates p-value $\leq$ 0.001 and 0.05, respectively. Letters indicate significant differences according to the Tukey test ($p < 0.05$). n.s. represents not significant.

The PCA plot allowed the distribution of the genotypes in the three dimensions. Within this context, four groups (A, B, C, and D) were well distinguished based on their interactions with the PCA axes, which are represented by the three extracted components PC1, PC2, and PC3. Group A encompasses the combination of Bb/Bb and Bb grown under IR50 and IR100. Particularly, these combinations differ for the higher values registered for the yield components (FP, FWE, and FPP) in comparison to the counterpart cultivated at IR200 (Figure 2). Conversely, group B included the genotypes showing the highest yield, which were all the combinations encompassing To/Bb and En/Bb. Furthermore, group C is represented by the combination Be/Bb grown in all the irrigation regimes (IR), which is well clustered due to its highest values of plant diameter at the grafting point (PDGP). Finally, group D was composed of the non-grafted and self-grafted genotypes grown by IR200 (Figure 2). This group was characterized by the worst agronomic performance recorded.

PC1 summarized the 44.452% of the total phenotypic variability. As a result, combinations To/Bb (C), En/Bb (D), and Be/Bb (E) under IR50, IR100, and IR200 were positioned along the first axis. This was due to their high values in vegetative traits such as PH and PBDR, as well as production-related traits like FWE, FPP, FLD, FP, and FTD. In contrast, the second component represented 16.396% of the total variability. Within this context, group B included the non-grafted Bb and the self-grafted Bb/Bb, both grown under IR200. Notably, this group was characterized by the lowest values of FPP. Group A was characterized by high values of WP and FUa* (Figure 2).

Table 11. Pearson's correlation among the evaluated traits.

	PH	PBDR	PBDS	PDGP	LL*	La*	Lb*	FUL*	FUa*	FUb*	FLD	FTD	FDM	FSSC	FP	FWE	FPP	WP
PH	1.000																	
PBDR	**0.542** *	1.000																
PBDS	**0.739** **	0.157	1.000															
PDGP	**0.544** *	**0.906** **	0.203	1.000														
LL*	−0.114	0.183	−0.281	−0.038	1.000													
La*	**0.684** **	0.055	**0.687** **	0.248	−0.256	1.000												
Lb*	**−0.630** **	−0.093	**−0.693** **	−0.312	0.434	**−0.835** **	1.000											
FUL*	−0.397	**−0.548** *	−0.352	**−0.599** **	−0.017	−0.191	0.285	1.000										
FUa*	−0.274	**−0.515** *	0.088	**−0.674** **	0.382	−0.127	0.211	0.027	1.000									
FUb*	0.023	−0.099	−0.100	−0.202	0.039	0.053	0.059	**0.767** **	−0.172	1.000								
FLD	**0.469** *	**0.457** *	0.182	**0.585** *	−0.055	0.392	**−0.456** *	**−0.679** **	−0.208	**−0.636** **	1.000							
FTD	0.183	0.314	−0.015	0.562 *	−0.099	0.260	−0.380	**−0.677** **	−0.278	**−0.670** **	**0.771** **	1.000						
FDM	**−0.764** **	**−0.641** **	−0.317	**−0.478** *	−0.123	−0.297	0.202	0.093	0.287	−0.245	−0.363	0.104	1.000					
FSSC	**−0.645** **	**−0.553** *	−0.316	**−0.629** **	0.390	−0.332	0.270	0.274	**0.704** **	−0.039	−0.258	−0.243	**0.494** *	1.000				
FP	**0.566** *	0.314	**0.482** *	0.294	−0.255	0.254	**−0.479** *	**−0.640** **	0.025	**−0.552** *	**0.706** **	0.344	−0.409	−0.202	1.000			
FWE	**0.839** **	**0.622** **	**0.580** *	**0.726** **	−0.132	**0.588** *	**−0.660** **	**−0.659** **	−0.314	−0.326	**0.791** **	**0.545** *	**−0.608** **	**−0.498** *	**0.694** **	1.000		
FPP	**0.760** **	**0.479** *	**0.579** *	**0.534** *	−0.225	**0.475** *	**−0.632** **	**−0.688** **	−0.144	**−0.469** *	**0.815** **	0.477 *	**−0.547** *	−0.369	**0.922** **	**0.917** **	1.000	
WP	**−0.554** *	**−0.845** **	−0.280	**−0.762** **	−0.203	−0.121	0.112	0.339	0.374	−0.203	−0.162	−0.072	**0.604** **	**0.491** *	−0.060	−0.563 *	−0.316	1.000

**, and * indicate p-values $\leq$ 0.01, and 0.05, respectively.

Table 12. Matrix of the three components extracted for the principal component analysis (PCA).

Trait	PC1	PC2	PC3
PH	0.842	−0.277	0.306
PBDR	0.703	−0.367	−0.529
PBDS	0.588	−0.002	0.621
PDGP	0.797	−0.259	−0.467
LL*	−0.198	0.021	−0.378
La*	0.607	0.000	0.620
Lb*	−0.669	−0.125	−0.576
FUL*	−0.691	−0.444	0.351
FUa*	−0.401	0.555	0.301
FUb*	−0.327	−0.792	0.344
FLD	0.791	0.446	−0.207
FTD	0.560	0.491	−0.385
FDM	−0.610	0.495	−0.045
FSSC	−0.621	0.513	0.090
FP	0.686	0.430	0.167
FWE	0.972	0.055	0.060
FPP	0.894	0.273	0.151
WP	−0.647	0.502	0.217
Variance (%)	44.542	16.396	13.939

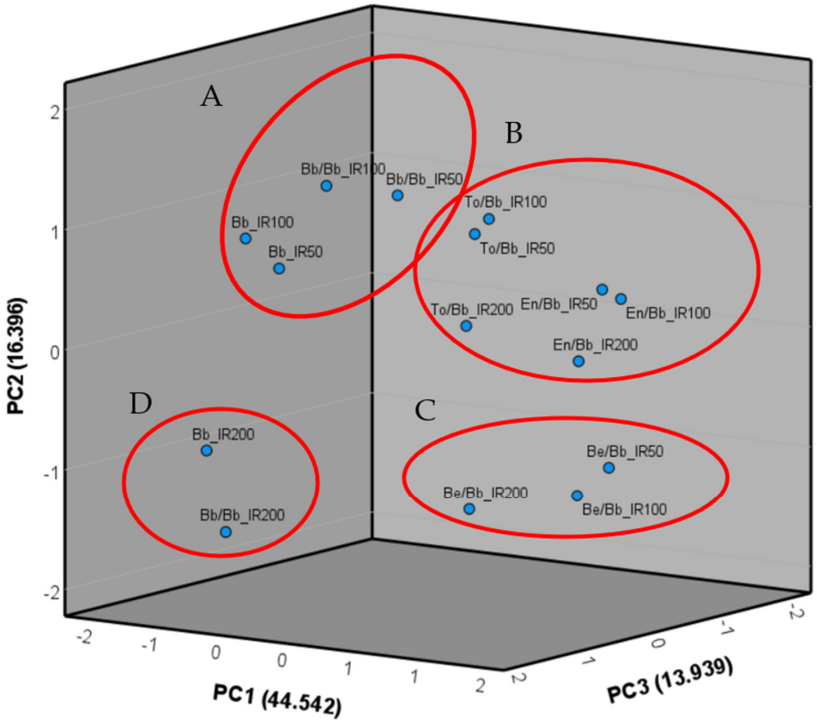

Figure 2. PCA plot showing the genotypes distribution based on the three axes, which are represented by PC1, PC2, and PC3. The different rootstock–scion combinations, along with their respective irrigation regimes, were grouped into four categories (A, B, C, and D). Group A consisted of the self-grafted and non-grafted eggplant cultivar Black Bell, grown under both ordinary and halved irrigation regimes (IR100 and IR50, respectively). Group B included the most promising combinations in terms of yield, cultivated under all irrigation regimes (To/Bb and En/Bb). Group C comprised the Beaufort F1 rootstock grown under all irrigation regimes, characterized by grafting incompatibility with the scion Black Bell. Finally, Group D encompassed the least favorable combinations in terms of agronomic performance, consisting of non-grafted and self-grafted Black Bell plants grown with double the usual water volume (IR200).

4. Discussion

The primary focus of this research was to determine the optimal rootstock–scion combination in response to varying irrigation levels. In line with this objective, this study aimed to identify the most effective combination capable of thriving with half the usual amount of water, enhancing water use efficiency (WUE) in agriculture, and ensuring optimal agronomic performance. Within this context, the combinations evaluated included the rootstock hybrids F1 Beaufort and Energy, which were already evaluated for their agronomic performance [38,39]. Beaufort F1 showed a robust agronomic performance but a low affinity when grafted with eggplant. On the other hand, Energy F1 showed less vigor in comparison to the interspecific rootstock but high mineral uptake.

In our study, the combination of the Black Bell cultivar grafted onto the Beaufort F1 rootstock (Be/Bb) exhibited the highest vegetative growth, as evidenced by parameters like plant height (PH), rootstock basal diameter (PBDR and PBDS), and grafting point diameter. This outcome aligns with previous studies where the Beaufort F1 rootstock has typically shown incompatibility with various eggplant cultivars, resulting in excessive vegetative development [40–42]. Conversely, the combination of the *S. torvum* rootstock with the Black Bell scion (To/Bb) displayed substantial vegetative traits, particularly a notable increase in scion basal diameter (PBDS). This can be attributed to the strong compatibility between *S. torvum* tissues, ensuring an efficient vascular connection capable of transferring nutrients and water without causing grafting imbalances [43–45].

The combination involving the self-grafted combinations consistently exhibited low values of plant height. This can be attributed to the longer tissue regeneration time of self-grafted plants compared to non-grafted ones. Specifically, self-grafted plants use a rootstock typically chosen for its production-related characteristics. Consequently, after the grafting stress, they may have greater difficulty in regenerating tissues and especially in forming a robust root system. In contrast, F1 hybrid rootstocks like Beaufort, Energy, and *S. torvum* have been selected precisely for their strong adaptability, facilitated by their powerful root systems. Within this context, in line with previous studies [46,47], it can be postulated that self-grafted plants require a prolonged adaptation period to achieve the same level of growth performance as their non-grafted counterparts. As a result of these hypotheses, self-grafted plants probably require more time to mitigate the issue of the grafting activity and to regenerate tissues at the grafting point. Building upon our thesis, in a previous work [48], the self-grafted combinations showed the lowest fruit yield in comparison to the self-rooted and the grafted plants.

It is noteworthy that the non-grafted Black Bell (Bb) exhibited the highest values of plant basal diameter of the scion (PBDS) at 21 and 42 days after transplanting (DAT). The larger stem diameter in non-grafted Bb can be attributed to the absence of stress caused by grafting. Conversely, Bb/Bb exhibited lower PBDS values due to self-grafting. However, after this initial period, PBDS in Bb was surpassed by other rootstock–scion combinations, as they overcame the grafting stress and exhibited greater growth. Furthermore, PBDS significantly increased in the To/Bb combination from 21 to 84 DAT, possibly due to successful grafting compatibility, promoting optimal vegetative growth. In contrast, plants grafted onto Energy and Beaufort F1 rootstocks displayed limited scion growth despite a large diameter at the grafting point (Figure 1). This could be attributed to the vigorous water uptake by the Beaufort rootstock, resulting in phenomena such as guttation and vitrescence, which we observed in Be/Bb.

Regarding the impact of different irrigation protocols on plant height, our study revealed minimal differences between plant heights under IR50 and IR100, which aligns with the findings of a previous study [49]. This minimal height difference can be attributed to the development of a robust root system capable of reaching deeper soil layers, ensuring sufficient water and nutrient absorption. In contrast, the excessive water content in the rhizosphere under IR200 may hinder root development, resulting in shorter plants. Similar trends were observed for basal diameters (PBDR and PBDS), which showed no significant

variations between deficitary (IR50) and normal (IR100) irrigation, as also noted by previous research [50].

Concerning flowering time, we observed a significant reduction in non-grafted plants compared to the self-grafted ones, which exhibited a considerable delay in flowering. Additionally, we noticed a significant reduction in flowering time for the deficitary irrigation regime IR50, as compared to IR100 and IR200. This can be attributed to the reduced water in the flower tissue that led the flower to a faster fruit-setting process. The significant reduction in flowering time for IR50 could have substantial advantages in terms of early fruit production.

Regarding fruit quality traits, grafted plants produced longer fruits compared to the non-grafted ones. This is likely due to grafting enabling a more efficient transport of water and nutrients to the reproductive organs. Specifically, the combinations with the *S. torvum* and Energy F1 rootstocks (To/Bb and En/Bb) showed the highest values for both fruit longitudinal and transversal diameters (FLD and FTD, respectively). However, there was no significant variation in FLD and FTD between the IR50 and IR100 conditions. Reduced values were observed only for IR200, which could be attributed to oxygen deprivation in plants grown under IR200, where the water regime was doubled compared to normal conditions. Oxygen deprivation is typically induced by waterlogging stress and significantly affects physiological and developmental processes, ultimately impacting biomass production [51,52].

Regarding the soluble solid content (FSSC), non-grafted plants exhibited significantly higher values. This could be attributed to the lower water uptake capacity of the non-grafted root system compared to the rootstock's, which might result in a dilution effect on the sugar content in fruits. Surprisingly, we did not observe an increase in FSSC under IR50, as the deficitary water regime we applied did not induce stress during the fruit-setting process.

Furthermore, the number of fruits per plant (FP) significantly varied among the different grafting combinations. Notably, the combination with *S. torvum* rootstock displayed the highest FP value. This higher FP value can be attributed to the strong compatibility between S. melongena grafted onto *S. torvum* rootstock, as supported by various studies [42,44,53]. Additionally, under IR50 conditions, we observed the highest FP value, which is likely due to the reduced vegetative growth of IR50 plants, which addressed a faster transition to the reproductive stage.

The trend in fruit weight (FWE) mirrored that of the number of fruits per plant (FP), with the combination involving *S. torvum* rootstock registering the highest value. Notably, the highest FWE value was observed under normal irrigation conditions (IR100), exhibiting a significant difference from both IR50 and IR200 conditions. This difference can be attributed to the enhanced water and nutrient uptake facilitated by grafting compatibility.

Regarding water use efficiency (WUE), we noticed a significant variation due to the different irrigation regimes applied. Interestingly, all combinations subjected to reduced irrigation (IR50) displayed the highest WUE values. This characteristic led to a significant reduction in water uptake from the soil, but there was minimal impact on fruit production per plant compared to the control (IR100). Among these combinations, To/Bb exhibited the highest WUE value, which is possibly attributed to the strong compatibility between the rootstock and scion, optimizing nutrient and water conversion into fresh produce. Additionally, the robust root system of the rootstock allowed for more effective exploration of soil layers, enhancing water absorption.

Conversely, our research revealed a notable decrease in WUE values for plants cultivated with double the amount of water (IR200). This can be attributed to the challenges plants face in water absorption due to reduced oxygen availability in waterlogged conditions.

Regarding the physiological analysis of water potential (WP), the highest values observed at A2h suggest that plants were transitioning to the reproductive phase, with resources being allocated to reproductive organs. Furthermore, this value was recorded

when plants were under stress, as this assessment was conducted within a temperature range of 27 °C to 32 °C.

Notably, Bb/Bb exhibited the highest water potential values at A2h DAT, while the grafting combinations with the rootstocks Energy F1 and *S. torvum* showed lower values due to their better grafting compatibility. Conversely, lower water potential values were recorded at A3i DAT, likely reflecting the differentiation of floral buds, as confirmed by flower observations. On the other hand, the higher water potential in IR50 may be explained by the increased pressure required for water to exit the leaves. This higher pressure requirement can be attributed to the reduced water content inside the leaf tissue, which is possibly related to the plants' efforts to support reproductive growth.

In light of these findings, it becomes imperative to conduct additional assessments that include the modulation of substrate temperature. This is necessary to overcome the limitation posed by the grafting incompatibility observed with the rootstocks F1 Energy and Beaufort. These particular rootstocks resulted in an excessive growth of the Black Bell cultivar used as the scion in the experiment.

5. Conclusions

The utilization of grafting techniques to ensure the application of the appropriate water volume for supporting plant growth and development processes has been strongly associated with the improvement of water use efficiency (WUE). In the current study, the significant affinity between *S. torvum* used as a rootstock and the eggplant cultivar Black Bell used as a scion was confirmed. This affinity was consistent across all irrigation regimes applied. Importantly, we observed a significant interaction between the rootstock–scion combinations and the irrigation regime, particularly in relation to water use efficiency (WUE) concerning the fruit production per plant. Notably, the highest WUE was observed under the IR50 irrigation regime, which involved replenishing 50% of evapotranspiration. Furthermore, significant differences in WUE were also observed among the various rootstock–scion combinations, with lower values for the non-grafted and self-grafted plants. It is worth highlighting that this study serves as a foundation for developing new strategies to enhance water use efficiency, especially in regions with limited water resources, where rootstocks can play a crucial role in accessing water from deeper soil layers.

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The Effect of Water Stress on the Glucosinolate Content and Profile: A Comparative Study on Roots and Leaves of *Brassica oleracea* L. Crops

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Abstract: Drought is one of the major challenges of global crop production, and its severity is increasing because of climate change. This abiotic stress is an important target for *Brassica* species, which are generally grown in arid and semi-arid climates. This study was conducted to investigate the effects of water deficit on a set of accessions belonging to the *Brassica* core collection of the EU H2020 BRESOV project, represented by *Brassica oleracea* L. crops and *Brassica oleracea* complex species (n = 9). In particular, the variation in the amount and profile of the glucosinolates (GLSs) compounds was analyzed on the root and the leaf tissues. The plant morphometric traits and GLSs amount and profile were detected for the plants grown in cold greenhouse in Catania (Sicily) during the autumn–winter season for ten weeks. The results showed a wide qualitative and quantitative variation among the *Brassica* accessions. The GLSs profile varied qualitatively and quantitively among both genotypes and portions of the plants (hypogenous-root and epigeous-leaf). Plants grown under drought stress, for the last two weeks of the growing cycle under consideration, showed a higher amount of GLS in their leaves (190.1 ± 8.9 μmol·g$^{-1\ d.w.}$) compared to their roots (17.3 ± 1.9 μmol·g$^{-1\ d.w.}$). Under water stress conditions, the highest increase in the glucosinolate amount was detected in broccoli (the accession BR1) with 85.4% and in cauliflower (the accession CV1) with 72.8% in the roots and leaves, respectively. Positive correlations were found between the major leaf and root GLSs identified. The selection of chemotypes allows for an important time reduction during the breeding programs after crossing accessions with the specific profiles of glucosinolates.

Keywords: drought stress; *B. oleracea* complex species (n = 9); glucosinolates; morphometric traits

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1. Introduction

Owing to world population growth and the rise of food security risk, global concerns for endangered water and land resources are increasing. The availability of water is one of the most common environmental factors that has a great impact on plant growth and on vegetable crop productivity [1]. Therefore, it is critical to understand how crops respond to water stress and what steps are possible to adopt for improving their drought tolerance [2]. In fact, species can adapt themselves to environmental change through different strategies, varying from extinction to resilience. On the other hand, plants may mitigate ongoing climate change by modifying their morphological and physiological traits [3]. It is important to note that the Mediterranean basin is most sensitive and hence more vulnerable to climate

change with longer and warmer summers, more frequent and severe heat waves, altered precipitation patterns, as well as less rainfall [4]. In addition, a complex of botanical species of agricultural and nutritional importance originated in the Mediterranean region due to the mild winter climate and to the fertility of its soils [5]. Sicily, due to its geographic isolation, is an important source of biodiversity for many *Brassica oleracea* L. crops for the genetic flux among them and for several populations of *B. oleracea* complex species ($n = 9$) which represent their wild relatives. In fact, *B. oleracea* crops are represented in Sicily by several varietal groups that are distinguished by various morphometric, biochemical, and genetic traits [6]. Nevertheless, before starting any plant breeding effort, each breeder must answer the crucial question: what are the detrimental consequences of abiotic stresses (i.e., drought, salinity, and water logging) on the crop that should be resolved?

B. oleracea crops, similar to other agricultural plants, are affected by both abiotic and biotic stressors which stimulate different organs to accumulate higher amounts of primary and secondary metabolites to boost their resistance [7]. Drought stress is an environmental stress that can cause physiological, biochemical, and agronomic damage to plants, such as less turgor, lower crop productivity, and reduced plant height and weight [8]. It can also affect the quality of the crops [9]. In addition, transpiration, the absorption of ions, carbohydrates, nutritional assimilation, and growth promoters are damaged as a result of arid conditions [10]. Water deficiency can produce an increase in reactive oxygen species (ROS) [11] and cause morphological and anatomical changes in the roots and leaves of many plant species [12]. In general, drought conditions can reduce the photosynthetic rate, accelerate the senescence of the leaves, but can also trigger an oxidative burst, accelerate the degradation of photosynthetic pigments, and damage the cell membrane by inducing the expression of antioxidant enzymes [13]. However, water deficiency causes the accumulation of proline, which is considered a compatible solute that protects cellular structures and maintains the pressure of turgor. Several studies have shown how the accumulation of proline increases the resistance of plants against numerous environmental conditions, activating antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) [14]. Water deficiency also affects the amount and profile of the metabolites present in the crops. Among the secondary metabolites that are affected by water, glucosinolates (GLSs) have received high attention as a bioactive compound mainly found in *B. oleracea* crops and in general of the species belonging to the *Brassicaceae* family, recognized for their distinctive benefits for human nutrition and plant defense.

Glucosinolates (GLSs) are commonly used as chemical markers in chemotaxonomy. Their distribution in *B. oleracea* crops is known to vary among crops and landraces and are very diversified for different populations of the *B. oleracea* complex species ($n = 9$) [15]. Many key roles in different physiological processes have been attributed to GLSs and their breakdown products (mainly isothiocyanates and nitriles), such as auxin signaling [16], flowering time [17], stomatal closure [18], water transport [19], environmental adaptations [20], plant stress alleviation, and growth–defense balance [21]. GLSs can be classified in three different groups depending on the amino acid from which their biosynthesis starts; aliphatic compounds are derived from one of the following amino acids: alanine, valine, leucine, isoleucine, and methionine, the indolic ones derive from tryptophan, and the aromatic ones derive from phenylalanine or tyrosine. The GLSs compounds are synthesized through a specific metabolic pathway that is influenced by different factors. The biosynthesis of glucosinolates involves three phases: chain elongation of selected precursor amino acids, formation of the glucosinolates structure, and secondary modifications of the amino acid side chain [22]. In the first step, for glucoraphanin (GRA), for example, aliphatic glucosinolates derived from methionine, the ELONG gene, regulates the elongation of the methionine chain. The pathway of methionine is regulated by the MYB28 transcription factor. CYP79F1, CYP83A1, and UGT74B1 genes regulate the formation of desulfo-glucosinolates which are further catalyzed into aliphatic glucosinolates by ST5b [23]. FMOGS-OX1 catalyzes glucoerucin into glucoraphanin, which is converted to gluconapin by AOP2 [24]. The biosynthesis of GLSs in plants has received much attention, and the accumulation and

profile of GLSs in plants are largely determined by genetics, but environmental and developmental variables also play an important role [25]. The aliphatic GLSs content is highly heritable and varies among *Brassica* species and cultivars. Indolic GLSs, on the other hand, are present in great quantities in *Brassica* vegetables, although their levels are influenced not only by environmental factors but also by growing, harvesting, and processing conditions [26]. Actually, great diversity was detected in both the amount and profile of GLSs for *Brassica oleracea* L. crops in comparison to *B. rapa* ones, for which the genetic diversity of the GLSs profile is extremely narrow. Similarly, each crop is distinguishable by the detection of similar major and minor glucosinolates [27]. Sinigrin, glucobrassicin, and glucoiberin have been identified as the major GLSs in kales and cabbages, while in broccoli, common GLSs are aliphatic glucoraphanin, indolic glucobrassicin, and neoglucobrassicin [28]. Roots showed the highest diversity and content of individual GLSs due to the complicated and stressful rhizosphere [29].

The profile and amount of GLSs are affected by abiotic and biotic stress. Stressors such as drought or the salinity concentration affect the GLSs concentration, determining their increase in stressed plants [30]. The irrigation frequency and amount of water and salinity provided to the plants can affect the GLSs concentration in different tissues and organs, as reported by Chorol et al. (2021) [31,32]. High levels of temperature increased the GLSs concentration in the plants throughout the growing cycle, according to Velasco et al. (2007) [33], whereas low temperatures reduced the GLSs content. Moreover, Ciska et al. (2000) [34] confirmed that high temperatures significantly increased the glucosinolate content of various Brassica plants. The aliphatic glucosinolate gluconasturtiin content is influenced by the photoperiod [35]. The amount of gluconasturtiin increased by about 30–40% for the plants cultivated during photoperiodic conditions of long days rather than for plants grown during short days. Plants cultivated at temperatures between 10 and 15 °C increased the amount of gluconasturtiin by about 50% more than plants grown at the same day length at 20–25 °C [36].

The tolerance to water stress observed in the different varieties studied is associated with the content of specialized metabolites that can serve not only as a defense mechanism against environmental stressors but also as a source of nutritional compounds for human health. We hypothesize that the accumulation (quantity) and the profile (type) of the glu-cosinolate depends on the genotype and the organ studied. The aim of this study is to investigate the GLSs variations in the roots and leaves of seventeen accessions of *Brassica oleracea* landraces (LRs) and a composite cross population (CCP) in relation to water stress practices to identify differences due to genetic and environmental factors (abiotic stress).

The detection of the GLSs concentration and profile in different crops of *Brassica oleracea* L. and the correlation analysis between the individual GLSs in relation to water stress could highlight the impact of stress factors on GLSs biosynthesis. Moreover, a chemotaxonomy approach based on GLSs composition was developed by calculating the molar percent of the different glucosinolates composition for each accession, further comparing the profile with and without water stress. Thus, comparing genotypes in a specific environment for evaluating and identifying the variation in the plant morphometric and biochemical traits allows for the individuation of the elite breeding lines for further breeding programs.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Brassica oleracea landraces (LRs) and one set of some *B. oleracea* composite cross populations (CCP, *Brassica oleracea* L. var. cross), established in the frame of the EU H2020 BRESOV project, are represented by the landraces of cauliflower (*Brassica oleracea* L. var. *botrytis*), broccoli (*Brassica oleracea* L. var. *italica*), and kale (*Brassica oleracea* L. var. *acephala*), and by the CCP F1 and F2 populations. The experimental design adopted was split by plot with two experimental factors: the first was the irrigation regime (IR), while the second was represented by the genotype (GE), and each thesis was replicated three times with ten plants for each elementary plot. The accession list included three accessions of kale (BH1-BH2-BH3), five

of broccoli (BR1-BR2-BR3-BR4-BR5), five of cauliflower (CV1-CV2-CV3-CV4-CV5), and four composite cross populations (CCP1-CCP2-CCP3-CCP4) (Supplementary Data Table S1). All the tested accession belonged to the *Brassica* collection of the Department of Agriculture, Food and Environment (Di3A) of the University of Catania (UNICT). Seeds were sown in cellular trays using organic substrate (Terri Bio, "Agro-Chimica S.p.", Bolzano, Italy) and placed under cold greenhouse conditions on the experimental farm of the University of Catania (Di3A) (south Italy 37°31′, 37°31′10″ N 15°04′18″ E; under natural light) at the beginning of the month of September. After one month, the plantlets were transplanted into 0.3 L pots filled with the same substrate utilized for the sowing. Four weeks after transplanting, we separated all the grown plants into two plots: the irrigated (IRR) as the control and the not irrigated (NIR). The IRR plants were irrigated until they reached the field capacity, whereas the NIR ones were not irrigated. After two weeks of drought stress, the plants were collected for registering the morphometric and biochemical traits. The leaves and roots samples were gently washed and dried and stored at −80 °C for one week before freeze-drying them for biochemical analysis. All the examined traits are listed in Table 1.

Table 1. Morphological and biochemical descriptors and their corresponding units.

Code	Descriptors
PW	Plant weight (g)
PH	Plant height (cm)
SD	Stem diameter (mm)
NL	Number of leaves (n)
SPAD	SPAD (0–99.9)
RW	Root weight (g)
MRL	Main root length (cm)
SIN	Singrin ($\mu mol \cdot g^{-1\ d.w.}$)
GRA	Glucoraphanin ($\mu mol \cdot g^{-1\ d.w.}$)
GNA	Gluconapin ($\mu mol \cdot g^{-1\ d.w.}$)
GER	Glucoerucin ($\mu mol \cdot g^{-1\ d.w.}$)
GBN	Glucobrassicanapin ($\mu mol \cdot g^{-1\ d.w.}$)
GAL	Glucoalyssin ($\mu mol \cdot g^{-1\ d.w.}$)
GBS	Glucobrassicin ($\mu mol \cdot g^{-1\ d.w.}$)
NGBS	Neoglucobrassicin ($\mu mol \cdot g^{-1\ d.w.}$)
SIB	Sinalbin ($\mu mol \cdot g^{-1\ d.w.}$)
GST	Gluconastrutiin ($\mu mol \cdot g^{-1\ d.w.}$)
GLST	Total GLSs amount ($\mu mol \cdot g^{-1\ d.w.}$)

2.2. Morphometric Traits

The characterization of the plants was done using the International Descriptors IBPGR (International Board for Plant Genetic Resources) and UPOV (the International Union for the Protection of New Varieties of Plants) morphological descriptors. The plant weight (g) and height (cm), stem diameter (mm), number of leaves (n), root fresh/dry weight (g), and main root length (cm) were registered among the main morphometric traits. The Single Photon Avalanche Diode (SPAD) was utilized for detecting the nutritional status of the plant and the SPAD index was utilized for three fully developed leaves for all the plants of each replicate, using a portable chlorophyll meter SPAD-502 (Minolta Camera Co., Osaka, Japan).

2.3. Glucosinolates Extraction

In the leaves (L) and the roots (R) collected, we detected the GLSs total amount and their profile. The extraction method of the GLSs was based on the International Standard Method ISO 9167-1, 1992 [37], which the European Commission has formally adopted (European Commission, 1990), with several modifications [38]. An amount of 200 mg of the freeze-dried samples was boiled in 5 mL of methanol 70% for 10 min at 70 °C to inactivate myrosinase, thereby preventing the enzymatic hydrolysis of the GLSs. The supernatant was collected after centrifugation at 12.000 rpm for 20 min at 4 °C. A total of 2 mL of the samples

was inserted into a 25 × 8 mm inner diameter column filled with 0.5 mL of an aqueous mixture DEAE-Sephadex A-25 resin 50% *w/v*, previously conditioned with a 0.02 M buffer of acetic acid and pyridine. The glucosinolates were hydrolyzed in the column to obtain their desulfoglucosinolates by adding 75 µL (5 U. mL^{-1}) of sulfatase E.C.3.1.6.1 from *Helix pomatia*. After overnight incubation, the desulfoglucosinolates were eluted with 1.5 mL of ultrapure H_2O and analyzed using high-performance liquid chromatography (HPLC, Agilent 1200 Series System) with a diode array detector.

2.4. High-Performance Liquid Chromatography (HPLC) Analysis

The GLSs content and profile were determined by the HPLC diode array detector technique, separating de-sulpho glucosinolates. Each of the ten intact glucosinolate standards at a 0.2 M concentration were dissolved in 2 mL of Milli-Q water to prepare the mixture of the stock standard solution. The mixture was further diluted to prepare calibration standard solutions of 0.1, 0.2, 0.4, and 1.0 µmoles mL^{-1}, respectively. All standard solutions were stored at 4 °C until use. The desulphoglucosinolate extracts were injected into an HPLC-DAD equipped with a Kinetech C18 (250 × 4.6 mm, particle size 5 µm) column with a mobile phase of ultrapure water (solvent A) and acetonitrile: water 20:80 (*v/v*) (solvent B), with a flow rate of 1.1 mL min^{-1} and an injection volume of 20 µL, with a binary gradient: 100%A–0%B for 5 min, increased to 70%A–30%B from 5 to 17 min, and then at 30%A–70%B for 3 min; the entire run lasted for 40 min. All the reagents used in the analysis were of HPLC grade. The chromatograms were recorded at 229 nm; the quantification was based on the calibration curves of the external standards by comparing each compound through the retention time (RT) and UV spectra. The results were expressed in micromoles per gram of the dry weight. The data are presented with the means and standard deviation (SD) of triplicate experiments. The GLSs standards were (in order of elution) SIN: sinigrin; GRA: glucoraphanin; SIB: glucosinalbin; GNA: gluconapin; GAL: glucoalyssin; GER: glucoerucin; GBS: glucobrassicin; GBN: glucobrassicanapin; NGBS: neoglucobrassicin; and GST: gluconasturtiin (Figure S1). All standards were purchased from ChromaDex (Santa Ana, CA, USA).

2.5. Statistical Analysis

Data are presented in terms of the means ± standard deviation (SD) and the statistical analysis significance was calculated in triplicate, two-way ANOVA by CoStat software version 6.4, followed by Tukey's multiple comparisons test, of which p-values < 0.05 were considered to be statistically significant. Data were transformed using the percentage rank of the analysed matrix. Statistical analysis was performed using the SPSS software version 27. Pearson's correlation coefficient was used to determine the correlation among the individual glucosinolates. The variation index (VI) describes the variation percentage of the morphometric traits of the NIR plants compared to the IRR ones, using the following formula:

$$VI = -(100 - (Stress/Control \times 100)).$$

We performed the principal component analysis (PCA) utilizing the relative data of the GLSs detected in order to make evident the contribution of each GLS detected in the percentage in relation to the total amount detected. The PCA was established to discriminate the different *B. oleracea* varietal groups and the main GLSs associated with them. The percentage calculated was normalized using the angular coefficient (DEGRES(ASIN(RACINE(x/100))). We elaborated the percentage of variation for leaves collected in the NIR plot in relation to those in the IRR plot. ((NIR/IRR) × 100).

3. Results

3.1. Agronomic Trait Analysis

During the growing cycle, the mean temperature registered was 22.4 ± 5.8 °C and the mean solar radiation was 5.9 MJ. m^{-2} d^{-1}. The morphometric data results highlighted several differences between the conditions under which the plants were tested and the genotypes analyzed (Table 2). The plant weight (PW) showed a significant interaction

between the two experimental factors and the values varied from 535.0 g to 112.0 g, for BR3 grown in the IRR main plot and for CV1 in NIR, respectively (Table 2). The genotypes which showed the positive variation index (VI) were CCP4 and CV3, varying from 33.1 to 8.9, and the lowest negative VI was observed for BH3, BH2, CV4, BR5, and BH1, fluctuating from −8.4 to −29.7; all the above-mentioned accessions showed a good resilience as the reduction in the PW was limited for the plants grown in the NIR plot compared to the IRR one (Table 2). Concerning the plant height (PH), we observed a significant interaction IRR × GE, and its value varied from 69.8 cm for BH2 in IRR to 13.7 cm for CV2 in NIR. With regard to the VI for the PH trait, it varied from −6.1 to −27.1, for BR2 and BR4, respectively (Table 2). The stem diameter (SD) exhibited a significant interaction between IRR and GE (IRR × GE) and the value ranged from 5.1 mm for BR1 grown in the normal irrigation system to 1.3 mm for BR1 and CCP4 both in the drought stress condition. The genotypes showed negative VI and the value varied from −20.7 to −29.4 for BH1 and BR5, respectively (Table 2). Regarding the number of leaves (NL), we observed a significant interaction IRR × GE, and its value varied from 15.0 leaves for CV3 in the IRR system to 4.0 leaves for BR2 and CCP2 grown by the NIR one. The VI value for the NL trait fluctuated from 0.0 for CV4, in which variation was not observed in comparison to the plants grown by drought stress application, to −28.6 for BR1 and CCP4 (Table 2).

The SPAD index displayed a significant interaction between the two experimental factors (IRR × GE); it varied from 62.8 to 35.1 for CV5 and BH2 grown by IRR and NIR protocols, respectively (Table 2). The VI value ranged from 0.0 to −28.5 for CCP2 and BH2, respectively (Table 2). Concerning the root weight (RW), the variation observed for IRR was not significant, but we noted a significant interaction between the two experimental factors studied (IRR × GE); the VI varied from 43.9 to 8.0 g for BR3 grown in the IRR plot and CV5 in the NIR system, respectively. The VI observed ranged from −0.7 to −28.2 for BH2 and BR1, respectively (Table 3). Regarding the main root length (MRL), we also observed a significant interaction between IRR and GE and its value varied from 21.0 to 2.8 cm for BH2 and BH3 in the IRR and NIR regime, respectively (Table 3). The VI value exceeded among all the genotypes was −30.0 and the variation among the genotypes was higher than the other traits analyzed (Table 3).

3.2. Comparison between the Total Amount of GLS between Roots and Leaves

The water stress affected the amount and the profile of GLSs in all the studied genotypes. With regard to the GLSs content of the roots, we observed a significant interaction between the two experimental factors (IR x GE) and its value ranged from 38.2 to 2.5 g^{-1} d.w. for BH2 grown in NIR plots and CCP1 grown in IRR ones (Table 4). The accessions that showed the highest concentration of GLSs in the roots were BR4, CCP3, CV1, and BH1, in decrescent order, respectively, varying from 38.2 to 11.8 g^{-1} d.w. for BH2 grown in NIR and IRR plots, respectively (Table 4). The total GLSs concentration detected was higher in the leaves than in the roots for all the accessions analyzed. The total GLSs amount was affected significantly by the interaction between IR and GE and its value varied from 578.9 to 35.8 μmol·g^{-1} d.w. for BR4 grown in the NIR plot and for CV3 grown in the IRR one (Table 4). In general, the accessions BR4, BR5, BR2, CCP3, and CV1 showed the highest GLSs content in the leaves, which varied from 578.9 to 111.8 μmol·g^{-1} d.w. for BR4 in the NIR plot and for CV1 grown in the IRR one, respectively (Table 4). The genotypes in both IR plots showing the lowest variation in the total GLSs content were BH1, CV2, and BR3, and their GLSs total amount fluctuated from 126.2 to 66.9 μmol·g^{-1} d.w. for BH1 grown in the NIR plot and CV2 grown in the IRR one, respectively (Table 4).

Table 2. Variation in the plant weight (PW), height (PH), stem diameter (SD), number of leaves (NL), leaves SPAD index, and the variation index (VI) in relation to the experimental factors studied.

Genotype	PW (g)				PH (cm)				SD (mm)				NL (n)				SPAD (0 to 99.9)			
	IRR	NIR	VI	Mean	IRR	NIR	VI	Mean	IRR	NIR	VI	Mean	IRR	NIR	VI	Mean	IRR	NIR	VI	Mean
BH 1	300.0 ± 2.0	211.0 ± 1.0	−29.7	255.5 ± 1.5	69.0 ± 1.0	26.8 ± 0.6	−61.2	47.9 ± 0.8	2.9 ± 0.0	2.3 ± 0.1	−20.7	2.6 ± 0.1	8.0 ± 1.0	7.0 ± 1.0	−12.5	7.5 ± 1.0	59.9 ± 0.3	48.4 ± 3.9	−19.2	54.2 ± 2.1
BH 2	165.0 ± 13.0	145.5 ± 3.5	−11.8	155.3 ± 8.3	69.8 ± 1.8	30.9 ± 0.7	−55.7	50.4 ± 1.3	3.8 ± 0.1	2.5 ± 0.2	−34.2	3.2 ± 0.2	9.0 ± 1.0	6.0 ± 1.0	−33.3	7.5 ± 1.0	49.1 ± 3.8	35.1 ± 3.4	−28.5	42.1 ± 3.6
BH 3	304.5 ± 55.5	279.0 ± 31.0	−8.4	291.8 ± 43.3	64.0 ± 1.0	35.6 ± 0.8	−44.4	49.8 ± 0.9	4.1 ± 0.0	2.4 ± 0.3	−41.5	3.3 ± 0.2	8.0 ± 1.0	8.0 ± 1.0	0.0	8.0 ± 1.0	60.1 ± 0.7	57.4 ± 2	−4.5	58.8 ± 1.4
BR 1	472.5 ± 38.5	192.0 ± 50.0	−59.4	332.3 ± 44.3	45.5 ± 6.5	31.1 ± 8.1	−31.6	38.3 ± 7.3	5.1 ± 0.3	1.3 ± 0.4	−74.5	3.2 ± 0.4	7.0 ± 1.0	5.0 ± 1.0	−28.6	6.0 ± 1.0	55.1 ± 0.1	45.7 ± 6.1	−17.1	50.4 ± 3.1
BR 2	369.0 ± 19.0	199.0 ± 23.0	−46.1	284.0 ± 21.0	42.5 ± 3.5	39.9 ± 1.7	−6.1	41.2 ± 2.6	4.2 ± 0.6	2.1 ± 1.1	−50.0	3.2 ± 0.9	10.0 ± 2.0	4.0 ± 1.0	−60.0	7.0 ± 1.5	57.6 ± 2	54.9 ± 2.3	−4.7	56.3 ± 2.2
BR 3	535.0 ± 15.0	212.0 ± 46.0	−60.4	373.5 ± 30.5	42.5 ± 1.5	33.6 ± 6.2	−20.9	38.1 ± 3.9	3.3 ± 0.2	2.4 ± 0.1	−27.3	2.9 ± 0.2	12.0 ± 0.0	8.0 ± 2.0	−33.3	10.0 ± 1.0	61.4 ± 0.4	46.4 ± 5	−24.4	53.9 ± 2.7
BR 4	508.0 ± 15.0	296.0 ± 16.0	−41.7	402.0 ± 15.5	49.1 ± 2.1	35.8 ± 4.4	−27.1	42.5 ± 3.3	4.7 ± 0.0	1.7 ± 0.3	−63.8	3.2 ± 0.2	13.0 ± 2.0	5.0 ± 1.0	−61.5	9.0 ± 1.5	56.4 ± 0.5	39.5 ± 0.6	−30.0	48.0 ± 0.6
BR 5	382.0 ± 42.0	288.0 ± 76.0	−24.6	335.0 ± 59.0	58.3 ± 3.3	45.8 ± 2.8	−21.4	52.1 ± 3.1	3.4 ± 0.0	2.4 ± 0.1	−29.4	2.9 ± 0.1	9.0 ± 1.0	7.0 ± 1.0	−22.2	8.0 ± 1.0	50.6 ± 9	47.4 ± 0.7	−6.3	49.0 ± 4.9
CCP 1	426.0 ± 76.0	170.0 ± 60.0	−60.1	298.0 ± 68.0	50.8 ± 1.8	35.5 ± 11.5	−30.1	43.2 ± 6.7	4.5 ± 0.6	1.9 ± 0.0	−57.8	3.2 ± 0.3	8.0 ± 1.0	5.0 ± 2.0	−37.5	6.5 ± 1.5	55.4 ± 5.5	48.2 ± 1.6	−13.0	51.8 ± 3.6
CCP 2	452.0 ± 48.0	138.0 ± 44.0	−69.5	295.0 ± 46.0	48.5 ± 7.5	24.2 ± 2.2	−50.1	36.4 ± 4.9	4.9 ± 0.1	2.8 ± 0.1	−42.9	3.9 ± 0.1	11.0 ± 0.0	4.0 ± 0.0	−63.6	7.5 ± 0.0	54.6 ± 0.9	54.6 ± 0.9	0.0	54.6 ± 0.9
CCP 3	300.0 ± 42.0	172.0 ± 8.0	−42.7	236.0 ± 25.0	36.5 ± 4.0	20.9 ± 1.5	−42.7	28.7 ± 2.8	4.8 ± 0.8	2.7 ± 0.5	−43.8	3.8 ± 0.7	10.0 ± 0.0	5.0 ± 1.0	−50.0	7.5 ± 0.5	62.1 ± 2.3	51.1 ± 2.7	−17.7	56.6 ± 2.5
CCP 4	192.0 ± 32.0	255.5 ± 1.5	33.1	223.8 ± 16.8	37.0 ± 1.0	34.6 ± 1.2	−6.5	35.8 ± 1.1	4.2 ± 0.5	1.3 ± 0.1	−69.0	2.8 ± 0.3	7.0 ± 1.0	5.0 ± 1.0	−28.6	6.0 ± 1.0	47.2 ± 1.6	46.8 ± 1.6	−0.8	47 ± 1.6
CV 1	413.0 ± 83.0	112.0 ± 4.0	−72.9	262.5 ± 43.5	47.5 ± 8.5	26.2 ± 4.0	−44.8	36.9 ± 6.3	3.5 ± 0.4	1.7 ± 0.1	−51.4	2.6 ± 0.3	10.0 ± 1.0	6.0 ± 1.0	−40.0	8.0 ± 1.0	56.9 ± 6.0	44.0 ± 0.5	−22.7	50.5 ± 3.3
CV 2	289.0 ± 48.0	177.0 ± 59.0	−38.8	233.0 ± 53.5	26.0 ± 3.0	13.7 ± 0.7	−47.3	19.9 ± 1.9	4.0 ± 0.8	2.2 ± 0.1	−45.0	3.1 ± 0.5	11.0 ± 3.0	5.0 ± 1.0	−54.5	8.0 ± 2.0	57.5 ± 1.9	44.8 ± 0.2	−22.1	51.2 ± 1.1
CV 3	333.0 ± 45.0	362.5 ± 17.5	8.9	347.8 ± 31.3	38.0 ± 6.0	16.8 ± 5.3	−55.8	27.4 ± 5.7	4.4 ± 0.4	1.7 ± 0.1	−61.4	3.1 ± 0.3	15.0 ± 3.0	5.0 ± 2.0	−66.7	10.0 ± 2.5	50.9 ± 3.8	41.7 ± 2.8	−18.1	46.3 ± 3.3
CV 4	401.0 ± 49.0	306.5 ± 18.2	−23.6	353.9 ± 33.6	44.5 ± 3.5	27.6 ± 9.6	−38.0	36.1 ± 6.6	4.5 ± 1.2	2.4 ± 0.5	−46.7	3.5 ± 0.9	6.0 ± 0.0	6.0 ± 2.0	0.0	6.0 ± 1.0	60.8 ± 3.9	51.7 ± 5.2	−15.0	56.3 ± 4.6
CV 5	525.5 ± 99.5	231.0 ± 45.0	−56.0	378.3 ± 72.3	34.0 ± 2.0	16.6 ± 1.4	−51.2	25.3 ± 1.7	4.2 ± 0.5	2.0 ± 0.4	−52.4	3.1 ± 0.5	13.0 ± 0.0	6.0 ± 2.0	−53.8	9.5 ± 1.0	62.8 ± 5.3	43.1 ± 4.6	−31.4	53.0 ± 5.0
Mean	380.0 ± 102.4	214.9 ± 64.5			47.3 ± 12.2	29.1 ± 8.8			4.1 ± 0.6	2.1 ± 0.4			9.8 ± 2.5	5.7 ± 1.2			56.4 ± 4.7	47.1 ± 5.7		
Significancy of the differences by ANOVA Student–Newman–Keuls																				
IR	**				**				***				**				*			
GE	***				***				**				***				***			
IR × GE	***				***				***				***				***			

*, **, and*** indicate respectively that the effect is not significant or significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Table 3. Variation in root weight (RW), main root length (MRL), and the variation index (VI) in relation to the experimental factors studied.

Genotype	RW (g)				MRL (cm)			
	IRR	**NIR**	**VI**	**Mean**	**IRR**	**NIR**	**VI**	**Mean**
BH 1	43.6 ± 7.4	25.1 ± 3.2	−42.4	34.3 ± 5.3	18.6 ± 1.9	6.0 ± 0.7	−67.7	12.3 ± 1.3
BH 2	14.2 ± 2.5	14.1 ± 4.1	−0.7	14.1 ± 3.3	21.0 ± 2.2	5.4 ± 1.1	−74.3	13.2 ± 1.7
BH 3	25.6 ± 7.1	15.0 ± 3.0	−41.4	20.3 ± 5.1	16.6 ± 2.5	2.8 ± 0.5	−83.1	9.7 ± 1.5
BR 1	39.0 ± 7.4	28.0 ± 2.0	−28.2	33.5 ± 4.7	13.6 ± 3.3	5.0 ± 0.0	−63.2	9.3 ± 1.7
BR 2	30.6 ± 3.5	29.0 ± 1.0	−5.2	29.8 ± 2.3	11.9 ± 1.2	7.6 ± 1.8	−36.1	9.8 ± 1.5
BR 3	43.9 ± 5.5	15.0 ± 5.0	−65.8	29.5 ± 5.3	12.6 ± 1.0	5.8 ± 0.5	−54.0	9.2 ± 0.8
BR 4	41.7 ± 5.2	13.5 ± 3.2	−67.6	27.6 ± 4.2	14.7 ± 1.2	3.4 ± 0.2	−76.9	9.1 ± 0.7
BR 5	31.6 ± 4.1	19.0 ± 1.0	−39.9	25.3 ± 2.6	17.5 ± 1.6	5.6 ± 0.4	−68.0	11.6 ± 1.0
CCP 1	35.0 ± 6.0	16.0 ± 2.0	−54.3	25.5 ± 4.0	15.2 ± 1.3	4.5 ± 0.3	−70.4	9.9 ± 0.8
CCP 2	37.4 ± 7.9	16.0 ± 8.0	−57.2	26.7 ± 8.0	14.5 ± 1.9	4.3 ± 0.3	−70.3	9.4 ± 1.1
CCP 3	24.9 ± 3.2	19.0 ± 1.0	−23.7	22.0 ± 2.1	10.8 ± 0.7	6.0 ± 0.4	−44.4	8.4 ± 0.6
CCP 4	16.5 ± 4.2	16.0 ± 4.0	−3.0	16.3 ± 4.1	10.9 ± 0.9	4.1 ± 1.1	−62.4	7.5 ± 1.0
CV 1	33.9 ± 6.4	28.0 ± 8.0	−17.4	31.0 ± 7.2	14.2 ± 4.0	5.0 ± 0.3	−64.8	9.6 ± 2.2
CV 2	24.5 ± 11.0	13.0 ± 3.0	−46.9	18.8 ± 7.0	7.6 ± 0.1	5.1 ± 1.3	−32.9	6.35 ± 0.7
CV 3	27.9 ± 6.5	24.0 ± 4.0	−14.0	26.0 ± 5.3	11.3 ± 1.2	5.5 ± 0.5	−51.3	8.4 ± 0.9
CV 4	33.3 ± 7.4	23.0 ± 13.0	−30.9	28.2 ± 10.2	13.3 ± 1.0	5.5 ± 0.3	−58.6	9.4 ± 0.7
CV 5	43.5 ± 12.4	8.0 ± 0.0	−81.6	25.8 ± 6.2	10.1 ± 0.6	4.3 ± 0.4	−57.4	7.2 ± 0.5
Mean	32.2 ± 9.1	18.9 ± 6.2			13.8 ± 3.4	5.0 ± 1.1		
Significance of the differences by ANOVA Student–Newman–Keuls								
IR	n.s.				*			
GE	***				***			
IR × GE	***				***			

ns, * and *** indicate respectively that the effect is not significant or significant at $p < 0.05$, and $p < 0.001$, respectively.

Table 4. Variation in the total amount of GLSs (μmol $g^{-1\ d.w.}$) in the roots and leaves in relation to the two experimental factors studied.

	Roots			Leaves		
Genotypes	**IRR**	**NIR**	**Mean**	**IRR**	**NIR**	**Mean**
BH 1	5.1 ± 1.0	9.7 ± 0.3	9.7 ± 3.6	122.7 ± 3.0	126.0 ± 3.7	124.4 ± 2.3
BH 2	11.8 ± 0.6	38.2 ± 1.6	38.2 ± 18.7	39.1 ± 2.5	82.5 ± 2.4	60.8 ± 30.7
BH 3	3.5 ± 0.2	5.1 ± 0.2	5.1 ± 1.1	48.4 ± 5.0	76.9 ± 1.7	62.7 ± 20.2
BR 1	4.7 ± 0.6	32.2 ± 1.8	32.2 ± 19.4	39.8 ± 2.8	72.7 ± 3.6	56.3 ± 23.3
BR 2	4.2 ± 0.0	6.2 ± 0.0	6.2 ± 1.4	184.3 ± 14.9	264.9 ± 4.2	224.6 ± 56.9
BR 3	8.8 ± 1.6	10.7 ± 0.1	10.7 ± 1.3	81.6 ± 3.2	98.2 ± 0.0	89.9 ± 11.7
BR 4	28.9 ± 5.4	36.1 ± 6.0	36.1 ± 1	291.4 ± 91	578.9 ± 33.5	435.2 ± 203.3
BR 5	9.2 ± 1.9	20.1 ± 1.9	20.1 ± 7.7	222.2 ± 17.9	336.7 ± 51.5	279.5 ± 80.9
CCP 1	2.5 ± 0.1	2.7 ± 0.2	2.7 ± 0.1	45.5 ± 1.0	66.2 ± 2.6	55.9 ± 14.7
CCP 2	4.3 ± 0.1	6.6 ± 0.6	6.6 ± 1.6	103.9 ± 67.3	138.7 ± 16.8	121.3 ± 24.6
CCP 3	19.6 ± 7.5	23.6 ± 5.3	23.6 ± 2.8	115.3 ± 35.9	400.1 ± 10.5	257.7 ± 201.4
CCP 4	5.2 ± 0.7	8.3 ± 2.1	8.3 ± 2.2	48.9 ± 0.0	99.2 ± 4.6	74.1 ± 35.6
CV 1	29.0 ± 0.1	32.1 ± 3.2	32.1 ± 2.2	111.8 ± 23.7	411.6 ± 5.1	261.7 ± 211.9
CV 2	4.9 ± 1.1	18.2 ± 1.2	18.2 ± 9.4	66.9 ± 7.2	78.6 ± 2.9	72.8 ± 8.3
CV 3	6.1 ± 0.9	16.6 ± 2.3	16.6 ± 7.4	35.8 ± 0.0	88.1 ± 0.0	62.0 ± 36.9
CV 4	10.9 ± 0.4	12.8 ± 1.6	12.8 ± 1.3	39.02 ± 2.4	66.9 ± 7.3	53.0 ± 19.7
CV 5	14.8 ± 1.1	15.3 ± 4.4	15.3 ± 0.4	114.6 ± 0.0	245.7 ± 0.0	180.2 ± 92.7
Mean	10.2 ± 8.4	8.4 ± 11.4		100.6 ± 72.7	190.1 ± 156.0	
Significancy of the differences by ANOVA Student–Newman–Keuls						
IR		**			**	
GE		**			**	
IR × GE		**			***	

** and *** indicate that the correlation is significant at $p < 0.01$ and $p < 0.001$, respectively.

3.3. Variation in Individual Glucosinolate in Roots

In the GLSs profile in the roots, each compound was affected by the significant interaction between the two experimental factors studied (Tables S1 and S2).

3.3.1. Aliphatic Glucosinolates

The singrin (SIN) content was affected by the interaction IR x GE and it varied from 10.8 to 0.0 for BR4 grown in the NIR plot and for BR2, CCP4, CV1, and CV5 grown in the IRR one, respectively; for CCP3, we did not detect SIN in the NIR plot, and in the IRR plot, we observed 2.1 μmol·g$^{-1\ d.w.}$ (Table S1). The highest values were detected in the cultivar *Brassica oleracea* var. *italica* BR4. SIN was not detected in CCP3 and CCP4 in both IR plots. The highest variation in the SIN content was observed for BR3, fluctuating between 5.5 and 1.0 μmol·G$^{-1\ d.w.}$ grown in NIR and IRR plots, respectively (Table S1).

The glucoraphanin (GRA) ranged from 7.1 to 0.0 μmol·G$^{-1\ d.w.}$ for CCP3 grown in the IRR plot and for BR4 and CCP2 grown in the NIR plot, respectively. For BR4, we have also not detected GRA in the NIR plot or in the IRR one (Table S1). For the genotypes BR3, CPP1, and CCP3, the GRA content increased by about 50% in the plants grown in the NIR and the IRR plots. The CCP4 roots increased the content of GRA by about 200%, from 0.3 to 1.0 μmol·g$^{-1\ d.w.}$.

The gluconapin (GNA) varied from 1.6 to 0.0. g$^{-1\ d.w.}$ for CCP2 grown in the NIR plots and for BR1 grown in the IRR plot, as well as for BH1, BR5, and CCP4 grown in the IRR plot, respectively. The GNA was not detected for BR2 and BR4 in both the IR studied (Table S1).

The glucoerucin (GER) content varied from 7.1 to 0.0 μmol·g$^{-1\ d.w.}$ for BR5 grown in the NIR plot and for BR1, BR2, BR3, CCP3, CV2, CV3, CV4, and CV5 in the IRR plot, respectively; for BH3 and CV1, any GER in the roots of the plants grown in both IR plots was not detected (Table S1).

The glucobrassicanapin (GBN) ranged from 12.5 to 0.0 μmol·g$^{-1\ d.w.}$ for BR4 grown in the NIR plot and for CV3, CV4, and CV5 in the IRR one, and for CCP2 grown in the NIR plot, respectively (Table S1).

The glucoalyssin (GAL) detected in the roots varied from 15.7 to 0.0 μmol·g$^{-1\ d.w.}$ for BR3 grown in the NIR plot and for BR5 and CV1 grown in the IRR one, respectively. We did not detect GAL in the roots of CCP1 and CCP4 grown in the NIR plot (Table S1).

3.3.2. Indolic Glucosinolates

The glucobrassicin (GBS) content varied from 11.7 to 0.0 μmol·g$^{-1\ d.w.}$ for CV3 grown in the NIR plot and for BR1 and CCP2 grown in the IRR plot, respectively. We did not detect GBS in BR2 in the plants grown in both IR plots studied, and in CV4 in the NIR plot (Table S2).

The neoglucobrassicin (NGBS) was detected in a lower concentration in all tested ac-cessions and its value fluctuated from 8.6 to 0.0 μmol·g$^{-1\ d.w.}$ for CV5 grown in the NIR plot and for BR4, CCP2, CCP3, and CV4 grown in the IRR one. NGBS was not detected in BH1, BH2, BH3, BR1, BR2, and BR3 in the IRR and NIR plots, and in BR5 in plants grown in the NIR plot (Table S2).

3.3.3. Aromatic Glucosinolates

The sinalbin (SIB) content ranged from 13.5 to 0.0 μmol·g$^{-1\ d.w.}$ for BR5 and for BR3, CCP1, and CV3 grown in the NIR plot, in decrescent order, respectively. For BR1, BR2, CCP2, CCP4, and CV1 we did not find SIB in the roots of the plants grown in both IR plots (Table S2).

The gluconasturtiin (GST) detected in the roots varied from 27.0 to 0.0 μmol·g$^{-1\ d.w.}$ for CV1 grown in the IRR plot, and for BH2, BH3, BR2, CCP3, and CV4 also in the IRR condition, respectively. We did not find GST in CCP4 and CV3 in the NIR plot. The GST was not detected in BH1, CCP1, CCP2, CV2, and CV5 in the roots of the plants grown in IRR or NIR conditions (Table S2)

3.4. Variation of Individual Glucosinolate in Leaves

The GLSs profile detected in the leaves showed a highly significant interaction between the two experimental factors studied (IRR × GE) for all compounds registered (Tables S3–S5).

3.4.1. Aliphatic Glucosinolate

The sinigrin (SIN) ranged from 185.7 to 0.0 μmol·g^{-1} $^{d.w.}$ for BR4 grown in the NIR plot and for CCP1 and CV3 also in the NIR condition, in decrescent order, respectively (Table S3).

The glucoraphanin (GRA) varied from 36.4 to 0.0 μmol·g^{-1} $^{d.w.}$ for CV5 in the NIR plot and for BR2, BR4, CCP2, and CV2 grown in the IRR plot. We did not detect GRA in the leaves of BH2, CCP1, and CV4 collected in the NIR plot or for BH3 and CV3 collected in both IRs studied (Table S3).

For all the genotypes analyzed, we detected low amounts of gluconapin (GNA) in comparison to the other glucosinolates analyzed. The GNA ranged from 83.1 to 0.0 μmol·g^{-1} $^{d.w.}$ for BR3 grown in the NIR plot and for BR4, CCP2, CCP4, CV1, and CV2 grown in the IRR plot, in decrescent order, respectively. The GNA was not registered for CCP3 and CV3 grown in the NIR conditions and for BH1, BR1, BR2, BR5, CCP1, and CV4 grown in both IRs studied (Table S4).

The glucoerucin (GER) content in the leaves varied from 331.8 to 0.0 μmol·g^{-1} $^{d.w.}$ for BR2, BR3, BR5, CCP1, CCP2, and CV4 grown in the NIR condition, in decrescent order, respectively (Table S4). The GER was not detected for CV2 in plants grown in the IRR plot, whereas BR4 was not registered in either IR studied (Table S4).

The glucobrassicanapin (GBN) content fluctuated from 100.6 to 0.0 μmol·g^{-1} $^{d.w.}$ for CCP4 in the IRR plot and for BH2, BR3, CCP3, and CV3 in the NIR condition, in decrescent order, respectively (Table S4). The GBN also was not detected for CV1 in the plants grown in the IRR plot and for BR1 grown in both IRs studied (Table S4).

The glucoalyssin (GAL) ranged from 77.8 to 0.0 μmol·g^{-1} $^{d.w.}$ for CCP1, BR3, BR4, CCP3, and CV3 grown in the NIR condition, respectively. The GAL was not detected for BH1, BR1, and CV1 in both IRs studied (Table S4).

3.4.2. Indolic Glucosinolate

The glucobrassicin (GBS) detected varied from 125.3 to 0.0 μmol·g^{-1} $^{d.w.}$ for BR4 in the NIR condition and for BR2 and CV2 in the IRR plot (Table S5). The GBS was not detected for BR3, BR5, CCP1, CV1, and CV4 grown in the NIR plot. For BH1, BR1, and CCP4, the GBS was not registered for the leaves of the plants grown in both IRs studied (Table S5).

The neoglucobrassicin (NGBS) varied from 63.6 to 0.0 μmol·g^{-1} $^{d.w.}$ for CV2 in the NIR plot and for BH1, CCP1, CCP2, and CCP4 grown in the IRR plot, in decrescent order, respectively. The NGBS was not detected for BH2, BR1, BR3, and BR5 in both IRs studied (IRR and NIR). For BH3, BR2, and CCP3, we have not detected the NGBS in the leaves of the plants grown in the NIR plot (Table S5).

3.4.3. Aromatic Glucosinolates

The sinalbin (SIB) ranged from 20.1 to 0.0 μmol·g^{-1} $^{d.w.}$ for BR4 grown in the NIR plot and for BH1, BR1, CV2, and CV5 grown in the IRR condition, in decrescent order, respectively (Table S5). The SIB was not detected for BR3, CCP1, CCP3, CV3, and CV4 grown in the NIR plot, and BR2 was not found in either IR (Table S5).

The glucosnasturtiin (GST) content varied from 65.0 to 0.0 μmol·g^{-1} $^{d.w.}$ for CV1, BR1, BR3, and CCP2 grown in the NIR condition. The GST was not detected for BR4, CV2, and CV4 in either of the IRs studied (Table S5).

3.5. Chemotaxonomy of the Different Accessions

We noted that the aliphatic GLS were predominant in the roots of broccoli (Brassica oleracea var. italica) and kale (Brassica oleracea var. acephala) with 61.8% and 55.3%, respectively (Figure 1). Under water stress conditions, the percentage of aliphatic GLSs changed: an increase to 65.3% was observed, with a decrease in indolic from 16.3% to 10.9%,

and the aromatic glucosinolates increased from 21.9% to 23.8%. While in kale, the aliphatic GLS decreased to 48.0%, an increase in the aromatic glucosinolates from 6.2% to 35.3% was noted, while the indolic GLSs decreased from 38.5% to 17.8%.

The indolic glucosinolate accounted for the major component in cauliflower (Brassica oleracea var. botrytis) with 66.0% of the total amount. Glucobrassicin represents 37.5% of the total glucosinolates; in relation to water stress, a decrease to 32.4% was observed while the neoglucobrassicin increased from 28.7% to 34.0%. In our study, the aliphatic glucosinolates represent 31.0% of the total GLSs in cauliflower, while the aromatic glucosinolates were found in a very low percentage.

The aromatic glucosinolates were predominant in the roots of CCP, Brassica oleracea var. cross with 50.0% in well-watered conditions and increased to 56.0% under water stress, while both aliphatic and indolic GLSs decreased from 31.0% to 28.6% and from 18.2% to 14.7%, respectively.

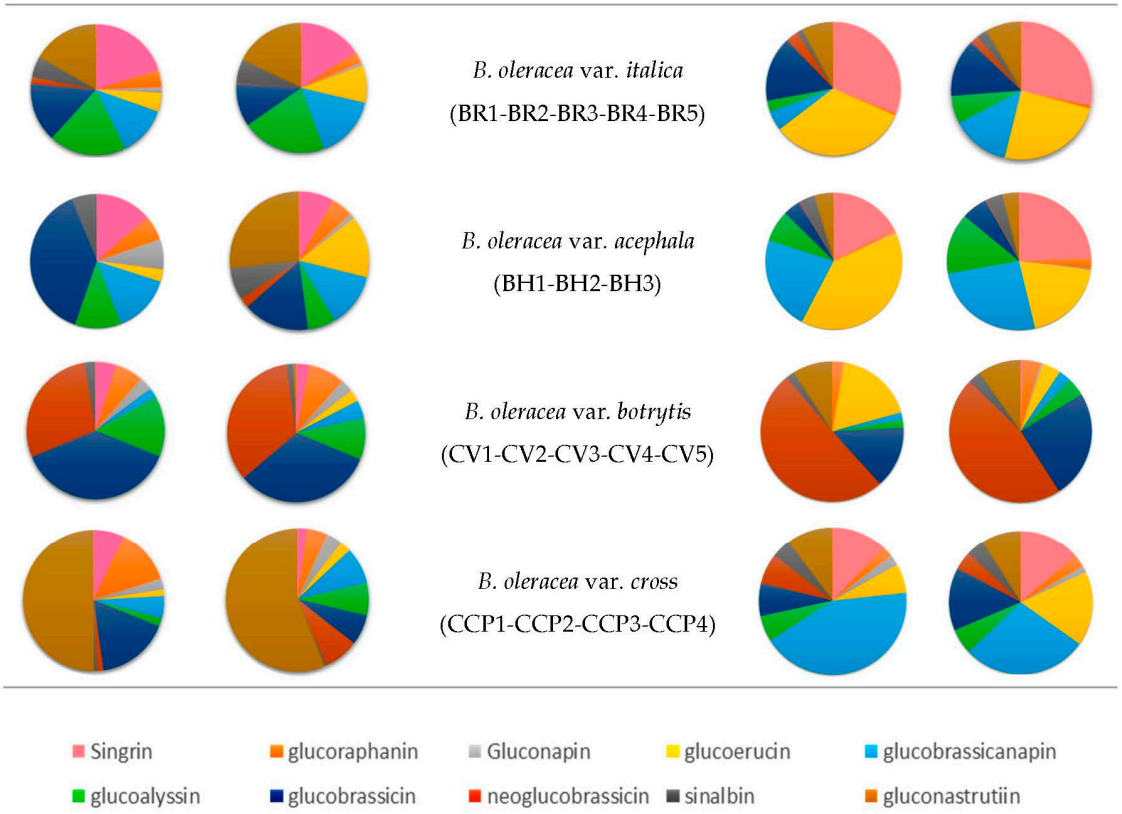

Figure 1. Chemotaxonomy of different glucosinolates found in the tested accessions in relation to water stress, grouping them by varieties (roots and leaves).

The aliphatic GLSs were found in a major percentage of the leaves of broccoli (*Brassica oleracea* var. *italica*) and kale (*Brassica oleracea* var. *acephala*), and, in particular, the glucoerucin and the sinigrin were the predominant glucosinolates with 39.6% and 37.4%, respectively, in broccoli (*Brassica oleracea* var. *italica*) and 17.3% and 39.4%, respectively, in kale (*Brassica oleracea* var. *acephala*) in well-watered conditions (Figure 1). The percentage of sinigrin under water stress conditions decreased in Broccoli (*Brassica oleracea* var. *italica*) (28.5%) and increased in kale (*Brassica oleracea* var. *acephala*) (24.2%). The glucoerucin under water stress conditions decreased both in broccoli (*Brassica oleracea* var. *italica*) and in kale (*Brassica oleracea* var. *acephala*) from 24.7% and 19.4%, respectively. The mean aliphatic GLSs found in *Brassica oleracea* var. *cross* was glucobrassicanapin with 42.6% of the total amount.

The indolic glucosinolates were predominant in *Brassica oleracea* var. *botrytis*; in particular, the percentage of neoglucobrassicin was 50.6% in normal conditions and 46.3% under water stress conditions. In other crops, the percentage of neoglucobrassicin was very low.

The glucobrassicin was the main indolic component found in *Brassica oleracea* var. *italica*, *Brassica oleracea* var. *botrytis*, and *Brassica oleracea* var. *cross*. The amount of glucobrassicin increased under water stress conditions from 14.1% to 24.5% in *Brassica oleracea* var. *botrytis* and *Brassica oleracea* var. *cross*, while in *Brassica oleracea* var. *italica*, the indolic GLSs decreased under drought stress from 18.9% to 13.6%.

The aromatic glucosinolates were found in low percentages in all crops.

3.6. Principal Component Analysis of Glucosinolate Profile in Leaves and Roots

We investigated the potential value of glucosinolate profiles as reference markers for the chemotaxonomic classification and distribution of the four varieties of *Brassica oleracea* (kale, broccoli, cauliflower, and composite cross population) analyzed. Principal component analysis (PCA) of the GLSs data was performed to better visualize the observed differences in the samples based on their differential GLSs profile (Figure 2). PC1 explained 25.6% of the total variation, clearly separating the crops according to the tissues (roots and leaves). The PC2, which explained 22.0% of the total variation, mostly corresponds to the different tissues of the leaves and roots. These differences between tissues and cultivars are due to the differences in specific GLSs, as is clear from the PCA-loading plots visualizing the distribution of the individual GLSs across the various cultivars. In the left cluster, all the control and stressed roots are grouped together and correlated with the majority of the glucosinolates, where we found them to be aliphatic, indolic, and aromatic. In the other part of this biplot, all the leaves are grouped together and correlated with the three aliphatic glucosinolate singrin, glucoerucin, and glucobrassicanapin, which are predominant in this part of the plant; except for the leaves of cauliflower in the control condition, it showed a positive correlation with the indolic glucosinolate neoglucobrassicin.

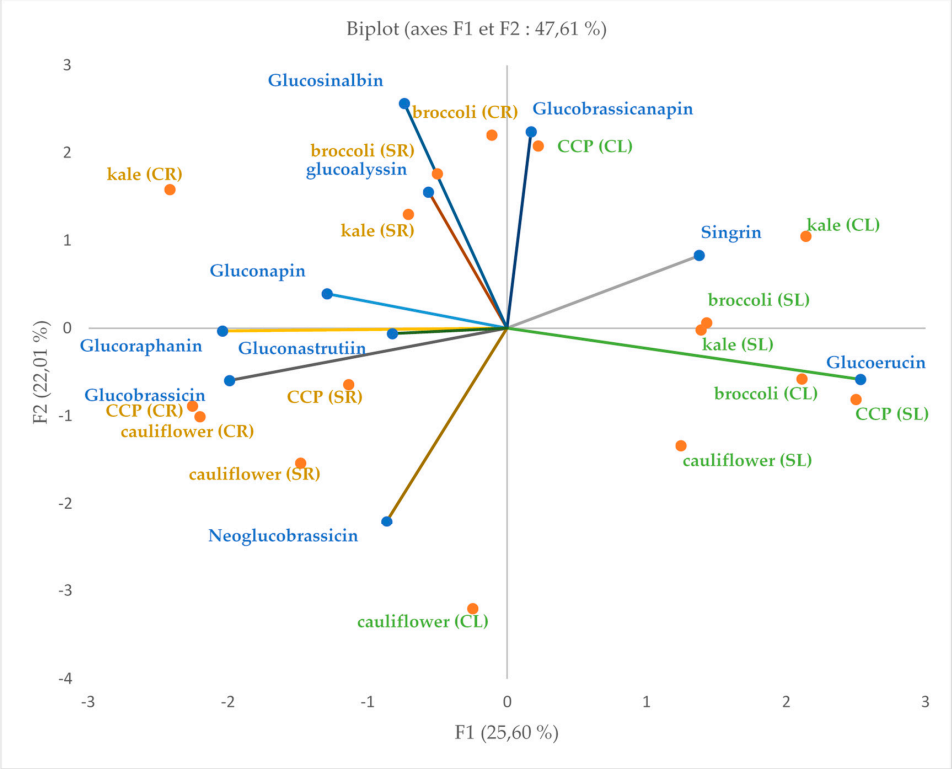

Figure 2. Two-dimensional principal component analysis (2D-PCA) of glucosinolates profile in leaves and roots. The first two principal component (PC) axes are labelled as PC1 and PC2, with the corresponding proportion of total variance in parentheses. SL: stressed leaves; CL: control leaves; SR: stressed roots; CR: control roots; different colors indicate various collard tissues (leaves in green and roots in brown). Kale (BH1, BH2, BH3); broccoli (BR1, BR2, BR3, BR4, BR5); cauliflower (CV1, CV2, CV3, CV4, CV5); composite cross population (CCP1, CCP2, CCP3, CCP4).

3.7. Variation of the Glucosinolates Compounds and Profile of the Leaves

The leaves represent the products of kale and sprouting broccoli landraces; as for the latter ones, their products are traditionally represented in the Southern most Italian regions by their small heads and small tender leaves present around the inflorescence, which are collected and utilized for preparing typical dishes. For this reason, we analyzed the data registered to bring to light the variation in the GLSs content and profile in relation to water stress and to individuate the set of genotypes which could increase the nutraceutical value of the products.

The correlation among the different GLSs detected in the leaves of the accessions of *B. oleracea* crops studied and their morphometric parameters was carried out to show the relation among the GLS profile and the GLS pathways variation in drought conditions (Table 4). The SIN showed a high negative correlation with the indolic glucosinolate NGBS (Table 5), as they had different metabolic pathways, so the biosynthesis of one affects the other. The aromatic glucosinolate SIB was positively correlated with the plant height (IH) and the GER showed a negative correlation with GAL and SIB. In addition, the GER showed a positive correlation with GST, and no other ones were observed with all the plant morphometric parameters detected (Table 5). A strong positive correlation was also observed between GBN and SIB and with the root weight (IRW) (Table 5). The GAL showed no correlation with all the glucosinolates detected, except for a significant positive correlation with the root weight (IRW) (Table 5). The positive correlation observed between GBS and the plant height (IW), and between GBS and the number of leaves (IL), could be of great interest for the health profile of the leaves, and also of the products of kale and broccoli crops (Table 5). No correlation was observed between GBS and the rest of the GLSs detected. Moreover, NGBS showed no relationship with all the GLSs, but a negative correlation with the plant height (IH) and number of leaves (IL) (Table 5). The NGB was correlated positively with the NGBS and root length (IRL), and the SIB was significantly highly positively correlated with the SPAD index. The plant weight (IW) showed a strong positive correlation with the root weight (IRW) and number of leaves (IL) (Table 5). The plant height (IH) showed a high positive correlation with the SPAD index; nevertheless, the root length (IRL) highlights a negative correlation with the number of leaves (IL) (Table 5).

Table 5. Pearson's correlation coefficients among the individual and total GLSs (detected in the leaves in NIR) and the index of morphometric traits identified in the studied crops.

	Correlations																
	SIN	**GRA**	**GNA**	**GER**	**GBN**	**GAL**	**GBS**	**NGBS**	**SIB**	**GST**	**IW**	**IH**	**ISD**	**IRW**	**IRL**	**IL**	**ISPAD**
SIN	1	0.064	−0.012	−0.246	0.071	−0.043	−0.043	**−0.478 ****	**0.342 ***	−0.225	−0.093	**0.483 ****	0.208	−0.151	−0.271	0.150	0.064
GRA		1	0.180	−0.157	−0.052	−0.149	−0.244	0.239	0.029	−0.210	−0.275	−0.068	−0.044	−0.311	0.283	−0.234	−0.152
GNA			1	−0.223	−0.151	−0.112	−0.125	−0.036	−0.089	−0.199	−0.197	−0.066	−0.307	−0.037	−0.062	−0.014	−0.051
GER				1	−0.214	**−0.459 ****	−0.202	−0.279	**−0.353 ***	**0.503 ****	−0.325	−0.240	0.228	−0.187	−0.208	−0.019	−0.200
GBN					1	0.302	−0.253	−0.223	**0.394 ***	0.134	0.204	0.128	−0.056	**0.460 ****	0.088	−0.006	0.215
GAL						1	0.083	−0.041	0.284	0.067	0.314	0.224	−0.289	**0.362 ***	−0.101	0.132	0.338
GBS							1	−0.127	0.335	−0.137	**0.347 ***	−0.090	0.075	0.144	−0.258	**0.373 ***	0.230
NGBS								1	−0.165	−0.293	0.089	**−0.361 ***	−0.128	−0.188	**0.431***	**−0.363 ***	−0.239
SIB									1	−0.211	0.321	0.215	0.118	0.336	−0.151	0.259	**0.449 ****
GST										1	−0.252	−0.115	−0.118	−0.103	−0.170	−0.212	−0.122
IW											1	−0.146	0.142	**0.415 ***	−0.136	0.373 *	0.116
IH												1	−0.297	0.146	0.162	0.016	**0.410 ***
ISD													1	−0.170	−0.064	0.330	−0.050
IRW														1	0.184	0.105	0.312
IRL															1	**−0.434 ***	−0.064
IL																1	0.204
ISPAD																	1

* and ** indicate that the correlation is significant at $p < 0.05$ and $p < 0.01$, respectively. SIN = sinigrin; GRA = glucoraphanin; GNA = gluconapin; GER = glucoerucin; GBN = glucobrassicanapin; GAL = glucoalyssin; GBS = glucobrassicin; NGBS = neoglucobrassicin; SIB = sinalbin; GST = gluconasturtiin; IW = weight index; IH = height index; ISD = index stem diameter; IRW = root weight index; IRL = root length index; IL = index number of leaves; ISPAD = SPAD index.

4. Discussion

Plants affected by drought will modify their morphology and biochemical composition for mitigating the water stress conditions present [39]. Drought stress tolerance is a complex trait and according to several studies, plants under abiotic stress can change their phenotypes to adapt themselves to unfavorable growing conditions [40]. The plant response to drought stress can be mitigated by a strong root architecture, and mainly by big and long roots parameterized in our work by the root weight (RW) and root length (MRL). Under water stress conditions, the resilience genotypes can quickly reach deeper layers of the soil and accumulate the plant reserves, increasing their RL and RW. In this study, we found that the morphological traits are affected by water stress (Tables 1 and 2), and that helped us to determine the genotypes which can much more readily mitigate drought conditions and to study the variation in the antioxidant status of the plant by the GLSs amount and profile. Our results showed a moderate to high decrease in the morphometric traits, with a difference among the accessions. These findings are in accordance with the study by Issarakraisila et al. (2007) [41], in which it was reported that water deficiency significantly reduced the leaf area, fresh area, and dry weight of Chinese kale by more than half. Similarly, Souza et al. (2018) [42] reported lower values for the plant height and leaf number in cauliflower cultivated under water stress at 40% ETc in comparison to higher irrigation levels. Meanwhile, the accession CV3 showed a plant weight higher in water stress conditions than in the control, indicating that the water deficit did not alter the biomass of this resilient accession.

The morphometric variation in the traits caused by drought stress allowed for the individuation of the accessions to be reduced on average by 30% of the data registered, such as BH1, BH2, and BH3 among the kale accessions, BR5 among the broccoli ones, CCP4 among the cross-composite populations, and CV3 and CV4 among the cauliflower accessions which were considered (Table 3). On the whole, this suggests that tolerance to water stress requires different morphological and biochemical characteristics and may reflect different underling stress tolerance mechanisms.

The GLSs amount and profile is influenced by the genotypes, environmental growth conditions, the growing methods, and the plant organs considered. The GLSs amount in plant organs depends on several environmental and developmental factors. The data registered in our study confirm the increment of the total GLSs amount in relation to drought stress, both in the leaves and in the roots analyzed, as reported by several authors [43–45]. In this study, ten glucosinolates were detected in the leaves and in the roots of the *B. oleracea* accessions considered, including six aliphatic, two indoles, and two aromatics GLSs. The set of plants analyzed showed a significant interaction of IR x GE both for the total amount and profile of GLSs (Tables S1–S5). The GLSs content was twice as high for the plants grown in the NIR plot than in the IRR plot (Tables S1–S5). We can say that drought stress increases the metabolism for GLSs biosynthesis and for its accumulation in different organs of the plant; this as a consequence of the plant's response to drought conditions through the process of osmotic adjustment, as confirmed by Schreiner et al. (2009) [46]. However, in the study by Khan et al. (2010) [47], the waterlogged plants had the highest levels of GLSs, whereas the plants under drought stress had the lowest levels. The genotypes can influence the GLSs patterns, and the accessions of the same varietal group can show significant differences for both the GLSs total amount and profile in relation to the genotype and to the environmental conditions [48,49]. Individual GLSs detected in this study varied between water stress treatments. The variation between the aliphatic and indolyl GLSs concentrations in the well-watered and drought-stressed plants was significant (about two-fold). Therefore, the majority of indolic GLSs, particularly indol-3-yl-methyl glucosinolate (I3M), 4-methoxyindol-3-ylmethyl glucosinolate (4MOI3M), and 1-methoxyindol-3-ylmethyl (1MOI3M), accumulated in plants cultivated in waterlogged circumstances and experienced a considerable reduction in the drought stress treatment; Wiesner et al. (2013) [50]. López-Berenguer et al. (2008) [51] observed a significant increment in the GLSs in broccoli plants in response to salt stress.

The selected accessions showed a limited variation in the morphometric traits in relation to drought stress, evidenced by a high and significant increment in the amount of the indolic glucosinolate GBS (for BH1, BH2, BH3, CCP4, and CV3) and NGBS (for the accessions BR5 and CV4). As GBS is related to NGBS, the conversion between them seems evident [52]. Hornbacher 2022 [53] reported on the importance of glucobrassicin for mitigating water stress, and he suggested the glucobrassicin could represent a source of auxin for Arabidopsis thaliana grown in drought conditions. According to our study, in the kale accessions, the most abundant glucosinolates were sinigrin, glucobrassicanapin, and glucobrassicin (Figure 1). Similarly, Kushad et al. (1999) [54] reported a high sinigrin (10.4 $\mu mol \cdot g^{-1\ d.w.}$) and a low glucobrassicin amount (1.2 $\mu mol \cdot g^{-1\ d.w.}$), but only a very small amount of glucoraphanin. However, in the same study, the broccoli accessions showed significant differences in the indole glucosinolates content, and the glucobrassicin appeared to be the most abundant indole glucosinolate for broccoli cvs analyzed, which contradicts our study where the aliphatic glucosinolate are predominant. Based on our chemotypes detected, there is not a specific glucosinolate related to the resistance to water stress, but the accumulation of NGBS may have a link to the resistance to water stress. Thus, the differences in glucosinolates between accessions could mean that the glucosinolates pathway is regulated differently depending on the crop and the expression of the genes involved. The inhibition of one class of glucosinolates resulted in a compensatory increase in another class. Some GLSs showed strongly positive correlations with each other because all these GLSs are carbon aliphatic GLSs and follow quite similar biosynthetic pathways (Table 4). A positive correlation can be based on shared biosynthetic pathways or common regulation factors. A negative correlation, on the other hand, indicates chemical trade-offs, as shown in the correlation analysis [55]. Several authors [30,56] have studied the variation between the GLSs concentration in roots and leaves, indicating the lack of correlation between the above cited organs. According to our results, the total amount was much higher in the leaves compared to the roots.. Regarding the concentration of GLSs in roots, we found that the total amount in the control condition was similar to the values reported by Li et al. (2021) [57]. A significant increase (41.4%) was observed under water stress conditions. According to the recently published work of Huang et al. (2022) [58], the total glucosinolates content of roots showed a decreased trend initially and then increased during development, which reached the maximum in the pod-setting stage. However, the result in the leaves was opposite to that of the roots. In the leaves, the total glucosinolates content increased at first and then decreased in the mustard' life cycle, which reached the maximum at the bolting stage.

To better visualize the relationship between plant organs and GLSs accumulation, a principal component analysis (PCA) of the GLSs data was performed (Figure 2). The results highlight the characteristics of glucosinolates in different organs, and it was concluded that the aliphatic glucosinolates were predominant in the leaves of Brassica, while the roots were correlated more with indolic and aromatic glucosinolate. Our result may be confirmed by the findings of Huseby et al. (2013) [59], in which the aliphatic and indolic MYB factors have been shown to be regulated differentially in Arabidopsis plants by the light cycling. This result was consistent with the correlation analysis of glucosinolates, providing a new insight into glucosinolate compounds in different organs and genotypes.

Based on the chemotypes detected, there is not a specific glucosinolate related to the resistance to water stress, but there is a wide range of glucosinolate levels and profiles that change depending on the genotype. The capacity to biosynthesize the glucosinolates has been used as a taxonomic indicator to support classification systems based on crop evolution [60].

Different breeding and selection procedures have been successfully performed for the GLSs content for different *B. oleracea* crops; for example, marrow stem kale was successfully improved by a low content in indole GLSs using a full-sib family selection program [61,62]. On the other hand, a divergent mass selection has been used as a useful tool in plant breeding to generate varietal groups within each of the *B. oleracea* crops that share the same genetic background but with very variable GLSs amounts and profiles in the different

organs of the plant. The perception that the *B. oleracea* products represent healthy food has increased in recent decades. Greater requests for these products in local and national markets in all EU countries requires additional accurate information on the best cultivars to use as well as the establishment of new cultivars with a high yield, are stress tolerant, and have a high nutritional value.

5. Conclusions

The results obtained showed a great variation in the main plant morphometric traits and the amount and profile of the GLSs in relation to both the drought conditions and the genotypes utilized. For all the variations obtained, we observed a highly significant interaction among the two experimental factors studied, and for that we have identified elite genetic materials to use for the organic breeding of *B. oleracea* crops. Among the accessions analyzed, we have individuated the kales BH1, BH2, and BH3, broccoli BR5, the cauliflowers CV3 and CV4, and finally the CCPP4. The accessions showed a low variation index for several of the morphometric traits observed. The prevalent presence of high amounts of GLSs in the leaves in comparison to the roots is of great interest for all the *B. oleracea* crops providing leaves as products, such as kale and sprouting broccoli. The GLSs value increased significantly as a consequence of drought conditions in accordance with the findings of previous studies, highlighting the importance of GLSs to increase the antioxidant status of the plant for controlling water stress. These results could be used to identify different accessions which could be utilized for a future breeding program aimed at creating genetic diversity in the local Brassica germplasm with a high value of a specific glucosinolate. Further research using a genomic and transcriptomic approach targeting some candidate genes is needed to confirm this finding.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13020579/s1, Figure S1: High performance liquid chromatography (HPLC) chromatograms of desulfo-GSLs standards; Table S1. Variation in the aliphatic glucosinolates content (μmol $g^{-1\ d.w.}$) in roots in relation to the two experimental factors studied. Table S2. Variation in the indolic and aromatic glucosinolates content (μmol $g^{-1\ d.w.}$) in roots in relation to the two experimental factors studied. Table S3. Variation in three aliphatic glucosinolates content (μmol $g^{-1\ d.w.}$) in leaves in relation to the two experimental factors studied. Table S4. Variation in other aliphatic glucosinolates content (μmol $g^{-1\ d.w.}$) in leaves in relation to the two experimental factors studied. Table S5. Variation in the indolic and aromatic glucosinolates content (μmol $g^{-1\ d.w.}$) in leaves in relation to the two experimental factors studied.

Author Contributions: Conceptualization: F.B., R.L.S. and S.M.; methodology: F.B., R.L.S. and H.B.A.; software: H.B.A., D.A. and S.T.; validation: F.B., R.L.S., N.F. and S.M.; formal analysis: F.B., R.L.S., D.A. and H.B.A.; investigation: H.B.A., N.F., M.C.D.B., S.T. and D.A.; resources, F.B.; data curation: M.C.D.B., D.A. and H.B.A.; writing—original draft preparation: F.B., D.A. and H.B.A.; writing—review and editing: F.B., R.L.S., H.B.A., S.M. and D.A.; visualization, F.B.; supervision: F.B.; project administration: F.B.; funding acquisition: F.B. All authors have read and agreed to the published version of the manuscript.

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