UNIVERSITÀ degli STUDI di CATANIA
Dipartimento di Agricoltura, Alimentazione e Ambiente
Di3A

UNIVERSITÀ DEGLI STUDI DI CATANIA

Agricultural, Food and Environmental Science

XXXVI Cycle

SELECTION OF A *RICINUS COMMUNIS* L. GENOTYPE AND IMPROVEMENT OF THE AGRONOMIC MANAGEMENT IN SEMI-ARID MEDITERRANEAN ENVIRONMENT

Valeria Cafaro

Advisor:
Prof. Salvatore Luciano Cosentino

Co-advisor:
Prof. Angela Roberta Lo Piero
Dr. Danilo Scordia

Coordinators:
Prof. Antonio Biondi
Prof. Alessandro Priolo

Ph. D. attended during 2020/2023

Acknowledgements

At the beginning the Ph.D. seemed to be a long journey, but looking back I actually realized how quickly time has passed and how much I have grown since the beginning.

I will always be grateful to the Ph.D. because it allowed me to meet wonderful people who encouraged and supported me during this path.

My deepest thanks go to my mentor, professor Salvatore Cosentino, a unique guide, always ready for any clarification, doubt and perplexity. His profound knowledge has been a source of constant admiration for me. I thank him for the support he gave me, for teaching me everything, and for accompanying me this far, I admire him deeply.

I also want to say thanks to Professor Giorgio Testa from the bottom of my heart. Although he is not my academic tutor, I consider him my moral tutor. I genuinely thank him for his constant presence, for his kindness, and reliability. I thank him above all for also being a dear friend, as well as an exceptional professor and human being.

A great thank is also addressed to the exceptional doctor Cristina Patanè. My most sincere thanks for the help she has given me through these years, for giving me the opportunity to learn from her profound knowledge, and for passing me her passion for the research.

I would also like to thank National Institute of Social Security (INPS) for allowing me to participate to an 'Industry 4.0' scholarship and for founding my research project

A special thanks goes to all my colleagues, Elena, Concetta, Alessandra, Rachele, Vivienne, Carmela, Simona, Andrea, Silvio, Alessio, Roberta and Luigi who together with professors Cosentino, Testa and Dr. Patanè, have become my second family.

I particularly thank my super colleague and doctor Antonella Iurato, who has illuminated this path for just over a year, but who has entered my life as a deep and sincere friend, and my great supporter.

I would also like to thank the beautiful souls I met during my time abroad. I thank Dr. Efhytimia Alexopoulou and Professor Luisa Trindade, who welcomed me into their institutions with unique kindness and professionality.

I thank Antonio, Mohamed, Ornela from the Netherlands, and my dearest Greek friends George and Kostas.

I also dedicate a thank you to my international friends, my biggest fans Elise and Yuka.

A special thank goes to my best friend Martina, my twin and supporter. I thank my dearest friends Anna, Giulia, Giulia, Ylenia, Francesca, Conni, Simona, Marika, Martina, Vera, Benni, Silvia and Giorgio. Thank you for being part of my life every day.

I would also thank a special person who has always been part of my life.

Lastly, I dedicate this thesis to all my family, you mean the world to me and without you I would have not been here. A thank to Rosy, Giulia, Gianluca, Dalila, Mariolina and Salvo.

I thank my mom, my model, who inspires me every day. I love you deeply.

I thank my sister, my half. I admire your strength and goodness endlessly. I love you.

A special thank to my dear dad, who always guides and protects me, I know you would have been proud of me, I love you and I miss you.

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Abstract

The current world scenario is characterized by an extreme climate crisis, a response to the overexploitation of natural resources. Greenhouse gas emissions, agricultural intensification, population growth, and demand, accentuate severe climate change. To overcome these increasing problems, the use of fossil fuels must be reduced to a minimum, and the enhancement of energy crops becomes a valid solution to the request of obtaining renewable energy.

In line with European policies, such as the Agenda 2030, and the Renewable Energy Directive (RED), aiming to increase the energy independence of the European Union and the obtainment of 'clean and green energy', the production of biofuels is a valid alternative to meet these ambitious goals.

In this context, the adoption of crops, cultivated in marginal lands, which does not compete with food production, and the possibility of exploiting these degraded areas, become one of the main topic on which to focus for further scientific research.

Within this framework, the present thesis focused on the selection and the improvement of the agronomic management of castor (*Ricinus communis* L.) in order to assess the adaptability and potentiality of this crop in the Mediterranean region.

Specifically, the research activities attempt to: (i) review the current knowledge on the adaptation capacity of castor in the Mediterranean environment; (ii) select a local genotype adapted to the Mediterranean climate; (iii) study of the germination temperature requirements of local and dwarf genotypes of castor; (iv) evaluation of the response to salinity stress in the germination of different castor seeds; (v) assess the best sowing date and comparison between the local genotype and dwarf hybrids.

Overall, the present research highlighted the best combination between genotypes and environment of cultivation, in relation to temperature and salinity. These results can provide a valid base for further studies and for the exploitation of castor in the Mediterranean.

Keywords: *Castor bean, seed yield, oil yield, oil content, cardinal temperatures, seed vigour, synchrony, phenology, radicle length, hydrotime model*.

Riassunto

L'attuale scenario mondiale è caratterizzato da una crisi climatica estrema, risposta allo sfruttamento eccessivo delle risorse naturali. Le emissioni di gas serra, l'intensificazione dell'agricoltura, la crescita della popolazione e la richiesta alimentare, accentuano i gravi cambiamenti climatici. Per superare questi incessanti problemi, l'utilizzo dei combustibili fossili deve essere ridotto al minimo, e la valorizzazione delle colture energetiche diventa una valida soluzione alla richiesta di ottenere energia rinnovabile.

In linea con le politiche europee, come l'Agenda 2030, e la Direttiva sulle energie rinnovabili (RED), volte ad aumentare l'indipendenza energetica dei paesi sviluppati e l'ottenimento di "energia pulita e verde", la produzione di biocarburanti rappresenta una valida alternativa al raggiungimento di questi obiettivi ambiziosi.

In questo contesto, l'adozione di colture, coltivate in terreni marginali, che non competono con la produzione alimentare, e la possibilità di sfruttare queste aree degradate, diventano uno dei temi principali su cui concentrarsi per ulteriori ricerche scientifiche.

In questo quadro, la presente tesi si è concentrata sulla selezione e sul miglioramento della gestione agronomica del ricino (*Ricinus communis* L.) al fine di valutare l'adattabilità e le potenzialità di questa coltura nella regione del Mediterraneo.

Nello specifico, le attività di ricerca tentano di: (i) rivedere le attuali conoscenze sulla capacità di adattamento del ricino nell'ambiente mediterraneo; (ii) selezionare un genotipo locale adattato al clima mediterraneo; (iii) studio delle temperature di germinazione richieste da genotipi locali e nani di ricino; (iv) valutazione della risposta allo stress salino nella germinazione di semi di ricino; (v) valutazione della migliore data di semina e confronto tra il genotipo locale e gli ibridi nani.

Nel complesso, la presente ricerca ha evidenziato la migliore combinazione tra genotipi e ambiente di coltivazione, in relazione a temperatura e salinità. Questi risultati possono fornire una valida base per ulteriori studi e per lo sfruttamento del ricino nel Mediterraneo.

Keywords: *Ricino, resa in seme, resa in olio, contenuto oleico, temperature cardinali, vigore, sincronia, fenologia, lunghezza radichetta, modello hydrotime.*

Aim of the thesis

The main topic of the current research is to evaluate and select innovative energy crops to be used in the Mediterranean environment, to enhance marginal areas and limit the use of fossil fuels, as required by European policies, responding to the incessant need to obtain 'clean energy', to be used in the years to come.

In this context, information regarding the possibility to increase the suitability of castor in Mediterranean environment, such as agronomic management, seeds germination requirements in order to improve the final yield and the oil content, the identification of the best period of sowing, the thermal thresholds and the effect of salt stress on the germination ability were investigated.

Within this framework, castor genotypes were evaluated to obtain either the best genotype, adapted to the climatic conditions of the Mediterranean basin, and the best agronomic practices to perform for castor cultivation.

In particular, the main research activities attempt to:

(i) Review the current knowledge on the adaptation capacity of castor in the Mediterranean environment;

(ii) Select a local genotype adapted to the Mediterranean basin;

(iii) Assess the best sowing date and the final yield by comparing local genotype and dwarf hybrids.

(iv) Study the germination temperature requirements of local and dwarf genotypes of castor;

(v) Evaluate the response to salinity stress in the germination of different castor genotypes.

1 The adaptability of castor in the Mediterranean environment or semi-arid environment: an emerging oil crop

1.1 Introduction

The production and consumption of renewable energy is constantly increasing, thanks to the policies implemented in many countries trying to increase their energy autonomy, contribute to the reduction of carbon dioxide emissions and support agriculture. It has been estimated that the renewable energy will grow by around 50% in 2050, becoming the major source of energy, as reported in Figure 1.1. In line with the Kyoto Protocol, the Agenda 2030 and the Conference of the Parties, the European Union has issued a series of directives aimed at combating the greenhouse gas emissions into the atmosphere (GHG), trying to reduce them by 80-95% by 2050 [1].

Trying to cope with the exponential increase in oil and coal consumption around 2050 [2], renewable energies fit perfectly into this scenario as the only possibility to guarantee energy sources continuously and constantly over time, and although the process of affirmation is difficult, there is no doubt that there is a need to obtain "clean energy". Scientific research and technological innovation will therefore be essential for the realization of these ambitious goals. Biofuels, such as biodiesel and bioethanol, deriving from the transformation of oil-bearing and starchy plants, appear to be able to fully satisfy this new need [3]. Their production could not only satisfy the demand for sustainable energy, but would also make it possible to enhance those marginal lands where no-food crops could be easily introduced, without depriving farmers of the land necessary for food production.

Since the economic boom, a great interest in the vegetable oil sector began to grow. Sunflower, soybean and rapeseed proved to be the main crops on which to conduct research for alternative crops. Even if castor bean was not immediately the protagonist of the Italian market, the situation began to change in the 1980s when the Ministry of Agriculture and Forestry recognized its countless uses in the

chemical, mechanical and pharmaceutical sectors, and issued some laws, aimed at evaluating the possibility of cultivating castor in Italy [4].

In addition, the growing unavailability of arable land due to constant climate change should also be taken into consideration. Marginal, abandoned lands, with fragile and disadvantaged soils in arid and semi-arid regions, consequently seem to be the best alternative to minimize competition in land use for agriculture and for trying to reduce the effects of greenhouse gas emissions and the loss of biodiversity (The World Bank, 2003). The enhancement of marginal lands in the Mediterranean environment for the production of biofuels and bioenergy is the new goal of global policies [5]. Thus, the aim of this review is to study the inclusion and adaptability of castor in the southern regions in order to improve its cultivation and its productivity in the Mediterranean landscape.

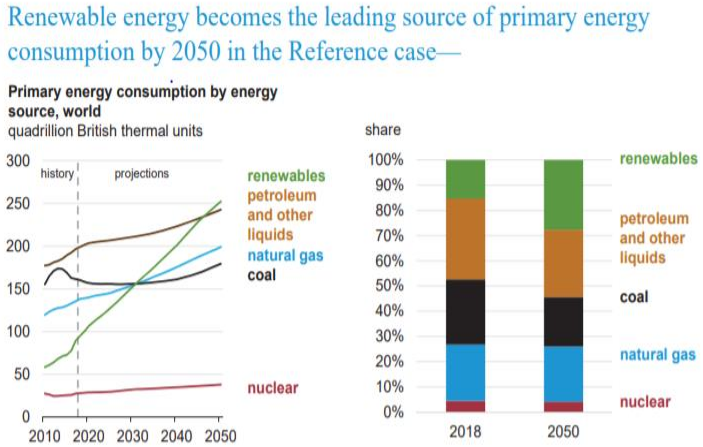

Figure 1.1 - Renewable energy increases planned for 2050
Source: U.S. Energy Information Administration, International Energy Outlook 2021

1.2 Establishing Ricinus as an advantageous oil crop in Europe: Economic importance

1.2.1 The oil world scenario

The annually production of lubricants is around 30 and 40 million tons. Lubricants have wide industrial applications such as reducing friction and heat, protecting against corrosion, transmitting energy and other activities. However, about 50%-70% of production is poured into the environment [6]. Lubricants, which are 95% oil-based, have uncontested, non-renewable environmental consequences [7] [8].

For this reason, in recent years there has been a substantial increase in the production of biofuels considered as the main source of energy that can be produced even in a geographical area with limited resources [9]. The goal of the scientific community is to develop new products with higher biodegradability and lower toxicity. Bio-based sources have developed exponentially since 2002 [10]. Indeed, the vegetable market for industrial oils was valued at USD 29.220.6 million in 2020, while it is expected to increase by 4.13% around 2021-2026 [11]. In this picture, Italy plays a major role in Europe, ranking third, after Germany and France, both for energy consumption from RES (21.9 Mtep), and for total energy consumption (107.6 Mtep). The GSE confirms that the share of energy consumption in Italy from renewable energy sources is 20.4%, thus exceeding the target set for 2020.

Hence, the recent increase of the vegetable oil market plays a very important role in Europe and the past twenty years have seen the introduction of different crops categorized by type, application, geography and above all, generation. In particular, according to the type of biomass used, crops can be divided into two groups of biofuels. First generation biofuels derive from food crops such as rapeseed, sunflower, and soybean; and second generation biofuels are produced

from non-food biomass such as miscanthus, Switchgrass, Willow and castor [12].

After the adoption of the U.S Renewable Fuel Standard in 2005, which set requirements for a minimum volume of renewable fuels in the fuel that has to be transported and sold in the USA, there has been a spread of first-generation biofuels. Their introduction to the market has given not only economic opportunities but also a more environmentally friendly approach necessary to limit the consumption of fossil fuel. However, biofuels production affects food prices and food security. In fact, the need for crops such as sugarcane and corn to use as biofuels has increased widely, influencing global food systems especially in developing countries. Other negative aspects include effects on greenhouse gas (GHG) emissions, air pollution, water consumption, pollution, deforestation, biodiversity loss, and rural development [13].

These concerns have increased the interest in second-generation biofuels that are more suitable to grow on land not used for food production, or in marginal and degraded land. Actually, the possibility of using second-generation biofuels has enormous vantages, for instance considering that they are perennial crops there is no need to replant them every year. As for *Miscanthus*, it is productive for years once established and contrary to annual crops, they require less water, fertilizer and pesticides [14].

1.2.2 Castor scenario

In this context, castor (*Ricinus communis* L.) fits perfectly as an industrial oilseed crop with ample possibilities, to be included in today's panorama. Indeed, castor oil content is around 35-65% (average of 50%) [15], which is really high in comparison with the oil content of other oilseed crops such as soybeans, sunflower seed, rapeseed and palm (Table 1.1) [16]. The range of variation of the oil in castor can vary because of the genetic difference of the cultivars

[17]. The oil contains 85-90% of ricinoleic acid (RA, 12-hydroxyl-cis-9-octadecenoic acid), an oleic acid derivate used in many industrial applications as a source of unique fatty acid. The RA content makes castor the only natural crop with such a high concentration, favouring its growing interest in the last few years.

Table 1.1 - Oil content of major oilseed crops

Crop	*Oil content %*
Castor bean	35-65
Palm	30-60
Rapeseed	30-50
Sunflower	25-48
Soybean	15-22

The rising use of castor has increased the market's volume, which is expected to reach US$ 1.54 billion in 2026. In 2018, castor oil was at 751st position as the most traded product, with a total trade of $1,72M. In the same year, the top exporter's countries were Mexico ($1.54 M), Egypt ($83,5K), Philippines ($46,3k), Kenya ($18,5k) and Ukraine ($17,9k). While the most importers were United Stated ($1.57m), Croatia ($62,5k), Uganda ($17,5k) and Kyrgyzstan ($9,86k), ranked 10th as the most imported product (https://oec.world).

Nowadays, China, United States and Europe are the main actors of the current market, where China represents the main importers with an import value of 41,4%. While India is ranked 1st as the main exporter with a value of export of 87,4% equal to $1,1B. India is then followed by some European countries such as the Netherlands (4,02%), France (2,96%), Germany (2,14%).

1.2.3 Castor's challenges towards the market

The growing use of raw material, the inclination towards plant-

based products, and the need to have less impact on the land used for agricultural productions seem to guide the market towards the growth of castor, although the percentages indicate that Europe is still heavily dependent on imports. This is attributable to different aspects that regard not only its cultivation but also its inner properties.

As a matter of fact, the presence of many toxins constituents like ricin, ricinine and certain allergens makes castor not widely used and exploited [18]. In particular, ricin is a powerful toxic protein that is present in the endosperm of the seeds and can lead to death, even in small quantities. Indeed, Ricin is considered one of the most poisonous substances existing[19]. Among seeds and leaves, which contains smaller quantities of the protein than the seeds, only the oil can be used for human needs because the protein is insoluble in the oil and any residual is discarded by the refining process [20](Severinoet al. 2012). Although studies have proved that high temperature can denature the proteins, the concern is still widely spread, even because of the enormous quantities of cases reported of people poised because of the voluntary or involuntary injection of ricin [19]. Another toxic protein is RCA (*Ricinus communis* agglutinin) which causes the coagulation of red blood cells.

Moreover, although castor oil has a great potential as biofuels, it is not widely marketed because of its hygroscopicity that causes a high water retention and can lead to algae growth, filtration and corrosion problems. Furthermore, problems during extraction and injection could be caused because of its high viscosity at temperatures under 50% and higher compressibility [21] that lead to incomplete combustion, atomization, deposition of carbon on the injector, cocking, higher engine deposits and engine problems during winter seasons [22].

Another impacting factor is due to the enormous handy work required by the crop. Castor seed's ripening is heterogenous, which is negative because prevents the use of the machine for the harvesting

that has to be carried out by hand. Manually harvesting increases costs for production and the relative by-products [23]. Furthermore, considering that the capsules are harvested when completely dry, their heterogeneity also causes problems of seed loss for shattering, as sometimes the greatest number of capsules is expected to be ripe before carrying out the harvest [24]. The intense labour work necessary makes the oil price of castor higher than other vegetable oils [25]. The biodiesel chain itself requires lower production costs because it is already affected by 70-80% from the cost of raw materials necessary [26]. This makes harder the competition that castor has to face with other more profitable and cheaper oilseed crops such as rapeseed and soybean.

1.3 Origin and distribution of the Palmus Christi

The history of castor is very ancient. The first literary appearances of this crop go back to the Book of Jonah "Then the Lord God provided a leafy plant and made it grow up over Jonah to give shade for his head to ease his discomfort(…)But at dawn the next day God provided a worm, which chewed the plant so that it withered."(Jonah 4:6-7) . Even though Jonah did not give any specific description of the plant, the Talmudists make it back up to the "oil of kik" which is the same "kikajon" described by Jonah.

However, it seems that its roots are even more ancient. Egyptians were used to keep a "Medical Papyri", also known as "The Papyrus Ebers", in which all the recipes and medical uses were collected by sages and philosophers. In these Hermetic Books, huge importance was given to the castor-oil plant, regarded as a magical drug because of its different and beneficial properties [28].

Numerous sources report the use of castor as a medicine in the early medieval period: it is reported that Albert the Great, Bishop of Regensburg, cultivated it in the mid-thirteenth century, but subsequently its cultivation went decreasing. It continued to be used

as an ornamental plant, until XVIII century in Europe where it was cultivated as a medicinal plant, although in small plots. More evidences are afforded by other ancient texts, which allow us to understand its indispensability within the common uses.

It is also interesting to outline the origin of the name. *Ricinus* is the Latin name of "tick", because the seeds have resemblance of the ticks' bodies, meanwhile *communis* is the Latin word for "common"; according to Linnaeus, this is the origin of *Ricinus communis*. Before the 18th century *Ricinus* was always introduced as "Christ's Palm" or "Palma Christi" because the leaves give resemblance of a hand and because its oil can heal wounds and ailments, as it is the Hand of Christ [28].

The name castor can be misguided because of the generic name of *Castor Canadensis*, the North American beaver. It is also the name of one of the two bright stars of the Gemini constellation - Castor and Pollux also known as the Gemini twins. Moreover, ancient Greek described Castor as one of the children born from the union between Leda and Jupiter. The latest version wants the name to have been coined by English traders, which mistook its oil for the oil of another crop [29].

As well as for the name, the centre of origin was not easily identified. In fact, the identification of the center of origin takes two distinct and separate factions. Some researchers are on the opinion that it is indigenous of Africa [30]. While other researchers claim that it is native from India [31]. One of the reasons why identify castor's origins is so difficult is because castor has 4 centers of diversity, based on morphological variation, (a) Ethiopian-East African, (b) North-West and South-West Asia and Arabian Peninsula, (c) Subcontinent of India and (d) China. [29]. Anyway, Ethiopian-East Africa is considered to be the probable site of origin because of the higher diversity present in it [32]. Moreover, given that the genetic material distributed in Eastern Africa has an arboreal phenotype, with a single

elongated trunk, a dehiscence capsule and small seeds, it is suggested that this represents the wild relative of the domesticated castor bean [33]. Once established, it spreads rapidly, which is why it is considered as an invasive crop in several countries (Weber 2003).

Nowadays, it is developed and it is produced easily in the African continent (221.130 ha), through the Atlantic coast to the Red sea, Tunisia to South Africa. It is also cultivated in the tropical and subtropical regions of America (162.203 ha) and Asia (1.151.200 ha) and in most of Europe [34], in particular this crop plays a huge role in the economic sector of Romania and Yugoslavia.

Germplasm studies reveal low levels of genetic variation and a lack of geographically structured genetic populations in castor oil. Therefore, seeking to broaden knowledge on the genetic diversity of castor oil, studying what genetic alterations occurred during domestication is essential. Xu et al., 2021 through a genome assembly study at the de novo chromosome level of the progenitor of castor, sequencing and analysing 505 accessions worldwide, confirms that the accessions found in East Africa are the wild progenitors of castor oil and domestication dates back to 3200 years ago. In addition, a notable climatic event that occurred about 7000 years ago in Turkana, could be the cause of the genetic bottleneck that led to significant genetic differences between populations in Kenya and Ethiopia.

Other researchers suggest, through sequencing studies, that a large amount of genetic variation is present in the genome of castor, contradicting what has been said so far about low genetic diversity [36]. Further studies on wild germplasm are required in the future.

1.4 Biological and genetic features

Belonging to the Euphorbiaceae family, *Ricinus communis* L. is a non-edible oilseed crop. Despite the fact that it is known as “Castor Bean”, the plant does not really belong to the Fabaceae family and the seeds are not true beans. Castor (2n = 2x= 20) is generally a cross-

pollinated diploid plant but actually it has a mixed pollination system that favours also self-fertilization by geitonogamy. Even favouring geitonogamy, it seems that castor bean prefers and has significantly higher production pollination results due to cross pollination which easily happens for anemophilia or entomophilia [37]. The cross-pollination rate is 50-80%, which depends on the genotype and the environmental conditions. In some dwarf cultivars, it may exceed 90% [38].

As for the sexual expression, it is normally recognised as a monoecious plant meaning that the pistillate flowers are on top of the panicle and the staminate flowers are on the lower part. However, it is a sexually polymorphic species and different forms of sexual expression can be shown. Aside from the normal form of monoecious plant, it can also be an interspersed monoecious with both the pistillate and staminate flowers interspersed in the rachis [29].

Furthermore, at the early beginning of its classification, the genus *Ricinus* included different species and subspecies. In particular, around 91 species were identified among its genus. Despite this belief, now it is considered that all the species and subspecies are included within the genus *Ricinus communis*. It is not possible to consider them as separate and different species, because all of them are easily intercrossable and produce fertile intermediates. Despite different researchers and studies confirm its diversity, actually the variability is not that high and never exceeds the characteristics of *Ricinus communis* and most of the actual variability is due to the uncultivated wild and semi-wild castor plants which are widespread in the world [39].

1.4.1 Botanical features

Castor is as an annual herb or a perennial shrub of around 1-7 m height, depending on the climate and soil types in which grows. Swetman, (2000) describes the plant as a perennial plant in semi-

tropical environments, even though the plant cycle tends to be multi-annual. However, in temperate environments, frosts and low temperatures cause the death of the plant, so it is preferable to grow the plant as an annual crop [16].

Castor's leaves are petiolate, stipulate and alternate, with the exception of the first node in which two opposite leaves are inserted, opposites, expanded, peeled, palmate-lobed with 5-11 lobes and ribs prominent on the lower page, equipped with nectariferous glands at the base of the lamina and petiole. The colour is normally green and varies from light to dark depending on the level of anthocyanin pigmentation. Moreover, the colour of the leaf is similar to the colour of the stem; therefore, if the stem is green the leaf lamina will have the same colour. According to the cultivar, the size of the leaf is different; some varieties have large leaves while others only have small leaves. The length ranges from 15 to 45 cm [41][29].

The root system is a well-developed taproot that can reach considerable depth, up to 5 m underground. From the main root, lateral roots develop characterized by an accretion limited to 90-120 cm in the soil. Tertiary roots do not reach lengths longer than 30-45 cm [33].

Kole & Rabinowicz, 2018 described the stem as erect, cylindrical, and branched. The stem is often fragile and is glabrous and glaucous. The main stem ends with an inflorescence. Once the main inflorescence is settled, from the first node below, the sympodial succession of ramifications begins and each branch ends with a secondary raceme that can originate other racemes. The recorded length ranges from 60 cm for dwarf varieties to 5 m. When the cob develops, 2-3 sympodial branches grow from each node. Development is sequential and the plant will have several inflorescences, according to the stages in which it is. Different colours have been recorded for castor stems. The typical colour varies from green to reddish depending on the variety (Kole & Rabinowicz, 2018).

The inflorescence is an erect monoecious raceme, of around 10-

40 cm length, depending on the cultivar. Female flowers have petals with a deciduous calyx of 3-5 sepals. The ovary is supero trilocular with three styles of lobosis of variable colour from red to yellow and with papillate surfaces. Male flowers consist of numerous branched stamens, each of which ends with a small yellow anther that will release the pollen, after which the male flowers fall. The plant becomes sexually ready in the first year after the sowing. Flowering occurs early, even after 40-70 days after sowing. African spontaneous castor blooms after 140 days from sowing [42] [40] [43].

Allogamy is promoted by anemophilous pollination, in fact the anthers discharge the pollen which is carried to the stigmas by the wind. After the pollination occurs, the dry stamens fall off and leave the pistillate flowers on, which become a spiny or spine-less capsule [44]. After the fruit set occurred, the development of the fruit start and in about 45 days, the plant reaches the physiological maturation; 25 more days are necessary to reach the harvest ripening. The fruit is a spiny capsule that can contain three ovoid seeds. The size of the seeds varies from 7 to 25 mm in length and from 5 to 15 mm wide. Seeds contain 40-60% oil, 20% substances like albuminoids, cellulose, rubber, resins, and salts, as well as a glycoprotein, ricin, and an alkaloid, the ricinin, highly toxic. Seeds may have a dormancy of a few months or even several years [43] [44].

The fruit is a spiny capsule, which contains three ovoid seeds, equipped with a white, orange, red or brown caruncle, depending on the variety. The maturation of the capsules on the raceme proceeds gradually, starting from the bottom to the top, but not homogenously. When ripe, the capsules become darker and open (dehiscence) or fall, especially in the wild types, much less in the cultivated forms [29]. The seeds' size varies from 7 to 25 mm in length and 5 to 15 mm in width. Some seeds can show a dormancy of a few months or even several years. A plant of 8 m in diameter can produce around 150,000, rather than a small of about 1 m in diameter that can produce around

1500 seeds. In some spontaneous varieties the capsules, once dried on the raceme, undergo a total dehiscence.

1.4.2 Phenology

Gervasio et al., 2016 described the phenological stages of castor in a phenological growth model by grouping them into 4 main development stages and 15 sub intermediates stages. The first stage (Stage1), corresponds to the initial slow growth and includes 3 sub stages that follow the sowing. Emergence (Ve) which occurs 15 days after the formation of the cotyledons, Seedlings (Vm) with the formation of two complete leaves, after about 38 days from sowing and 85 days after sowing, the last sub stage, differentiation (Vo) is reached, representing the end of the first phase of growth [46]. The second stage of development, which corresponds to an accelerated growth of the plant, includes 5 sub stages, of which in reality only F1 inflorescence appearing (F1), anthesis (A1) and fruit set (G1) are the main ones, while the others correspond to the subsequent formation of secondary racemes. F1, formation of the main inflorescence at 100 days DAS. A1, main raceme flowering, G1 beginning of fruit set of the main raceme. The third stage corresponds to the productive or intermediate stage, it includes 6 stages of which end of fruit formation (G2, and main racemic ripening (M1) are the main ones. While the other sub stages correspond to the overall formation of the secondary and tertiary racemes. The last stage, phase 4, includes only M3 maturation, indicating that the maturation of all the racemes of the plant are ripe and ready for harvest and is clearly indicated by the plant that is beginning to lose its leaves (Figure 1.2).

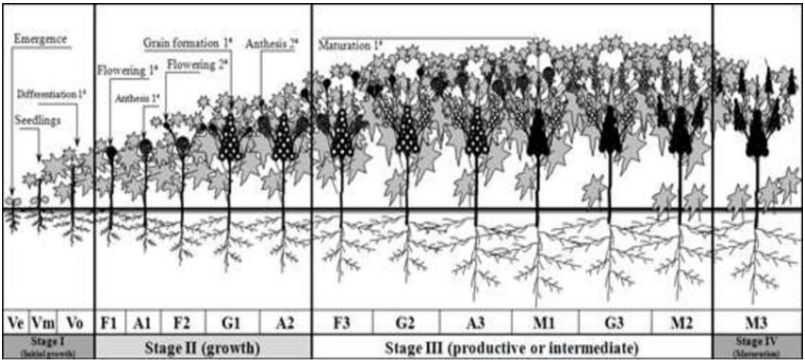

Figure 1.2 – Phenological stages of growth in castor

1.5 Ecology

Ogunniyi D.S. 2005 [47] states that in geographic areas characterized by temperate climates, to obtain a regular ripening and a high percentage of oil in the seeds, castor must be grown in well-sunny environments and located at moderate altitudes. While others authors affirm that castor is a rustic species and a warm season crop and as such is able to adapt moderately well to a varied amount of environments and climatic conditions [29][48].

1.5.1 Temperatures requirements

Castor is commonly grown in countries with 40 ° S to 52 ° N, even if in Russia some varieties can be grown till 52 ° N [29]. The cultivation area provides for altitudes ranging from about 1500 to 2500 m, even though the major factor limiting its growth remains the low temperatures, which is why in areas with severe frosts its cultivation is restricted to 500m [42]. The seed germinates in 7-14 days with soil temperatures of 16-17° C at a depth of 10 cm. The optimum for growth is between 20 and 26 °C. The optimum temperature for flowering is between 24 and 26 °C. Temperatures below 15 °C significantly reduce pollen vitality. Temperatures around 40 °C can cause flower's desiccation. The warmer the area, the higher the productivity of the plants and the percentage of oil in the seeds. However, it seems that

the oil obtained in temperate environments have a better quality [49]. Further studies are requested.

1.5.2 Light requirements

Castor is a long diurnal plant, but adapts to conditions of quite different length of the day, even if with a reduction in the production of seeds. Studies carried out in Russia indicate that the species develops normally with a duration of the day of 12 hours up to 18 hours [42].

1.5.3 Pedological requirements

Castor grows well in any land it is cultivated, it has no particular pedological needs, which is why it is considered an ideal plant to be used in marginal land [50]. However, it is preferable to grow castor in fairly deep, well-drained, and fertile soils with acidic conditions and avoid clayey, poor drainage and marshy soils. The pH of the soil that best fits the acid requirements of castor is around pH 5.0-6.5 but also tolerates pHs of 4.5 and 8.3. However, when the pH is above 8, the cultivation of castor is particularly limited due to the structure of the soil and its physical properties [29]. It tolerates dry arid conditions and grows moderately in conditions where the soil's water retention capacity is not maximum. Contrary to high crops, overly fertile soils are undesirable as they favour vegetative growth to the detriment of seed growth [33] [51]. It grows well in the sandy soils of North and South Africa, Australia and India, Central Africa and Brazil. For this reason, castor is cultivated in marginal areas, in which other crops cannot grow and this characteristic gives a high economic value. The high fertility of the soil promotes excessive vegetative growth and determines a lengthening of the biological cycle.

1.5.4 Water requirements

Castor grows during the rainy season in the tropics up to the arid areas of the subtropical (ideal rainfall 750-1000 mm), but in South Africa high yields are obtained even with a water availability of 375-500 mm. However, if the crop is grown as annual at least 100 mm of water is needed during the 4 months of growth. The critical stage of growing is the flowering stage. Soil humidity (or moisture) is needed in order to obtain a quick germination (7-14 days), while in the Mediterranean environment, the rains of late summer and autumn favour the emission of new racemes, causing harvesting problems. In marginal areas and wild environments, castor is able to adapt to long periods of drought and dry soil conditions [52].

1.5.5 Nutrition requirements

Castor bean appears to be a crop that stresses and exhausts strongly and quickly the soil. It has been estimated that for a seed production of about 1.8 and 2 t, castor extracts from the soil 60-80 kg/ha N, 10- 32kg/ha of K_20, 10-18 kg / ha P_2O_5, 5-13 kg / ha CaO, 6-10 kg/ha MgO [18] [53]. Doz, 2012 stated that the nutritional requirements are higher in the capsule formation phase. Despite the rusticity that distinguishes castor, some studies have estimated that the use of nitrogen increases the yield by about 114% compared to untreated plants [50]. Similarly to [54] who found an increase of about 500% when a 120 kg N ha^{-1} of nitrogen was used.

Phosphorus plays an important role in root development and therefore in the ability of the plant to collect soil water; the use of this element by the crop depends on its availability near the roots themselves (Figure 1.3). It is recommended to distribute it along the rows. The extent of phosphate fertilisation should be subject to soil analysis, since some soils in arid regions have an abundance of this element. Potassium is absorbed by the roots, translocate, and used for the formation of flowers and fruits; a potassium deficit seriously compromises the production. Depending on its availability, sometimes

its use is unnecessary.

Very often castor is fertilized using manure, ranging from 400 to 500 q/ha in pre-sowing and coverage. The most commonly used fertilizers are minerals, which require about 4-5 quintals of mineral superphosphate, 2 quintals of ammonium phosphate and 2 quintals of potassium sulphate. However, binary and ternary fertilizers, more complex than minerals, are more effective and cheaper.

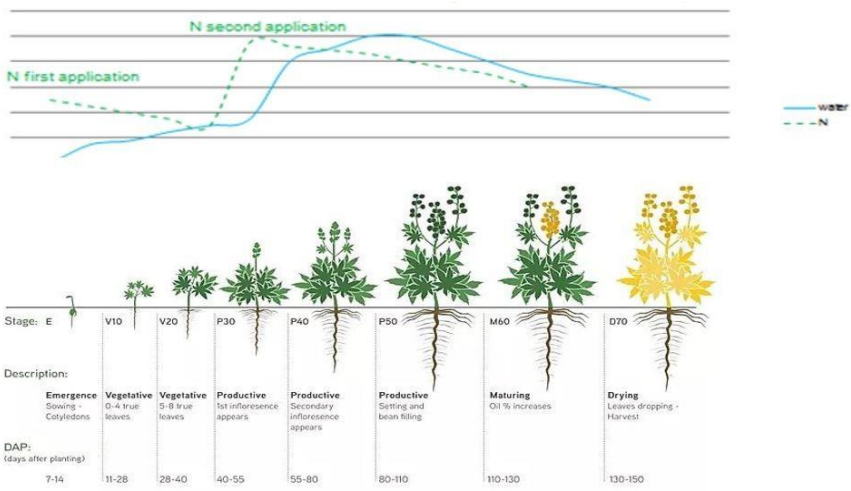

Figure 1.3 – Castor fertilization supplying

1.6 Breeding and variety development

Different researches, experiments and studies have been forwarded to castor's breeding programs in order to increase its adaptation, production and agronomic traits. Domingo, 1953 was

already highlighting how all the imported castor bean planted were not commercially useful because of the excessive plant height, stem size, low yield and the shattering of the capsule reaching the physiological maturity. Thus, back in the days, was already clear the necessity of having an efficient variety.

1.6.1 Dwarf varieties

One of the focus of research is the harvest, which is challenging because of the perennial growth attitude of the crop and because of the absence of proper harvest machine that avoid cracking, and splitting of the seeds. Therefore, manual harvest is the alternative even if the cost of production of castor oil and its by-products increase. Some mechanization trials have been done and are still going on, since castor is largely spreading in the market; however, there is still a large lack on dedicated machine.

The harvest has to be carried out quickly after ripening, to avoid fruit drop and the consequently fruit loss. However, the crop biological structure, the presence of different branches and racemes, their heterogeneous development and the different heights between the varieties [56] make it necessary to select highly performing genotypes. Most of castor programs are aimed on the selection of traits to be incorporated in new cultivars.

Baldanzi et al., 2015 conducted a study in order to select industrial genotypes, comparing 69 normal genotypes to 22 dwarf ones. The dwarf internode was studied as a morphological trait useful for mechanization. The dwarf genotypes have smaller branches and after the formation of the first one, two-three other racemes appear, reducing the height to around 65 cm making the mechanical harvest easier as the harvest height required has to not exceed 150 cm. Another study (breeding strategy) tries to select dwarf genotypes with high quality traits such us high grain yield, higher oil content and high physiological seed quality (related to germination and vigour). The

lines H4, H5 and H11 have an yield of 1400 kg/ha, resulting suitable for a direct selection based on agronomic and morphological traits: insertion height, number of racemes and stem diameter[58].

The adoption of dwarf varieties can be overcome by using Plant growth regulators (PGR), products used in agriculture as growth and development retardants. These products mostly act on the hormones concentration and specifically on the inhibition of gibberellin and auxin transport [59]. Campbell et al., 2014 studied the effect of gibberellic acid inhibitor with mepiquat chloride (MC) as an active ingredient. Based on previous studies conducted in cotton, in which this ingredient has a wide use as growth retardant, on castor, it was applied foliarly in two different stages of growth. However, the results showed that it was not effective in interfering with the plant height. Oswalt et al., 2014 confirmed, by studying the effect of applications of two different growth retardants Stance® and Pix®, how the effectiveness of PGRs on castor depends on too many factors and it is different among the species. Further studies are thus required.

1.6.2 Breeding improvement

Conventional methods and techniques have been performed for the improvement of castor traits such as mass selection and pedigree methods, but also genetic improvement can be exploited as an alternative and new frontier in its breeding. Indeed, mutations, hybridization and biotechnology are used for creating genetic variation and development.

In order to perform breeding programs, having knowledge of the germplasm is a key-factor. The uncertain center of origin makes it difficult to study the diversity of castor. Kallamadi et al., 2015 tried to figure out the genetic diversity of 31 accessions of castor from seven geographic areas by using random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR) and start codon targeted polymorphism primers (SCoT). Different levels of diversity resulted

from the study, probably due to factors such number of markers, genome coverage and natural evolution in which castor underwent through the years. However, the research concluded that the global level of variation is low even if the accessions studied come from different areas. Another study using different molecular markers, RAPD (randomly amplified polymorphic DNA), ISSR (inter-simple sequence repeats), AFLP (amplified fragment length polymorphism) and SNP (single nucleotide polymorphism) showed a low level of genetic diversity [63].

Auld et al., 2009 and Bertozzo et al., 2011 used mass selection to obtain high quality and heritable traits. Crops such as castor give successful results because of their reproductive system, which with the proper device, prevents cross-pollination and controls open pollination. Bertozzo et al., 2011 developed a line "NES" with a higher frequency of pistillate. Mass selection has been used for removing heterogenous traits from its genetic, such as flowering and number of capsules per raceme (Reddy et al., 1999).

The University of Catania has also conducted a study using mass selection for obtaining annual crop, short and compact, and with higher production from the main raceme and a multi-year cycle plant, with low insertion of the first raceme and long ramifications inserted in the basal part. The seeds of the genotypes used were collected from 162 Tunisian plants of the "polyracemal" type, with an average height of 157 cm and 64 plants of the monoracemal type, with an average height of 170 cm. From the total 226 plants, 55 genotypes were cultivated in the Mediterranean, carrying out the classic tillage of the soil, fertilization with 60 ammonium sulphate, 100 mineral superphosphate and 100% ETm. A further selection was carried out considering the ripening period of the racemes, selecting early racemes, and by selecting the plants from a productivity point of view, such as the number of seeds per raceme and their weight. From the initial 55 genotypes, 28 genotypes have been used in the final stage of

the selection. In general, the results obtained allowed the selection of genotypes with lower insertion height and earlier age. The height of insertion before the selection was 68.8 cm, decreasing to 62 cm in the new racemes, while the raceme length also decreased from 30.9 cm to 27.7 cm. While for the biological cycle, the recorded cycle is on average 119 days, with inflorescence appearing at 56.8 days after sowing and flowering at 60.

Among conventional methods, the back cross technique was used to set traits such as the dwarf habit, no spiny capsules etc., while studies using pedigree selection selected varieties with higher yield. The later selection provided homozygous genotypes through consecutive inbreeding for five or six generations. However, pedigree selection is known to create inbreeding depression within crops, given its nature of cross-pollinated crops, actually it can suffer from repeatedly and consecutive crossing. Even if there are divergent opinions regarding inbreeding, more studies are required.

Through crossing, genetic diversity is generated in castor collections. By the breeding of high quality sources of germplasm, cultivars like Dawn, Hale, Bakers 296, Cimmaron, Campinas and Lynn are created and then respectively used in other improvement programmes [66].

Service et al., 2002 obtained through intercrossing an open pollinated population, TTU-LRC. Eight parental lines were selected for reduced levels of ricin and *R. communis* agglutinin and for a dwarf internode, resulting in a valid source for other breeding lines. Another study focus on the harvest index (HI), which was really low, 0,1 [68], while in other crops can vary around 0.4 and 0.6.

The selection from germplasm accessions and from a line A74/18/10 has developed a natural mutant of castor, OLE-1, with an industrial relevance because of a low level of ricinoleic acid, around 140 g/kg, 6 times less than in the common wild cultivars. While for the oleic acid, the level is increased to 780 g/kg, 19 time more than in

common varieties [69]. The individuation of a natural mutation with such a high level of oleic acid, meaning higher oxidative stability could have wide and rich use in the industrial chain, making it essential the introduction of this mutation within the commercial castor genotypes [70].

550Gy r-rays gamma irradiation was used on seeds to induce mutations in a particular pistillate line of *Ricinus*, DPC-9, susceptible to leafhopper. Generations from M1 to M10 have been evaluated, leading to wilt-resistant pistillate lines like M-574 and M-619 [71].

The development of hybrids is possible thanks to the availability of sterile male lines. Indian and Israelian researchers have worked on the creation of proper hybrids to be used in agriculture. Indian hybrids, mainly selected for windy environments, have not been tested in the arid and semi-arid Mediterranean environments. On the other hand, the Israelian hybrids have been grown on experimental plots in Italy (Cadriano (BO) and Catania) and in Greece, with interesting results. An Israelian company, Kaiima, has commercialized and sold in the market five hybrids: Ariel, KS2020, KS2030, KS2135, KS2136. However, one of the main problems of using these hybrids is their high cost and the possibility of doing a mechanical harvest.

Currently, most of the studies are focusing on molecular adaptation mechanisms. Protoplast are thus studied for understanding the overall functions of genes and proteins. Bai et al., 2020 used the seeds of the cultivar Tongbi 5 for protoplast isolation. After growing the seeds on Murashige and Skoog agar in a growth chamber, cotyledons and leaves were used for obtaining the pellets with the protoplasts through a digestion process. To better investigate protoplast, a label GFP fluorescence is transferred and used for studying gene expression. Xu et al., 2016 is also studying plant specific GRAS gene families for their functions in regulating growth, development and stress responses. Based on genome-wide studies 48

specific GRAS have been identified in castor and validated through RNA-seq. The resulting expression showed how some genes were lost during the evolution (Os4, Os19, Os43 and Rc_Gras); while two genes 29661.m000923 and 30073.m002204 are strongly present in root tip tissue and related to root development. Other genes of the PAT 1 expressed in the leaves are involved in the phytochrome signal transduction [74]. Members of LISCL subfamily and from DELLA family were also identified, both involved respectively in the microsporogenesis process and the growth development [75].

Plant development is also controlled by transcriptional factors that involve epigenetic mechanisms of regulation. DNA methylation, histone modifications, gene expressions, cellular and physiological traits are frequently changed by epigenetic. Through a complex analysis on the transcriptome sequencing Han et al., 2022 provided an overall knowledge on seed-specific genes and the molecular basis for seed development, in particular DMV'S (DNA methylation valleys) are highly conserved and associated withthe evolutionary process of seed and leaves formation in many crops. The study was performed on different castor tissues and involved the identification of many tissue specific genes, around 1162. Different regulators were also identified within DMV's region, AGL61, AGL62, LEC1, LEC2, ABI3 and WRL1.

1.6.3 Stress adaptability

Mechanisms of tolerance are adopted by the plant during stresses. Due to climatic change and human activities, drought stress is one of the main reasons for soil marginality and it is described as the main limiting factor to crop productivity. To measure how castor is impacted by drought stress, researches evaluate if the overall productivity of the crop is reduced. One of the main topics of research is thus to select genotypes resistant to this stress. Nikneshan et al.,

2019 evaluated six ecotypes by using eight different stress indexes calculated analysing the yield, and four different moisture levels: no stress (30% water depletion), mild water deficit (45%), medium water deficit (60%), and severe water deficit (75%). The results show that one among the six ecotypes was tolerant to normal, mild and severe stresses, meaning that only one ecotype was still able to produce even under stress conditions. Another study evaluated how 45 castor genotypes respond to the stress. By using five different water levels and PEG (poly ethylene glycol) used to induce water deficit, the stress tolerance ability was evaluated. Through the research, the seed germination and seedling stage were confirmed as the most vulnerable, and it was not found relation between the germination rate and the subsequent yield.

The plant act on mechanisms of tolerance to survive and escape from hostile environments and to keep on with the growth. Mechanisms of drought tolerance are described as biochemical or morphological and include the maintenance of turgor pressure, the increase in elasticity of the cells, the decrease in cell size, the preservation of the photosynthetic activity and its apparatus, and stomatal closure. Considering the necessity of water during some developmental and physiological stage of castor, understanding how the plant behaves in these circumstances is important. Dos Santos et al. (2017) [78] conducted a study evaluating two different environmental conditions, semi – humid and semi – arid area, in Brasil. The vapor pressure deficit between leaf and air was calculated to study the stomatal response by calculating the difference between the saturation and the real air pressure, showing how in the semi – humid area was higher at 2:00 p.m and in the semi – arid area was higher at 12:00 p.m. Indeed, the VPD was higher in the semi – arid region because the plant kept the stomatal partially open. Stomatal conductance was low in both areas in the afternoon, and it was strictly related to transpiration, in fact in the semi – arid area the correlation

was high till the afternoon, in opposite with the semi – humid area in which it was high till midday. This seems to have a correlation in castor bean, as the vapour pressure deficit increase in the afternoon; the stomatal conductance is negatively impacted and there is intrinsic water use, enabling the plant to absorb more carbon and decrease the loss of water. Other studies, found that the plant regulates its transpiration in order to reduce water loss [79]. Moreover, the research highlighted how even if the temperature reaches a high value, the leaves of castor are still able to grow having mechanisms to adjust cellular homeostasis [80].

It is possible to affirm that castor bean shows tolerance ability to water stress conditions. Even if a severe lack of water and moderate lack of it, cause a decrease in plant growth. Moreover, the leaf water potential and the stomatal closure are related together as a response to tolerance mechanisms [81].

1.7 Agronomic management in the Mediterranean environment

Considering that castor is an oilseed crop, it is essential to obtain high yields for seed production. Seed yield depends on the number of racemes present per plant, the number of capsules per raceme and the weight of the seeds. Naturally, castor has many racemes due to the high quantity of branches present in the plant. The number of capsules per raceme depends on the number of female flowers present. The proportion between female and male flowers changes by raceme, genotype and sexual expression and it is mainly influenced by the environment [82].

In geographic areas characterized by mild climate, in order to obtain a regular ripening and a high percentage of oil, castor must grow in very warm and sunny environments located at moderate altitudes. Studies show that in the tropics and Mediterranean area, castor is a rustic species, capable of adapting to different conditions

pedoclimatic and that even in different environments castor respects the same parameters [47]. Studying and understanding which climatic, pedological and water characteristics castor prefers will allow an increase of seed yields and production.

1.7.1 Germination

The sowing of castor is carried out during the spring, between April and May, when the general temperatures are higher. In spite of that, several studies accomplished aim to verify the germination capacity of the plant even during different soil temperatures, analysing also how the plant reacts to autumn and winter sowing.

From previous studies performed on castor, it is evident that seedlings require a long period to emerge, referring to the time from sowing to the beginning of flowering on the first raceme. The differences found in the time of emergence are associated with the soil temperatures. A study conducted in Greece between 1995 and 1997 evaluated how many days it takes for castor to germinate. During three years of experimentation in two different farms, it was seen that in the area where the average temperatures were higher fewer days were needed for germination, ~2-19 days earlier than in the area with the lowest temperature. In particular, the microclimate had a higher average humidity (RH), an index of the climatic requirements of this crop [82].

Severino et al. 2012 highlighted how the slow, irregular and cold-sensitive germination of castor could be problematic and affect significantly castor's production. In fact, adverse climatic conditions, such as low soil temperatures, are the main consequences of the slowing down and inhibition of castor's germination in the regions in which it is cultivated.

Furthermore, divergences have been reported for the climatic requirements of castor. In the Mediterranean and warm temperate environment, castor is an annual spring-summer crop in fact the

minimum germination temperature required is between 14-15 ° C, while the optimal temperature is 31° C, with a maximum temperature of 36 ° C [18]. While Cheema et al., 2010 noted that 25°C is the optimum temperature for obtaining rapid germination of seeds. In general, germination and growing season are influenced by the sowing day and appear to vary according to the temperature of the soil: at 19°C, 10 days are required to 23 days at 10 °C Weiss (2000). While, 25 days are necessary with an average temperature of 12° C and 9 days are enough when the temperature is 22 °C [85]. Similar results were obtained from tests carried out in the laboratory by Cafaro et al. (2023) in which dwarf hybrids had a germination percentage higher than 80% with a temperature range from 25 to 32°C.

Different results have been shown with winter sowing. In fact, winter sowing occurred in November have a growing season of around 240 days, compared with spring sowing occurred in May in which the growing season is about 99 days. The interval between sowing and emergence also appears to be longer in winter sowing (~77 days) and shorter in spring sowing (~ 6 days). This is explained by the physiological germinative needs of the seed, which requires temperatures of about 15°C for its germination. It is possible to assume this temperature as the minimum thermal threshold for the germination of castor [48]. Laboratory experiment on castor germination confirmed that the lowering of temperature extend the germination time and that warmer temperature allow castor to germinate earlier. As reported by Cafaro et al. (2023) in which at temperature of 16°C the mean germination time for castor germination is about 30 days and at higher temperature (32°C), the MGT is 2.8 day approximately.

However, Anastasi et al., 2014 showed that in regions where the minimum daily temperature during winter is high enough, autumn sowing could give interesting results. In fact, it would improve the vegetative development of the plant, increasing its productivity and

improving the ripening of the seeds that could take place earlier. In particular, autumn sowing would allow the plant to be cultivated as a semi-perennial in coastal areas. In this case, rainwater would be the main water source for the crop. The study confirmed that the cultivation of castor bean during winter is feasible if the cultural needs of the plant are respected.

1.7.2 Soil preparation

Castor has to be treated differently if cultivated as an ornamental or as a crop for economical purposes. If grown as ornamental, the plant can directly be planted in late spring. Otherwise, it is possible to cultivate it as an indoor by planting it 6-8 weeks before the last winter frost, and then transplant it when the weather becomes warmer.

The soil has to be ploughed, harrowed and furrowed during summer and before the sowing, in order to arrange the soil and prevent the growing of weeds. The seeds' depth is around 7-9 cm and the space adopted for the sowing is 90-120 cm between rows and 40-60 cm between plants in the row. Approximately this would give a plant population of around 40.000-60.000 plants/hectare. The sowing is performed during the spring, which allows reaching higher temperature, requested for castor seeds' germination, between April and June. The sown is performed with a seed-drill or by hand dibbling. Moreover, it is suggested to use osmotic preconditioning of the seed with PEG (poethylene glycol), which promotes germination.

The soil preparation is carried out for the preparation of the seed bed and follows the following 4 steps: 1. Soil tilling before sowing; 2. Main soil preparation; 3. Complementary preparation and 4. Consecutive soil preparation.

1. The first soil preparation regards soils that have never been used before. The activities consist in the removal of bushes

and other spontaneous vegetation and in the possible levelling of the soil. This work can be carried out using bulldozers.

2. Considering the possible sandy nature of the soils, the main processing may be carried out by means of vertical dissolving by grubbers not exceeding 35 cm, so as not to introduce an excess of air into the soil resulting in the destruction of the little organic substance present. It can also be sufficient the minimum processing with surface dissolve or, in some cases (poor spontaneous vegetation) the non-processing, limiting the interventions on the soil to the strip interested in sowing, always with surface dissolve through cultivators (25 cm) or harrows (15 cm). It is possible to mix the minimal processing along the row, followed by sowing (strip-Tillage). In the case where the soil is compact, it is possible to use machines that operate a series of vertical cuts about 20-25 cm deep that facilitate the subsequent sowing operations. This processing can be carried out with growers.
3. Processes necessary to level the soil after the main work. In sandy soils they are not necessary.
4. Sometimes it may be necessary to work the soil in the interlocks to eliminate any weeds and reduce water loss by evapotranspiration. In this case, it is necessary to use harrows or cutters.

1.7.3 Sowing

The choice of the sowing period is related to the thermal needs of the crop. It is suggested to sow castor in spring (from March to April) in order to obtain the first seed production during the year (generally from July).

The air temperature must exceed 15°C to obtain germination of more than 90%. Sowing from June to September is possible but will provide a lower seed production. Sowing at a temperature higher than 40°C is not recommended.

Sowing is carried out manually or by seeding machines. Moreover, if cultivated as an annual crop, between the row the distance is 100 cm and within the row 30 – 50 cm, in order to obtain 20.000 – 30.000 plants/ha^{-1} and the quantity of seeds used is around 8 and 18 kg/ha^{-1}. While, if cultivated as perennial, the distance within the row can vary around 1,5 – 2 or 2,5 m and respectively between the row 2 – 2,5 or 3 m and the quantity of seeds can be between 2,7 e 1,1 kg/ha $^{-1}$. The seeding depth varies between 5 and 9 cm.

1.7.4 Irrigation requirements

The warm-arid environment of the Mediterranean favours the cultivation of castor, which has to take into consideration not only the high temperatures required by the crop but especially its high water requirement. In fact, water is the main environmental factor influencing the plant's growth and limiting its yield. The water supply of castor, unlike other crops such as oilseed rape, assumes a different meaning in relation to the biological stage of the plant. Considering that castor produces racemes at different times and each raceme can adjust the number and weight of seeds depending on the environmental conditions, under restricted water conditions, it is necessary to reserve water for periods of greatest need [86]. Whereas, when the volume of irrigation meets the crop's water needs, crop growth, flowering and pollination are prolonged. As a result, there will be an increase in the overall seed and oil yield [87].

Although castor is a crop with high drought resistance, the yield is reduced under water stress levels. Despite the conditions of water shortage, castor still manages to produce but with significantly lower yields. Castor oil consumes an average of 400 litres of water per kg of dry matter. In areas with summer rainfall, 500 mm of rain may be sufficient for the cultivation of dwarf varieties. However, its productive potential is reached with water availability of 600-700mm; even if interesting productions were obtained in South Africa with

precipitation of about 375-500 mm [88].

Studies conducted in the Mediterranean have shown that the intervals of castor production cycle are influenced by different regimes of irrigation. Copani et al. (1987) [89] showed that by supplying water from flowering to the maturation of the main raceme and by returning 100% of water (ETtm 100%) to the first 40 cm of soil, there was an extension of the cycle duration (about 87.4 days). While when the crop was irrigated until flowering the cycle was ~84.3 days and it was 78.3 days when the crop was irrigated only at sowing. The formation of the first-order raceme is also influenced by the amount of water supplied, highlighting how prolonged water stress can affect the formation of racemes, as demonstrated previously in the study [88] in which 70% of plants have not produced racemes of the first order. Thus, water deficiency can lead to cycle reduction but can also affect the formation of secondary racemes and higher yields are obtained with a prolonged supply of water. As confirmed by Calcagno et al. (2023) in which low levels of water induced a delay in the anthesis and the relative seed ripening in castor.

Moreover, the yield seems to be particularly influenced by the volume of irrigation supplied. Increases of 50% occurred when the water intake was increased from 1700 to 3200 m3/ha. The highest yield is mostly due to the number of racemes per plant, which, due to the effect of the volume of irrigation, increases significantly (Abbate, 1987b). More recent trials in the Mediterranean environment confirm that higher yields and hence the increase in biomass are achieved when external inputs are increased. In addition, if water stress is imposed during seed filling there will be a significant reduction in the number of seeds per capsule, number of seeds per plant and yield of a single plant. By applying water stress, Reza Sadeghi-Bakhtavari & Hazrati, 2020 achieved a yield of 1839.75 kg/ ha, compared to a full irrigation supplied that led to a yield of about 2600.33 kg / ha, as a result of the greater number of seeds and capsules per plant. Sowmya et al., 2019

reported that the number of seeds per plant is more reliable than the number of capsules per plant to determine yield. In addition, if water stress is applied during the seed-filling phase, there will be a reduction in the number of seeds and weight, which cause a decrease in yield.

Similar results were reported by Calcagno et al., 2021 showing that by irrigating at sowing and by irrigating from emergency until the maturation of the main raceme yields of about 15 t/ha are obtained, demonstrating and confirming how irrigation is one of the main factors influencing crop yield.

Considering the optimal environment of cultivation, the water use efficiency (WUE) has also to be considered for a complete adaptation. de Araújo Nascimento et al., 2022 studied the soil water balance components, which include the storage and the variation of water, the internal drainage, the capillary rise, irrigation depth and the rainfall. Soil aeration, soil water retention and water availability are influenced by the bulk density of the soil. The study of the soil is also an indication of the water retention of the soil. In soils where the percentage of clay is around 30%, retention will be greater, also due to a greater quantity of organic matter. Furthermore, the study highlights how the production potential of castor oil is fulfilled when there is at least 500 mm of water in the soil. The efficiency of the root system also affects the water content of the soil, particularly in the study the EBDA MPA 11 and BRES cultivars had, compared to the other cultivars, a quantity of humidity lower at 0.4 depth, an index of greater efficiency radical. However, the most interesting result is given by the water storage, which in the initial stages is low due to the demanding needs of the plant for production. Consequently, the lowest KC (crop coefficient) is recorded during the harvesting and physiological maturity phases, in which the culture no longer requires water.

1.7.5 Weed management

The vegetative growth of castor is very slow during the development phases, increasing the negative aspect caused by weeds, which are particularly competitive for the crop (Severino et al., 2012). The latter appears to be particularly sensitive to this competition [93] affecting yield and mechanized harvesting [94]. According to a study of Costa, Sofiatti, Maciel, Poletine, & Sousa, 2014 , the yield seems to decrease by 86% due to weeds. Furthermore, the sensitivity is maximum only in the first year of planting. Once the complete development of the crop has been reached, the fully developed aerial apparatus guarantee the inhibition of weeds [43].

The degree of interference is influenced by different factors related to a) pest community such as specific composition, density, distribution and b) culture as species or genotype, spacing and density c) time and period of coexistence between species; d) climatic and edaphic conditions of the crop [96].

Several studies try to establish the best time to manage weeds. Vitorino et al., 2012 specify that the critical period for preventing interference (CPPI) is between 9 and 41 days AED. While (Costa, Sofiatti, Maciel, Poletine, & Sousa, 2014) has identified the same period by indicating it around the second and 12th week after the emergency.

Different approaches are used against weeds, such as mechanical and chemical control which seem to be the most appropriate. Even though agricultural practices still being the most widely used. Crop management control provides for weed control through adequate soil preparation, crop rotation, intercropping and by choosing plant population [97]. Studies on the population to choose are still necessary, however dwarf plants, by reducing the weeding density, the space between the plants and consequently the use of chemical products, are able to reduce competitiveness with weeds. Despite their effectiveness, the use of herbicides is not common and used to combat weeds, due to the need to choose particularly specific

herbicides that do not interfere with the development of castor [95]. On the other hand, even though mechanical control is the most widely used and widespread method for controlling castor, due to its high costs, it is recommended to use it only for small properties.

Other external aspects negatively affect the growth of castor. Either direct factors such as competition for water, light, nutrients, physical space, allelopathy or indirect factors such as insects, diseases and nematodes [95]. Also the seeding density is a limiting factor, represented by plants that are too distant that leave room for weeds to develop (3.0 x 1.0 m for cultivars with large sizes and 1.0 x 1.0 and 1.0 x 0.5 m for dwarf cultivars)[98]. Thus, reducing the plant density is a very valid alternative[99], in fact using a density of 0.5 x 0.5 manages to reduce the CPP1 critical time from 3 to 25 DAE.

In the following years, more studies will be necessary in order to find the right weed management [95]

1.7.6 Diseases

Even if castor is considered a tolerant crop, many diseases affect its growth and consequently influence its economical production due to the consistent loss in crop productivity. Mostly bacteria and fungi are the pathogens affecting the crop's development. Under high humidity, temperature (around 25°C) and rainfall conditions, the spikes of castor are susceptible to gray mold (*Botryotinia ricini* described by Godfrey. G.H, 1919 or *Amphobotrys ricini* described by Buchw, 2021 in its anamorphic stadium), a fungal diseases spread worldwide [102]. The mold, first recorded in 1919 [100], is an Ascomycota belonging to Sclerotiniaceae, which attacks the inflorescence and the capsules in any development stage of the crop, that will lead to a total disorganization and breakdown of the infected tissue [103]. Other common diseases have been reported, such as Vascular wilt (*Fusarium oxysporum* f.sp. ricini Nanda and Prasad), first described in Morocco in 1953 by [104]. In particular, under

stressed irrigated conditions, all the parts of the plant, especially during the flowering and the spike formation, are affected by wilt. The fungus causes loss of turgidity and causes a lost in yield of about 85% [105]. Another disease is *Macrophomina phaseolina* (Tassi) Goid [106] , better known as charcoal rot, a fungus, which causes stem and root rot and seedling blight. Mainly, the disease is developed under high temperatures (about 30-35°C) and low soil moisture (<60%), and as well as the other pathogens, cause consistent yield losses [107]. Others fungi such as *Cercospora coffeicola* [108], *Phytophthora palmivora* [109], *Leveillula taurica* [110], and the bacteria *Xanthomonas ricinicola* [111] are known to affect castor production but have a lower importance in the economic value of the crop.

Using resistant cultivars seems to be the only solution that can appropriately be used against these diseases.

1.7.7 Pests

The current world scenario of global warming and intensive cultivation production has contributed on increasing pests and diseases for crop. Different pests have been described as responsible for attacking castor cultivation. There are 107 species of insects, six species of mites and around 150 diseases [112]. Most insects are native to the eastern hemisphere and attack castor from sowing till the harvest [112]. They are divided into categories according to the soil they inhabit, the portion of the plant they attack and the damage they cause [113].

Among the soil dwellers are described Sugarcane white grub, *Holotrichia consanguinea* Blanchard (Coleoptera: *Scarabaeidae*) and the subterranean termite, *Odontotermes obesus* (Rambur) (Isoptera: Termitidae) that are mainly present in sandy soils. The root system is completely destroyed by these insects and the emergence of seedling is highly compromised [113].

While, among Leaf sap-sucking insects there is Rose jassid /

leafhopper, *Empoasca flavescens* Fb. (Hemiptera: Cicadellidae). The nymphs and adults cause a gradual leaf loss favored by the injection of a toxin, which also causes the edges of the leaves to curl, which dry up, and fall, suck the leaf sap. A major pest in all castor-growing regions is White fly Trialeurodes ricini Mishra which sucks sap from leaves which leaves the plant stunted.

Within the defoliators there is castor semi looper Achaea janata Linn., a Lepidoptero present during the summer season between July and September, in some regions it persists until November. Younger larvae feed on the outer tissues of the leaf causing holes in the leaves which can sometimes cause total crop devastation [112] Another important one is and castor Shoot Borer Dichocrosis punctiferalis Guene, that is the main cause of crop loss in India, mainly between October and December in rainfed castor. The attack begins during flowering and continues until maturity. Moreover, it is described American serpentine leaf miner, Liriomyza trifolii (Burgess) (Diptera: Agromyzidae), the leaf miners form tunnels between the leaves consuming the green tissue. In severe cases, the leaves stop growing and fall off.

In addition, tobacco caterpillar Spodoptera litura is an important polyphagous pest, which cause "skeletonized" leaves. The larvae feed on the lower surface of the leaf leading to skeletonization [113].

IPM or Integrated pest management could be a strategy to combat the attack of the pests. IPMs include 1. Consider the level of attack and any economic threshold to be addressed; 2. Preventive cultivation practices; 3) monitoring of the environment to limit the outbreak of parasites; 4) mechanical control; 5) biological control; 6) chemical control [112].

Furthermore, pest management practices include intercropping based on the principle of reducing pests by increasing the diversity of the ecosystem under consideration [114].

In recent years, breeders have been developing new

mechanisms of resistance and tolerance to environmental, biotic and abiotic stresses. The natural resistance of the host plant represents the most exhaustive method of struggle in the fight against pests. Obtaining resistant cultivars through breeding is therefore one of the cheapest and most valid methods to use, although it seems that resistance to parasites and insects is only temporary. New breeding programs are needed.

1.7.8 Harvest

When all the capsules of castor are totally dried and the leaves start to fallen from the plant, the crop is ready to be harvested. Considering that the production of one, two or more racemes is progressive during the production cycle of the plant, it is not possible to plan the harvest because of the ripening that will not be simultaneous for all the racemes. This reason influences and impacts mechanized harvesting which cannot be carried out adequately. The scale of production therefore implies the choice of a manual harvest of ripe racemes, which are selected and cut manually. Delay in harvesting can lead to loss of seeds by shattering [115]. The scale of production makes it necessary to have more harvests per year, increasing the production costs of oil and sub-products [23]. The production costs are indeed, largely influenced by the harvest which also affects the quality of the biomass. Moreover, the potential presence of green racemes and still unripe capsules slows down the production and the relative harvest [115]. In some trials, modified combines equipped with headers were used, generally used for corn; however, several trials and speed tests are needed to avoid seed loss and damage. These combines have a cutter bar, which, by cutting the stem of the plant at a certain height, involves the loss of a certain amount of biomass. Another approach involves the use of a vibrating system instead of the cutter bar, in order to collect only the capsules [116]. However, more studies are needed about the development of

harvesting machines and a lack in the literature is still notable regarding castor harvesting. The racemes must then be shelled to free them from the woody shell of the capsules. This second operation can be carried out mechanically. There are machines on the market (in the USA) able to carry out both operations (collection and ginning) of dwarf types.

1.8 The wide potentiality of castor

As Mutlu & Meier, 2010 stated, "Castor oil is considered as one of the most promising renewable raw materials for the chemical and polymer industries because of its manifold uses and to different series of well-established industrial procedures that yield a variety of different renewable platform chemicals". Then later was confirmed by Mubofu, 2016 who testified how the increase in production of castor witnessed in the past centuries, is due to its very wide range of applications and the possibility of using it in various sectors (Figure 1.4).

Already in the past, castor was strongly used mainly for its oil, which was considered a strong laxative. While, nowadays, the oil has a wide range of applications [119] ranging from agricultural uses to the pharmaceutical industry, but also textile, paper, rubber, cosmetics, paints, inks, additives and especially lubricants and the production of biofuels.

Figure 1.4 – Castor oil applications

1.8.1 Potential feedstock

1.8.1.1 Castor oil

Like other vegetable oils, castor oil is also composed of triglycerides of various fatty acids and about 10% glycerin. The fatty acids present are approximately 80-90% ricinoleic, 3-6% linoleic, 2-4% oleic and 1-5% saturated fatty acids. The high content of ricinoleic acid involves a great versatility of uses of the oil. Due to the high presence of ricinoleic acid, the oil results particularly viscous as well as poisonous due to the presence of ricin and ricinin. Toxicity is a positive factor in the competition of use of the oil itself, between food and industrial uses. The oil also has good stability and shelf life. Although castor oil has considerable toxicity, its fields of uses are broader than most other vegetable oils [18]. The oil comes used in obtaining many industrial chemical products thanks to its unique composition structure. In fact, this oil is one of those vegetable oils, which has found use in different processes of the chemical industry. The production of castor oil generates two important by-products, the extraction cake and the residues of the capsules and racemes. For every ton of oil, 1.31 t of residues and 1.13 t of deoiled cake are produced. The extraction cake is the most important by-product, rich in N and P, which finds its main use as a fertilizer. The N content of

castor cake (7.54%) is similar to cotton cake (8.21%)[120]. The oil extraction cake can also be used, after detoxification, as a protein supplement in the diet of some ruminants.

1.8.1.2 Biofuels

In the upcoming years, biodiesel, made from vegetable oils, will become one of the most important replacements for petroleum based diesel fuel. Today's context requires low impact production and alternatives to the commonly used fossil fuel [121] [50]. By using natural and renewable raw materials, the environmental impact caused by fossil fuels is reduced because of the consequent decrease of greenhouse gases and hydrocarbon emissions and lower particulate released in the atmosphere. Biodiesel is non-toxic, aromatic-less and, the one made with castor, has a lower cetane number (CN;43,7) than diesel (CN;51) [122][123], high miscibility, low iodine content and low freezing point (-14°C) [124]. Moreover, it has a higher content of oxygen (>10%) that helps through the combustion process allowing the biodiesel to burn cleaner, and it is also considered an environmental-friendly product because of the absence of sulphur, which usually causes the production of sulphur oxides [125] which negatively impact the environment. Albuquerque et al., 2009 highlighted how pure castor biodiesel's properties do not match the European requirements for specific gravity (0.860-0.900) while castor's value is 0,920 and viscosity (3.5-5.5) in which castor has 13, 5 cSt. Nevertheless, by blending the pure castor biodiesel with contents up to 60% vol of soybean, the value of specific gravities meets the European requirements, on the contrary of contents of 60% volume of cotton and canola that keep exceeding the value. As well as for the viscosity, that needs a blending of 200g kg^{-1} with cotton seed or soybean diesel to fall into the EU specifications[126][18].

Biodiesel oil made from castor is produced by the extraction of the oil from castor's seeds. The extraction is either mechanical or by

solvent extraction, alternatively both extraction methods can be combined. Even if fuels, obtained by vegetable oils, could be used as such without refining, due to their high viscosity it is necessary to investigate and apply some processes. Viscosity is a property that determines the ability of biodiesel to mix [127], affecting also the spray quality [128] and the production of new reactants [129]. High viscosity can be avoided by using microemulsions [130], thermal cracking, blending with diesel [131] and the most used process the transesterification. The latter also named alcoholysis, consists of a chemical reaction that happens in the presence of a catalyst, of an alcohol with an esters producing esters and glycerol. The presence of the catalyst is essential in order to make the process shorter, hence the cost of the overall production of biodiesel will increase [132].

1.8.1.3 Organic fertilizer

Even if castor is mainly used for the production of oil, the relative by-products formed are widely used in the industry-chain. Husks and meal are the two major by – products generated during the oil production process. Specifically, the capsule husks are the outer residual portion that covers the fruits, while the meal is produced after the oil's extraction [120]. Considering the high amount of N found in the meal, some authors have used it as a valid replacement in animals' diets [133][134]. In spite of using it merely as an animal feed, the industry chain appears to be more interested in the use of the meal as an organic fertilizer because of its wide range of applications. Indeed, organic fertilizer can be used for supplying micro and macronutrients to the soil, improving the soil physical properties, immobilizing toxic elements and promoting microorganism's activity [120].

Definitely, in a point of view in which is important to reduce the impacts of environmental factors in the agricultural industry, using the residual parts of castor could lead to positive vantages such as improving soil productivity, yields of agricultural crops, and recycling

nutrients.

1.8.1.4 Medicinal and pharmaceuticals

Minor uses of castor oil are associated with medicinal and pharmaceutical products and activities. Antibacterial activity of leaf extracts of *Ricinus communis* has been proven against bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *K. pneumoneae*, *Streptococcus progens* [135]. Moreover, chronic diseases such as heart diseases and cancer can be prevented by using natural antioxidants as tested by Iqbal et al., 2012 who showed *Ricinus communis'*s hydrogen and free radical scavenging ability acting as antioxidants. While for the roots, their ethanolic extraction seems to lower the glucose level of fasting blood, acting positively as antidiabetic [137].

The various applications within the medicinal and pharmaceuticals field range also from anti-inflammatory activity [138], central analgesic activity [139], anticancer [140] and antifertility activities [141].

1.8.1.5 Phytoremediation

The demographic expansion followed by the industrial revolution has increased the heavy metal pollution within the environment through the direct discharge of pollutants into water and soil [142]. The use of pesticides, commercial fertilizers, industrial and in general all kinds of human practices are strictly responsible for the high amount of polluted land, over 75% of the worldwide land surface [143].

In this contest, phytoremediation represents a valid alternative for restoring these degraded and contaminated lands and using oil crops in the phytoremediation process is outrageously beneficial because of the double possibility of decontaminating the soil by using crops that are not fated to the food industry and that can still be

productive [124]. As for achieving good results, the choice of a crop with high biomass production is essential. Moreover, the crop should be perennial, non- edible, and tolerant to biotic and abiotic factors. Crops such as C*annabis sativa, Zea mays, Thelypteris palustris, Chenopodiaceae Typha latifolia, Nicotiana tabacum*, and *Helianthus annuus* L. are described as highly efficient in the phytoremediation process (Tripathi et al., 2021). However, the requirements, such as the unpalatability, are more represented by crops such as *J. curcas*, *I. carnea*, *C.procesa*, *L. camara*, etc. [144] [145].

Recently studies have proved the consistent validity of *Ricinus communis* as a crop able to extract heavy metals and tolerate their high concentration [54]. The ability of growing in wasteland soil, its perennial attitude, its tolerance to salinity, drought and heavy metals keeping its oil yield make castor more suitable for the process morethan other crops [146]. A comparison study on the potential of phytoremediation of Cd between castor and *Brassica juncea* showed that castor was much more resistant to the accumulation in roots and shoots than in Indian Mustard. The accumulation of Cd and Pb in the roots and shoots has also been documented by [147]. In particular, with regard to the Pb, castor turns out to be a hyperaccumulator, capable of extracting and accumulating more than 10.54-24.61 g Pb kg^{-1} dry in its tissues.

Another study indicates the possibility of accumulation of Ni [148], increased by the use of organic manure. *Ricinus* is also used for the accumulation of (Ba) and other heavy metals [149].

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2 Breeding of a castor genotype to be exploited in the Mediterranean basin

2.1 Introduction

The rapid and growing climate change, caused by the release in the atmosphere of GHG emissions, the overuse of fertilizers, herbicides, and pesticides, and the human influence have caused an alarming loss in agricultural production [1]. The role of agriculture in the coming future has become uncertain due to the severe climate worldwide crisis, which causes alterations in temperatures and precipitation affecting crops, livestock, plant growth, and overall food security and people's livelihood [2].

The Mediterranean area has strongly been damaged, demonstrating a great decline in mean precipitation and a rise in the variability during the dry season, which in most cases, leads to a strong area aridification [3]. This phenomenon is already spread in many regions, such as Spain, Greece, and Israel, and it is extending also to many regions of Italy [3] [4], making clear the huge need to rapidly adapt to these major climate changes.

Trying to cope with these goals, the interest towards the use of energy crops, with great potential to fulfil energy needs, and to obtain renewable energy as requested by the Directive 2018/2001/UE (RED-Renewable Energy Directive) [5] and by the Agenda 2030 [6], is increasingly growing. Within this framework, the use of marginal, abandoned, and degraded lands could be a valid alternative to both re-evaluate these soils and avoid competition with land used for agricultural purposes [7].

Castor (*Ricinus communis* L.) seems to meet these sustainability requirements, being a valid alternative energy crops to be included in today's scenario [8]. The wide range of applications of castor make

this crop captivating for the industrial, chemical and pharmaceutical sectors. Specifically, being a non-edible oilseed crop, it is principally an oil's producer (35-65%), with a particular composition, given by ricinoleic acid (85-90%), making it particularly appropriate for biodiesel production, and consequently for obtaining renewable energies [9], thus limiting the overexploitation of fossil sources [10].

Moreover, castor is a rustic, easy-grown crop, native from Africa and well adapted to different pedo-climatic environment [11], behaving as perennial species in tropical and sub-tropical regions and as an annual crop in the Mediterranean basin . However, incorrect timing for sowing [12], temperatures alterations during germination [13], together with frosts and cool winters can lead to the plant death.

In this regard, the aim of the present research is the breeding of a genotype for the Mediterranean environment, that has improved morphological and qualitative-quantitative traits, a higher seed yield, and high oil content, a proper plant development, and that is suitable for cultivation in semi-arid environments.

2.2 Materials and Methods

2.2.1 Field Trial Description

The Department of Agriculture, Food and Environment (Di3A) of the University of Catania started a plan for the breeding of a heterogeneous genotype of castor (*Ricinus communis* L.).

Seeds, coming from a pool of plants having genetic material deriving from a collection of germplasm from Brazil, Tunisia and Sicily, which has intercrossed over the years, were sown in 2019 in Gafsa (Tunisia), in an area named as 'Gafsa1'. The germplasm used had a great variability in the color of the capsules, seeds and leaves, in the earliness of ripening of the racemes, in the time of differentiation of the ramifications, and consequently of the first order and successive

racemes.

The aim of the breeding program was the selection of a perennial and productive plant with earliness production. Thus, in 'Gafsa1', 55 plants with numerous ramifications and low insertion of the main inflorescence (< 100 cm) have been identified and selected for further investigations. The selected plants were chosen because of the presence of satisfactory production characteristics and uniform colour of the capsules (green).

The seeds collected from these plants in Gafsa1 were brought to Italy in order to start a genetic program in Catania. The breeding had the following steps, in depth described in Table 2.1:

Table 2.1 - Mass selection steps for the breeding program from May 2019 to September 2021

Steps	Location and period	Activities
I	Gafsa May-June 2019	Seed collection (genotypes) from 55 selected plants on 24 ha
II	Catania July 2019	Sowing of 55 genotypes on an area of about 1 ha
III	Catania July – December 2019-2020	Phenological scoring on the 55 genotypes and collection of mature racemes
IV	Catania 2020	Within the previously selected genotypes (55 genotypes of step I), 28 genotypes were selected for the earliness of maturation of the main raceme and their seed weight greater than 80 g
V	Catania June 2020	Sowing of the 28 selected genotypes in two ha divided into three plots (called L, M, R). Phenological scoring during the entire growth cycle of the plants.
VI	Catania July – September 2021	Seed collection of the 28 genotypes which constitute the reference pool of the new genotype

The field experiment on the 55 genotypes has been conducted

at the experimental farm of the University of Catania, Italy (10 m a.s.l., Catania (37°24'31'' N; 15° 3'33'' E) in a typical xerofluvent soil, over the period May 2019-September 2021. The characteristics of the soil are as follows: clay 32.3%, silt 11.8%, sand 55.9%, organic matter 1.4%, pH7.6, total N 0.2%, available P_2O_5 46.1 mg/kg, and exchangeable K_2O 293.3 mg/kg.

The soil was previously ploughed to carry out proper sowing, which has been done manually. A total amount of 60 kg/ha of nitrogen was applied using, before sowing, 60 kg/ha of ammonium sulphate, and at the flowering stage, while 100 kg/ha of simple superphosphate (18%) was supplied at sowing.

Each genotype was sown in 18 m row length and replicated 4 times. In each hole, a single seed was sown with a sowing depth of 3–4 cm. The plant density was 6666 plants/ha, and the rows were 150 cm apart with an interval within the row of 100 cm

Within the 55 genotypes at the recorded emission of the inflorescences, the plants that did not respond to the desired characteristics (colour of the capsules and leaves), those characterized by the insertion height of the first raceme (>71 cm), and those with short main raceme (<30 cm) and low weight (<80 g) were eliminated.

The selected 28 genotypes were sown in June 2020 on three different plots called: 'L', 'M' and 'R'. The soil had the same characteristics of the previous experiment.

The plant density was 5555 plant m^2, in each hole a single seed was sown at 3-5 cm depth and the rows were 1.80 m apart with an interval within the row of 1 m.

For both experiments the irrigation water was supplied by a drip irrigation system. The volume of irrigation was determined as the maximum amount of water available in the soil, in a depth of 0.4m, in which the root system is developed. Irrigation volume was determined by the following formula:

$$V = 0.6 \text{ X } (FC\text{-}WP) \text{ X } \Phi \text{ X } D \text{ X} 10^3$$

in which V = water amount (mm); 0.66 = readily available water not limiting for evapotranspiration; FC = soil water content at field capacity (27% of dry soil weight); WP = soil water content at wilting point (11% of dry soil weight); Φ = bulk density (1.1 g cm^{-3}); and D = rooting depth (0.6 m).

To schedule irrigation, the sum of daily maximum crop evapotranspiration (ETm) had to match the mentioned volume (V). Rainfall events were subtracted from the calculation.

The daily ETm was estimated according to:

$$ETm = E0 \text{ x } Kp \text{ x } Kc$$

Where ETm where ETm corresponds to the maximum daily evapotranspiration (mm); E0 is the evaporation of class-A pan (mm); Kp is the pan coefficient, equal to 0.80 in the semi-arid environment. The crop coefficients (Kc) were determined by previous observations: 0.4 from the emergence phase till the 4-leaf stage; 0.7 from the 4-leaf stage till the flowering; 1.2 from the beginning of flowering to complete capsule development of the first raceme and 0.55 from the first raceme to complete capsule ripening.

2.2.2 Measurements and Determinations

Considering the subsequentials growth of primary and secondary racemes, and the consequential subsequent ripening, two different harvests were carried out manually. The first on 10 December 2019 collecting the main racemes and secondary racemes already ripened, and the second harvest on 24 January 2020 on main and secondary racemes left.

After the harvest the number of capsule of main racemes, the

capsules weight, the seed weight were measured, and the number of total ripened racemes and total racemes per plant were counted.

2.2.3 Oil extractions and Determination

The seeds were analysed to evaluate the oil content and oil yield. GM200 blade mill (GM200 blade mill (Retsch, GmbH, Haan, Germany) was used to crush seed into a paste (cake). The oil extractions were performed according to AOAC (Association of Official Analytical Chemists) [150]. The SER 148/6 solvent extractor (produced by Velp Scientifica Srl, Usmate Velate (MB), Italy) was used, and by an evolution of the Randall method, as reported by Lovkis et al. (2018) [151], the oil was obtained.

In the current work, the preparation of the samples involved the filling of the crucibles (porous cellulose fibre thimble) with 3 g of the ground seed samples, immersed in 70 mL of boiling solvent (boiling temperature of 130 °C) and placed into a Soxhlet extractor. An immersion and washing phase of 60 min each followed to obtain the oil. After the first two stages, most of the solvent used is recovered in the recovery step. At the end of the procedure, the extraction vessels were placed in an oven at 105° for 30' to ensure the evaporation of any solvent residues. The vessels were cooled in a desiccator and weighed to calculate the total fat percentage.

The oil content of castor seed was calculated as Danlami et al. (2015) [152] described:

$$\text{Oil content (\%)} = \left(\frac{Weight\ of\ the\ extracted\ oil}{Weight\ of\ total\ sample}\right) \text{x } 100$$

The oil yield was calculated by the multiplication of the oil content (%) by the seed yield (kg/ha).

2.3 Preliminary study on genetic variability within castor population

2.3.1 Plant material and DNA extraction

DNA was extracted from 55 genotypes of castor, comparing 28 genotypes selected in 2019 to the same ones evaluated in 2020 (Table 2.1 - Mass selection steps for the breeding program from May 2019 to September 2021) using the method described by Doyle and Doyle (1990) [17] with minor modifications (such as quantity of leaf tissue taken, concentration of NaCl, and incubation duration and temperature). Fresh leaf tissues (20 mg g) were ground in liquid nitrogen and taken into a 2 ml microcentrifuge tube. To the ground sample 300 µL of extraction buffer [2% cetyl trimethyl ammonium bromide (CTAB), 100mMTris–HCl, 3.5M NaCl, 20mM ethylenediaminetetraacetic acid (EDTA), 0.2M -mercaptoethanol, and 2% polyvinylpyrrolidone (PVP), pH 8.0], was added and incubated at 65 °C for 90 min. The above sample was extracted with equal volume of chloroform: isoamyl alcohol (24:1) and supernatant was transferred into a new tube. The sample was treated with RNase (2.25 units) and extracted with Tris saturated phenol. The supernatant obtained was taken and extracted further with chloroform:isoamyl alcohol (24:1) twice more, and the DNA was precipitated with 80% ethanol. The pellet was air dried and resuspended in 100 µl of Tris–EDTA (TE) buffer. Each sample was diluted to 20 ng/µl with TE buffer (10mM Tris–HCl, pH 8.0 and 0.1mM EDTA, pH8.0) and stored at 4 °C.

DNA concentration was estimated by measuring UV absorption at 260 nm and 280 nm to assess DNA purity using a Nanodrop1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

2.3.2 Simple Sequence Repeat (SSR) amplification

The isolated DNA was used for PCR analysis. A total of three SSR primers (Table 2.2) were tested, according to the SSR fragments

found by Bajay et al. (2009) [153]. Amplifications were performed in a reaction mixture of 10 µL, including: 2 µL of genomic DNA at variable concentration (i.e., no dilutions were made after DNA isolations), 1× DreamTaq Buffer (Thermo Fisher Scientific,Waltham, MA, USA), 0.16 mM dNTP, 0.16 µM forward and reverse primers and 0.5 U DreamTaq DNApolymerase (Thermo Fisher Scientific, Waltham, MA, USA). PCR was performed in a thermocycler. The cycling program foresees: an initial denaturation step of 94 °C for 3 min, followed by 35 cycles of 94 °C for 45 s, an optimal annealing temperature of 60-62 °C (as suggested by Bajay et al. (2009) [153]) for 45 s, 72 °C for 1 min and a final extension at 72°C for 15 min.

Amplifications products were electrophoresed in 1.5% agarose in 1 x TBE buffer, and and SYBR safe (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) was used as intercalant.

Table 2.2 - Characteristics of 3 microsatellite from *Ricinus communis* L. forward (F) and reverse (R) primer sequence, repeat motif and size range in base pairs (bp).

Name	Primer nucleotide sequence (5'-3')	Size range (bp)
Rco09	F: CCAACTCCCTTGTCTGCAA R: GTGAATGGCAAGCAGCAAT	170-194
Rco13	F:GGTGCTTCCAGAAATTCAGTT R:GGAGGGGAAAGACAGGATTC	226-254
Rco15	F:CACGCACGTTAAAGCAAACT R: GCGAAGAAACCAAAATGGAG	220-230

2.4 Results

2.4.1 Phenological scoring of 55 genotypes

The data collected, referring to each individual plant were averaged for each of the four replicates to finally obtain a value for each genotype. Within each genotype, the existing variability was expressed as a standard deviation (Table 2.3). Table 2.3, Figure 2.1

and Figure 2.2 show the average values.

The genotype ‘61’ resulted as the genotype with the highest number of capsules (107), followed by genotypes ‘53’ and ‘85’ (103 and 100, respectively) (Figure 2.1). These were the only genotypes with a number of capsules >100.

Another important parameter is seed weight per genotype (Figure 2.2). The highest weight was measured for ‘61’ with 105 g, followed by ‘93’ and ‘106, both 101 g. On the other hand, the genotype with the lowest value was ‘99’ with 33 g.

Table 2.3 - Values (n=4) of the agronomic traits measured on 55 genotypes

Genotype	N. capsules of main raceme	Capsules weight (g)	Seed weight	N. ripened racemes per plant	N. total racemes per plant
3	63 ±27	118 ± 9	77 ± 37	6,7	8,9
5	79 ± 14	116 ± 25	80 ± 19	8,6	9,1
6	58 ± 15	92 ± 31	57 ± 24	8,3	9,7
8	76 ± 12	111 ± 21	77 ± 16	10,5	11,1
9	92 ± 19	133 ± 31	96 ± 25	8,4	8,5
11	77 ± 22	112 ± 36	81 ± 27	6,9	7,5
25	79 ± 12	121 ± 24	77 ± 19	12	12,9
27	79 ± 21	118 ± 33	63 ± 17	12,5	13,5
32	70 ± 16	110 ± 28	67 ±21	8,8	9,4
33	72 ± 17	104 ± 26	73 ± 18	12	12,5
35	78 ± 18	114 ± 31	85 ± 24	9,3	10
36	69 ± 18	101 ± 35	72 ± 25	8,2	8,6
38	77 ± 19	110 ± 31	78 ± 24	8,3	9,5
39	75 ± 14	112 ± 27	80 ± 19	10,3	11,2
40	65 ± 17	90 ± 27	68 ± 20	9,1	9,6
43	96 ± 19	135 ± 31	98 ± 19	6,6	7,2
47	87 ± 18	133 ± 26	85 ± 16	10	10,6
51	74 ± 19	107 ± 31	78 ± 25	8,9	10,3
52	82 ± 14	113 ± 26	82 ± 20	14,6	16,4
53	103 ± 22	140 ± 34	98 ± 26	9,4	10
55	88 ± 22	129 ± 42	83 ± 30	12,2	13
61	107 ± 13	156 ± 18	105 ±	10,2	12,4
62	93 ± 19	133 ± 27	94 ± 25	8	8,9
65	55 ± 24	86 ± 46	55 ± 32	8,1	8,9

66	68 ± 18	111 ± 34	70 ± 23	9,4	9,5
68	52 ± 19	72 ± 29	56 ± 25	5,5	7,2
69	76 ± 14	112 ± 27	82 ± 24	5,3	6,2
70	80 ± 14	115 ± 27	85 ± 20	10,3	11,1
74	70 ± 18	108 ± 38	70 ± 23	8,9	9,6
77	75 ± 20	109 ± 37	75 ± 26	10,5	11,6
79	73 ± 16	118 ± 24	79 ± 17	10,8	11,7
80	76 ± 19	116 ± 35	76 ± 24	8,9	9,8
83	67 ± 14	87 ± 24	63 ± 17	14,1	15,3
84	77 ± 19	113 ± 33	77 ± 23	8,9	9,5
85	100 ± 21	143 ± 31	97 ± 24	11,8	12,3
91	83 ± 23	127 ± 44	90 ± 32	6,2	6,6
92	71 ± 20	103 ± 32	72 ± 23	12,7	13,7
93	92 ± 19	145 ± 35	101 ±	10,6	11,5
94	77 ± 17	122 ± 31	86 ± 22	5,9	6,9
95	86 ± 17	121 ± 30	88 ± 23	8,4	9
97	81 ± 18	114 ± 33	85 ± 26	10,5	11,6
99	44 ± 12	70 ± 22	44 ± 13	10,6	11,5
100	77 ± 12	122 ± 23	82 ± 21	10,2	10,6
101	72 ± 20	115 ± 38	76 ± 27	11,4	12,8
105	85 ± 14	122 ± 22	86 ± 18	9,9	10,7
107	77 ± 14	116 ± 25	78 ± 19	8,2	9,6
115	85 ± 19	131 ± 35	84 ± 24	11,4	11,7
116	96 ± 16	139 ± 27	101 ±	12	12,7
118	75 ± 21	122 ± 37	81 ± 26	9,7	10,8
119	88 ± 19	136 ± 31	90 ± 22	9,2	10,6
120	76 ± 17	106 ± 28	78 ± 20	12,7	13,5
122	83 ± 19	114 ± 32	80 ± 23	8,9	9,6
128	81 ± 26	119 ± 40	81 ± 30	11,1	11,5
129	69 ± 13	115 ± 22	74 ± 16	9,4	10
131	82 ± 18	112 ± 26	80 ± 20	8	8,5
Mean	77.9±6.3	115.8±	79.5±6.	9.6±0.7	10.4±0.8

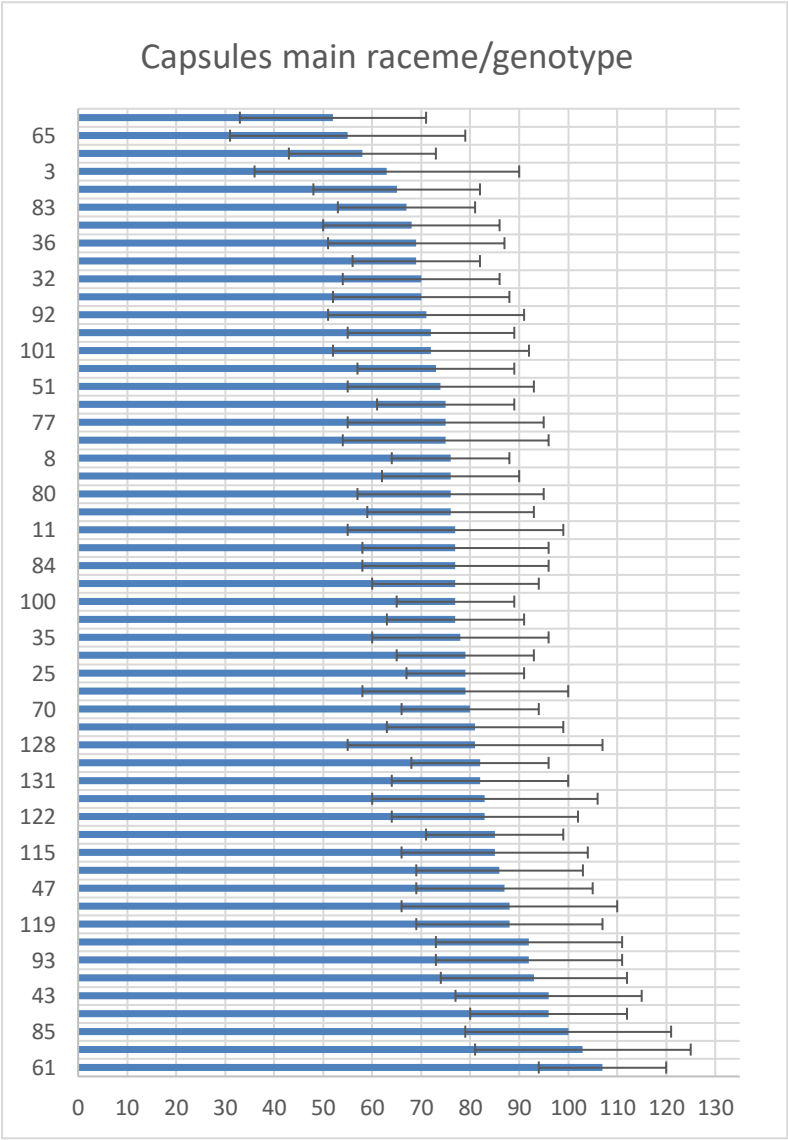

Figure 2.1 - Capsules of the main raceme of the 55 genotypes evaluated. Each line represents the average of the main racemes evaluated of the same genotype.

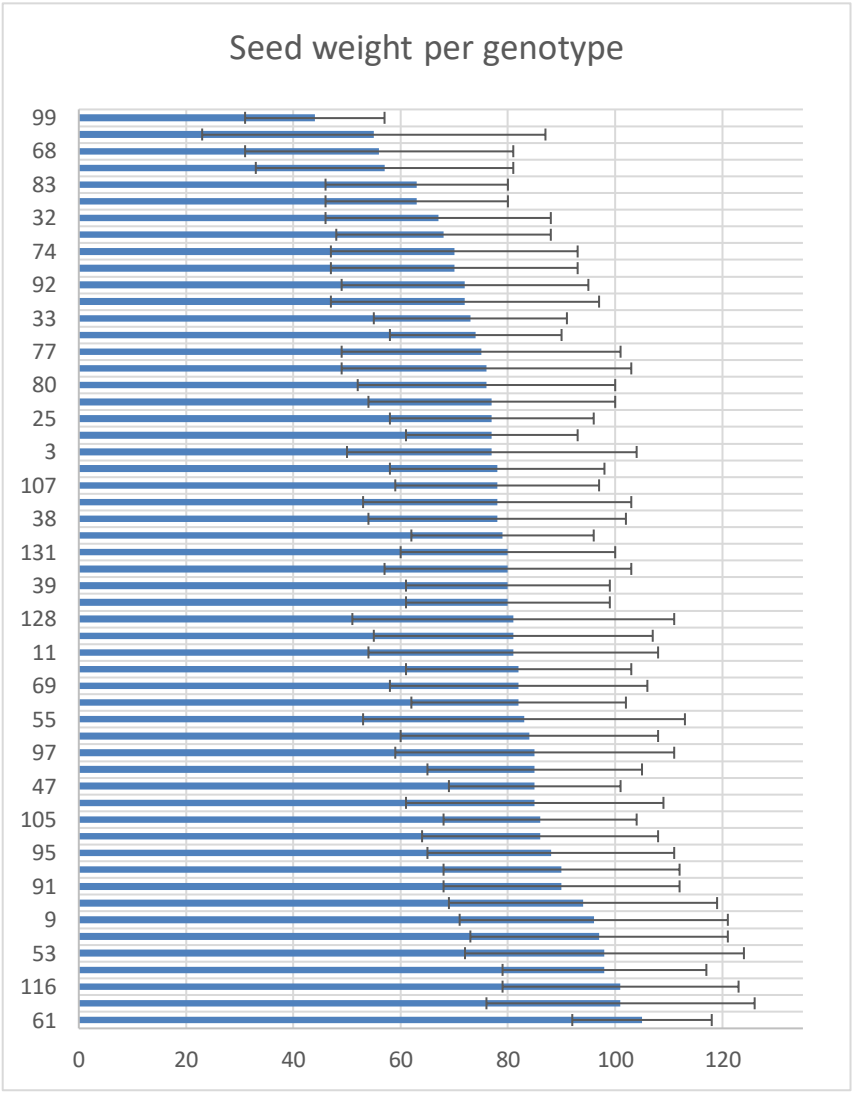

Figure 2.2 - Seed weight (g) of 55 genotypes evaluated. Each line represents the average of the main racemes evaluated of the same genotype.

2.4.2 Production characteristics (distribution over time, seed and oil yield, oil content)

As shown in Figure 2.3, the total yield obtained from the 28 lines genotypes is 3000 kg/ha, and it derives from the sum of 3 collections carried out between autumn-winter 2020 and summer 2021; the first ones (December 2020-February 2021) contribute for 30% of the overall production. The largest share of production (70%) belongs to the ripening of the following summer (2021). The genotype with the highest value resulted '100' with 4454.5 kg/ha of yield. The lowest yield was found in '35', achieving just 1392.5 g/ha. The results highlighted the great impact of secondary racemes in the final seed yield composition.

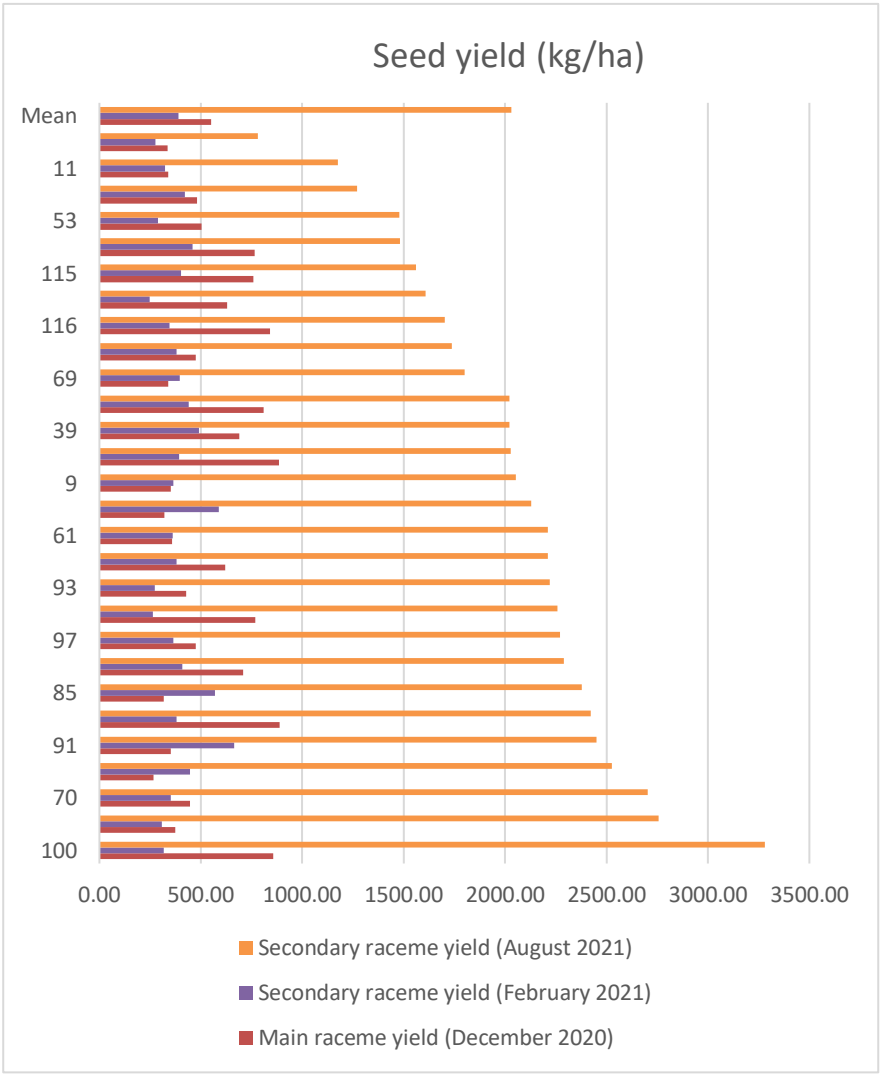

Figure 2.3 - Total yield (kg/ha) of 28 genotypes. The yield is result of three harvest occurred in December 20320 for main racemes, and on February and August 2021 on secondary racemes.

This is confirmed in Figure 2.4 and Figure 2.5, in which the order of secondary racemes harvest in August is 15th higher than the secondary racemes harvest in February. In particular, 25 racemes were counted in ‘105’, contrary to ‘11’ which had only 8 racemes.

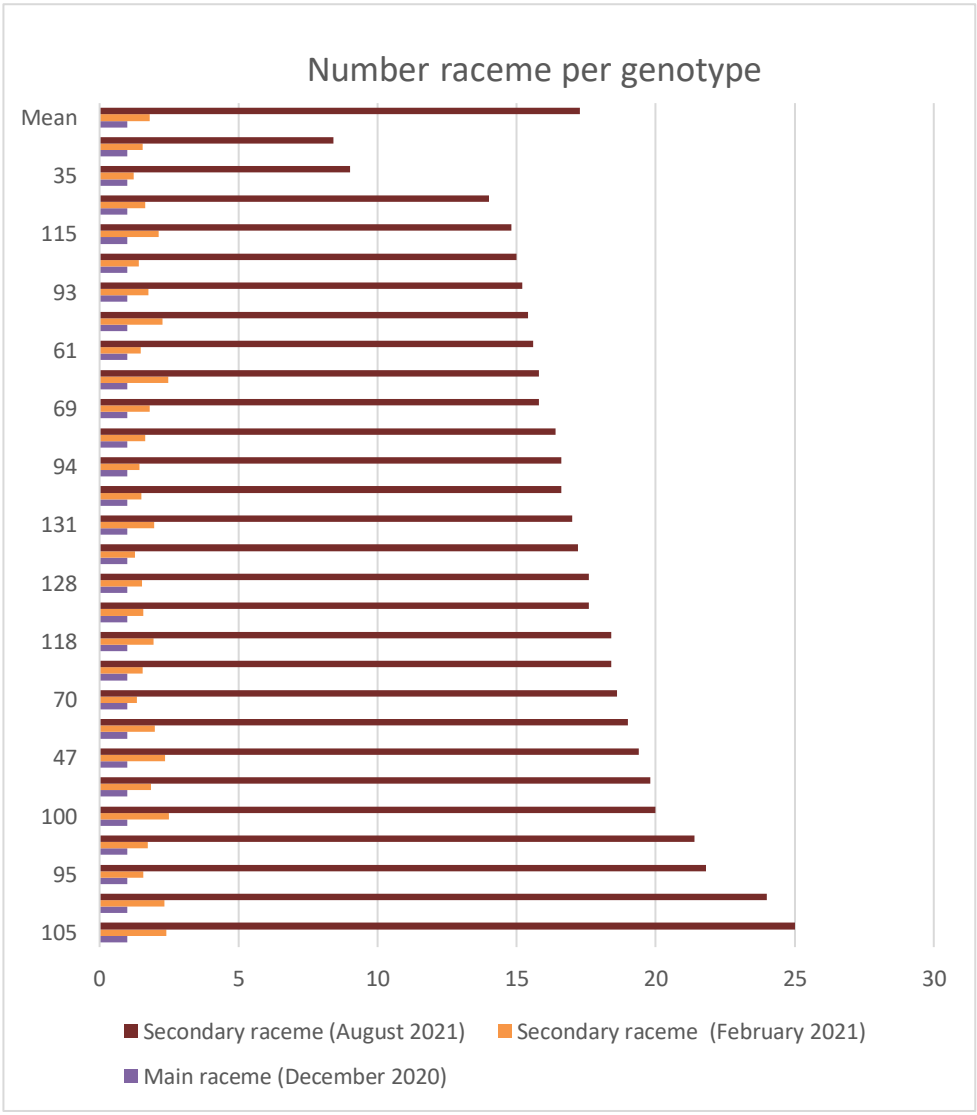

Figure 2.4 - Number of racemes per genotype of the 28 genotypes. The number of racemes derived from three harvest occurred in December 2020 for main racemes and on February and August 2021 on secondary racemes.

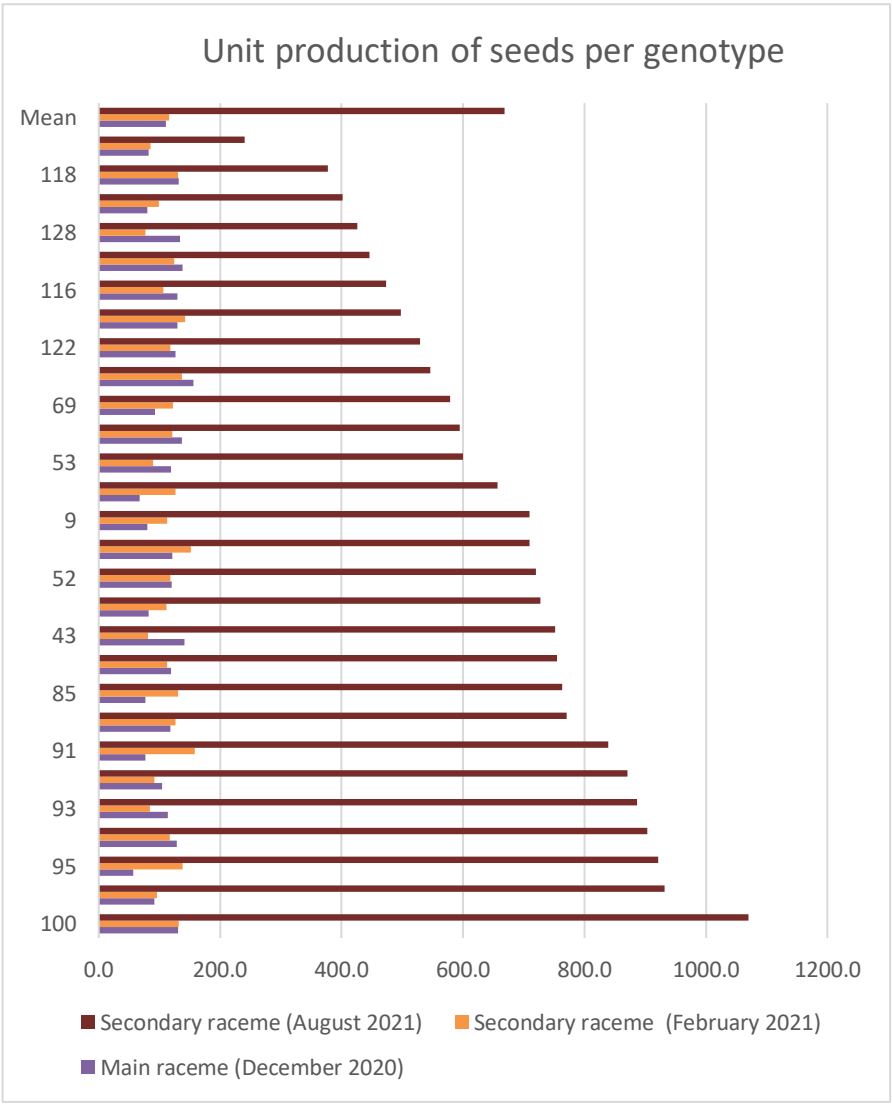

Figure 2.5. Seed yield per plant (g/plant) of the 28 genotypes. The seed yield derived from the collection of seeds harvest in December 2020 for main racemes, and on February and August 2021 on secondary racemes.

The oil content showed differences in relation to the genotype (Figure 2.6). The average oil content of the seeds is 38.4%, being the highest in genotype '11' (45.3%) and the lowest in '122' (30.6%). As for the oil yield (Figure 2.7), the average is 1137.4 kg/ha, being '119' the highest (1512 kg/ha), and the lowest in '9' (605 kg/ha).

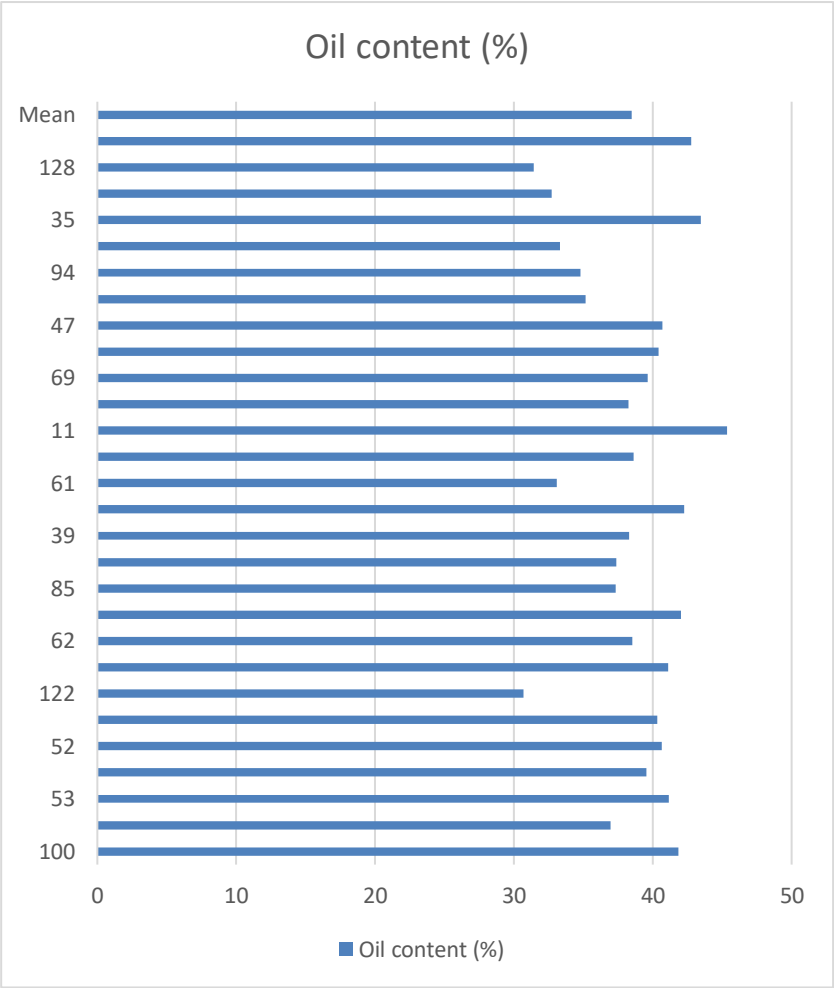

Figure 2.6 - Oil content (%) of the 28 genotypes.

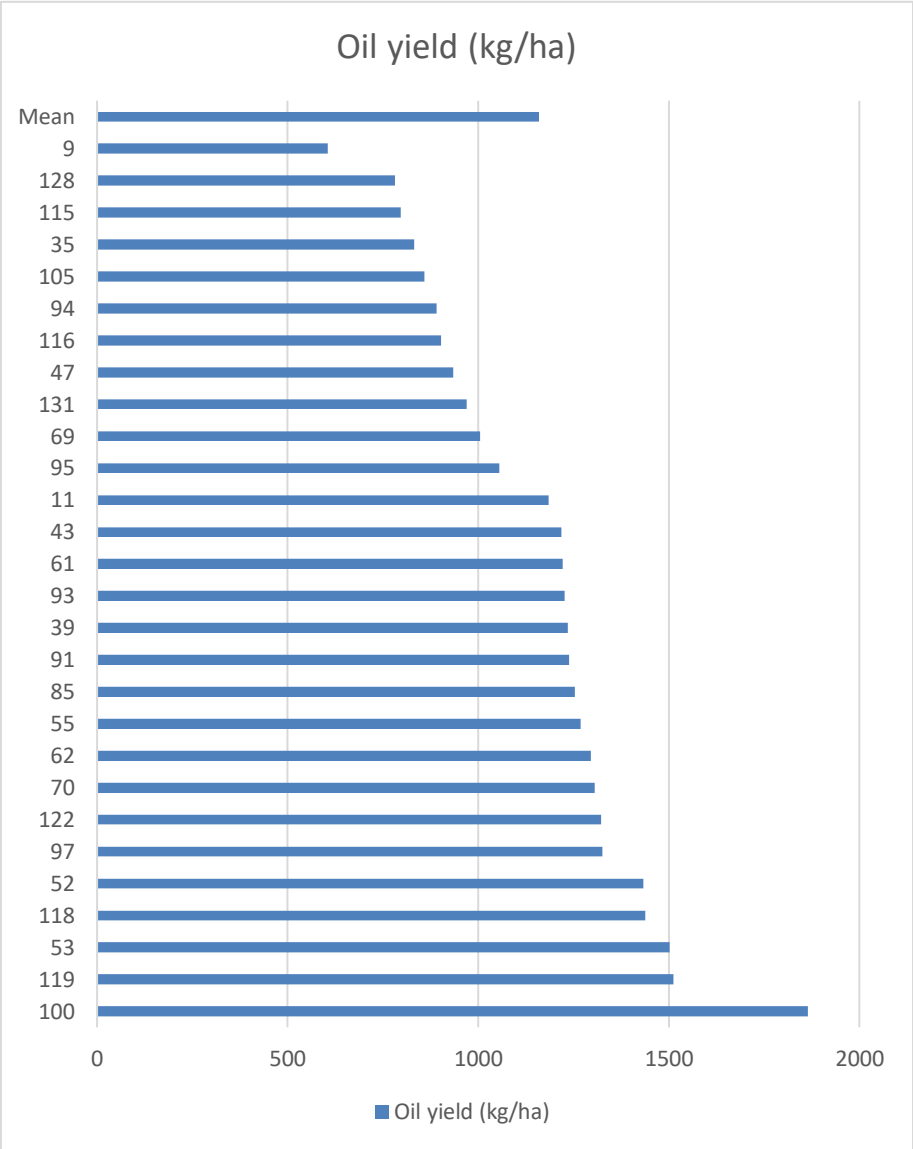

Figure 2.7 - Oil yield (kg/ha) and oil content (%) of the 28 genotypes.

2.4.3 Preliminary results for SSR analysis

The SSR bands were scored as present (1) or absent (0). The different intensity of the bands, when presents, was not taken into account, as shown in Figure 2.8. For each primer used, genotype-specific bands were observed. A band was considered polymorphic when the scoring changed from absent to present, and vice versa, between the two years field experiments (2019 and 2020). The 3 SSR primers produced 37 polymorphic bands across 28 genotypes for 2019, and 41 polymorphic bands across 28 genotypes for 2020.

The assumption is that the polymorphism between years is an index of genetic variability, and therefore an acquisition or loss of a specific trait.

Specifically, a loss in variability was found for the first primer 'RCO9', which had 12 bands in 2019 and 6 bands in 2020. Contrary, an acquisition was found in 'RC013', in which 10 bands were scored in 2019 and 17 bands were found in 2020, similarly to 'RCO15' in which 15 and 17 bands were scored in 2019 and 2020, respectively. In addition, as reported in Materials and Methods (Table 2.2), the regions of amplifications of these two primers are distant, being 170-194 bp for 'RC09', and 226-254 bp for 'RC013', indicating that the different regions analysed are differently susceptible to an acquisition or loss of a trait.

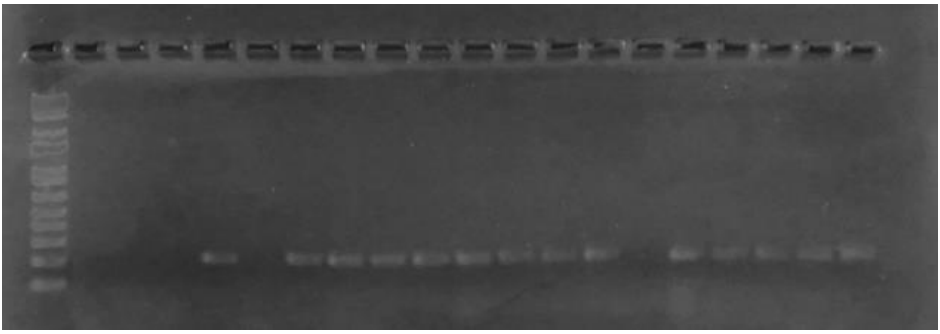

Figure 2.8 - PCR amplification products of 19 genotypes of castor produced with SSR primer.

2.5 Biometric features of genotype n.100

Table 2.4 presents a first-year type plant breed in Catania (Catania 2020).

In relation to the unitary investment adopted (5555 plants/ha), at the ripening, occurred in August, the main raceme measures over 200 cm. The main raceme has its grafting point on the main stem at a height of 44-62 cm from the ground (A). The first order racemes are inserted at a height of about 95-140 cm from the ground (A+F/G) and the secondary racemes at a height of about 200 cm from the ground. The main raceme measures 25–62 cm (B) and the first ramification, at least three, 20-34 cm (L, H).

The main raceme appears when the plant has differentiated about 12 nodes. When the main raceme has almost completed the flowering setting, new shoots appear in the axils of the underlying nodes (from 2 to 3) which will give rise to ramifications 50-74 cm long (F); these, in turn, differentiate the first order raceme at the apex. Other ramifications can also grow from the first basal nodes. The growth rate of this plant is sympodial: both the main stem and each branch, in a certain phase of their development, change the apex from vegetative

to reproductive, forming the inflorescence; therefore, the elongation of the plant continues thanks to a lateral shoot.

Table 2.4 - Catania August 2020. Biometric characteristics of a plant-type of the breed genotype

A : height of main raceme insertion 44-62 cm
B : main raceme length 25-62 cm
C : length main raceme 9-26 cm
D : insertion height 1st branch 4-5 cm
E : insertion height 2nd branch 11-36 cm
F : 1st order raceme insertion height 50-74 cm
G : 1st order raceme insertion height 51-80 cm
H : raceme length 1st order 20-31 cm
I : rachis length raceme 1st order 15-24 cm
L : raceme length 1st order 23-34 cm
M : rachis length raceme 1st order 8-28 cm

2.5.1 Biological characteristics

Table 2.5 shows the main biological phases scored: sowing in early summer (June 16th); emergence scored 7 days from sowing; 56

days from the sowing, appearance of the main inflorescence (F1); and at 60 days anthesis of the first female flowers. The maturation of the capsules (M1) takes place 106 days after sowing. In terms of thermal accumulation, the seeding-ripening interval of the main raceme required 1885° degree days (DD °C). The maturation of the I order racemes ended about 30 days after that of the main raceme (137 days from sowing) with a total of 2167° degree days. The achievement of the phenological phase presents an increasingly marked variability in the final phases of the cycle (ripening), with average gaps between 5 and 20 days.

The maturation of the main raceme and that of the first order take place with a considerable difference, about 30 days. Sowing in June places ripening between the end of August and September, when the average air temperature begins to drop. It is possible to verify that by anticipating the sowing period in March or April, the thermal accumulation for the ripening of the raceme is reached by the end of August or the first ten days of September, and the delay in the ripening of the main racemes and first order is shortened to about 15 days.

Table 2.5 - Catania August 2020. Biological characteristics of a plant-type of the

breed genotype

Phenological phases of the main raceme		Number of days since sowing	Thermal sum °C
Sowing	16/06/2020		
Emergence (E)	23/06/2020	7	116
Flowering appearance (F1)	11/08/2020	56	982
Anthesis (A1)	15/08/2020	60	1063
Beginning of Fruit set (G1/1)	19/08/2020	64	1142
End of Fruit set (G1/2)	06/09/2020	82	1487
Ripening (M1)	30/09/2020	106	1885

Phenological stages of I order racemes		Number of days since sowing	Thermal sum °C
Flowering appearance (F2)	28/08/2020	73	1310
Anthesis (A2)	03/09/2020	79	1419
Beginning of Fruit set (G2/1)	14/09/2020	90	1599
End of Fruit set (G2/2)	29/09/2020	105	1827
Ripening (M2)	31/10/2020	137	2167

The genotype that enclosed all these parameters was identified in the genotype n. ‘100’, which was then used in other research trials.

2.6 Conclusion

The aim of the study was the breeding of a genotype improved morphological and qualitative-quantitative traits, a higher seed yield, and high oil content and a proper plant development.

The selection plant started from the evaluation of 55 genotypes, selected from a Tunisian environment. From the evaluation of biometric and agronomic characters, such as the number of ripened and immature plants, the insertion height, the length of the racemes, the number of capsules, their weight, the seed weight, a mass selection programme started, allowing the further selection of 28 genotypes.

The seed yield, the oil yield and the oil content had a great variability within the population studied, depending on the genotypes.

The variety selected at the end of the two-year period of genetic breeding activities, made it possible to select a genotype characterized by:

- Greater uniformity for biometric (plant height) and morphological (caps and leaf color) characters;
- Reduction of the insertion height of the main raceme (important for mechanized harvesting);
- Reduction of the duration of the sowing period and maturation of the main and first order raceme (earliness);
- Yield close to 3 t/ha over a twelve-month period (from summer sowing to summer harvesting of the following year's main racemes, first and second order).
- High oil content (41.8%) and high oil yield (1864.2 kg/ha)

The genotype that enclosed all these parameters was identified in the genotype n. ‘100’, which was then used in other research trials.

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3 Effects of sowing dates and genotypes of castor (*Ricinus communis* L.) on seed yield and oil content in the South Mediterranean basin

This chapter is based on the published version of the article:

Cafaro et al., (2023) Effects of sowing dates and genotypes of castor (*Ricinus communis* L.) on seed yield and oil content in the South Mediterranean basin
Agronomy 2023, 13(8), 2167
https://doi.org/10.3390/agronomy13082167

Effects of sowing dates and genotypes of castor (*Ricinus* communis L.) on seed yield and oil content in the South Mediterranean basin

Valeria Cafaro [1*], Silvio Calcagno[1], Cristina Patanè[2], Salvatore Luciano Cosentino [1] and Giorgio Testa [1]

[1] Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli Studi di Catania, via Valdisavoia 5, 95123 Catania, Italy
[2] CNR-Istituto per la BioEconomia (IBE), Sede secondaria di Catania, Via P. Gaifami 18, 95126 Catania, Italy

***** Correspondence: valeria.cafaro@phd.unict.it

Abstract

To evaluate the performance of dwarf castor hybrids (‘C1012’, ‘C857’, ‘C856’), compared to a local selected genotype, in four subsequent sowing dates (SW1, SW2, SW3, SW4), a trial was conducted at the experimental farm of the University of Catania (Sicily, Italy). The length of the growing season decreased with the increase of the sowing date in the average genotypes from 160 to 94 days, respectively, for the first and the last sowing date. According to the RED—Renewable Energy Directive, the genotype ‘C856’ was the earliest (112 days), resulting in suitability as a catch crop for biomass production. The results showed that early spring sowings negatively impact dwarf hybrid production (1.2 and 1.5 Mg ha−1 in SW1 and SW2, in the average of the three hybrids), which reached the highest yield in the third sowing date (2.0 Mg ha−1), preferring warmer temperatures for the germination of seeds. On the contrary, the ‘Local’ genotype reached the highest yield (1.6 Mg ha−1) in the first sowing date and linearly decreased in the subsequent ones. Nonetheless, the third sowing date positively influenced the oil content and the oil yield in all dwarf genotypes except the ‘Local’ genotype, which showed the highest oil yield in the first sowing date.

Keywords: phenology; seed germination; oil yield

3.1 Introduction

Castor bean (*Ricinus communis* L.) is an oil seed crop belonging to the Euphorbiaceae family, originating from Asia or Africa [1]. The latter seems the most likely centre of origin because of the ample genetic diversity [2]. Nowadays, the main area of cultivation is India, which ranked 1st as the main exporter, followed by China, Brazil, and the African continent [3–5].

In the past decades, an increasing interest has grown in the market towards the use of this crop, especially for the high seed oil content (35–65%), which has wide applications within the industrial and pharmaceutical sectors [6]. Indeed, castor oil has a unique composition, having up to 90% of ricinoleic acid, 3–6% linoleic, 2–4% oleic, and 1–5% saturated fatty acids [7]. Through oil extraction and, consequently, purification and transesterification process, it is possible to obtain biofuels, such as biodiesel, which is non-toxic, aromatic-less with high miscibility, it has a lower cetane number (CN; 43.7) than diesel (CN; 51) [8,9], has a great biodegradability and it is renewable [10]. Moreover, biodiesel reduces SO2 emissions because of the high viscosity present, which avoids the addition of sulphur compounds, and it burns cleaner than other fuels due to its higher oxygen content [11].

These advantageous characteristics perfectly meet the requirements that must be satisfied within the global challenges of limiting the use of fossil fuels, as required by the law (UE) 2021/1119 [9,12]. They also represent the goal settled in the European Green Deal, which encourages the use of biofuels in Europe; the increase of national biofuels production, then reducing importations; the stabilisation of fossil fuel prices; the reduction of GHG emissions by at least 55% by 2030, and the possibility of having biofuels as a supplementary income for the primary sector [13].

Moreover, castor cultivation enhances the use of marginal

lands, allowing not only a re-evaluation of these degraded soils but also avoiding the competition with valuable lands used for agricultural purposes [14], coping with the goals settled in the Agenda 2030 (ONU, 2015) [12,15]. According to Directive 2018/2001/UE (RED-Renewable Energy Directive) [16,17], it is also necessary to verify if the crop is suitable to be cultivated as a catch crop between the two main crops in crop rotation.

Hence, to fulfil these goals, it is important to improve the production of castor and its agronomic traits. Therefore, in today's panorama, castor seems to be an interesting oil crop to use due to its rusticity and capacity to adapt to different pedo-climatic conditions. Being a warm-season crop, it behaves as a perennial species in tropical regions and sub-tropical regions and as an annual crop in the Mediterranean basin. However, frosts and cool winters can lead to the death of the plant, giving a reason for the selection of dwarf hybrids that are cultivated as annual crops and have higher yield potential, uniformity, and precocity in comparison with other castor genotypes.

Different studies confirm that to obtain high germination percentage, proper ripening, and consequently a high level of oil, castor must be cultivated in a warm environment, and the sowing has to be carried out during the spring when the general temperatures range from 25 °C to 30 °C [18,19].

In this regard, the aim of the present study is the identification of the best period of sowing; to avoid low temperatures, try to take advantage of the water stored in the soil during the rainy period, exploiting warm temperatures to ensure germination and seedling establishment and extending the growing season. Therefore, annual dwarf hybrids of castor were compared on four different sowing dates from April to July in a Mediterranean environment.

3.2 Materials and Methods

3.2.1 Field trial description

The field experiment was conducted at the experimental farm of the University of Catania, Italy (10 m a.s.l., Catania (37°24′31″ N; 15°3′33″ E) in a typical xerofluvent soil, over the period April-November 2021. The characteristics of the soil are as follows: clay 32.3%, silt 11.8%, sand 55.9%, organic matter 1.4%, pH7.6, total N 0.2%, available P2O5 46.1 mg/kg, and exchangeable K2O 293.3 mg/kg.

Two experimental factors were studied, genotype and sowing date. The sowing dates were four: 1 April 2021 (SW1); 30 April 2021 (SW2); 1 June 2021 (SW3); 8 July 2021 (SW4).

Four different genotypes were evaluated: three dwarf hybrids (C1012, C857, C856) and a local selected population (named local genotype). The local genotype of castor used as a control was mass-selected for good adaptation to the South Mediterranean environment from a wild Tunisian population at the Department of Agriculture, Food and Environment (Di3A) of the University of Catania. The other genotypes analysed are dwarf hybrids provided by Kaiima Company (Campinas—SP, Brasil). Dwarf hybrids are characterised by an early ripening cycle and are more suitable for mechanised harvesting due to the height reached by their stem. A split-plot design with three replicates was applied, assigning the sowing date to the main plot and the genotype to the subplot.

The soil was previously ploughed to carry out proper sowing, which has been done manually. A total amount of 120 kg/ha of nitrogen was applied using, before sowing, 60 kg/ha of ammonium sulphate, and at the flowering stage, the same amount was applied in the form of ammonium nitrate, while 80 kg/ha of simple superphosphate (18%) was supplied at sowing.

The plant density was 3 plants m^{-2}, and the rows were 1 m apart

with an interval within the row of 0.33 cm. In each hole, a single seed was sown with a sowing depth of 3–4 cm.

Irrigation water was supplied employing a drip irrigation system. The irrigation volume was determined as the maximum amount of available soil water content in a depth of 0.4 m soil, in which the root system is mostly developed.

The irrigation volume was calculated according to:

$$V = 0.6 \times (FC\text{-}WP) \times \varphi \times D \times 10^3$$

in which V = water amount (mm); 0.66 = readily available water not limiting for evapo- transpiration; FC = soil water content at field capacity (27% of dry soil weight); WP = soil water content at wilting point (11% of dry soil weight); φ = bulk density (1.1 g cm−3); and D = rooting depth (0.6 m).

To schedule irrigation, the sum of daily maximum crop evapotranspiration (ETm) had to match the mentioned volume (V). Rainfall events were subtracted from the calculation. The daily ETm was estimated according to:

$$ETm = E0 \times Kp \times Kc$$

where ETm corresponds to the maximum daily evapotranspiration (mm); E0 is the evap- oration of class-A pan (mm); Kp is the pan coefficient, equal to 0.80 in the semi-arid environment [20]. The crop coefficients (Kc) were determined by previous observations: 0.4 from the emergence phase till the 4-leaf stage; 0.7 from the 4-leaf stage till the flowering; 1.2 from the beginning of flowering to complete capsule development of the first raceme and 0.55 from the first raceme to complete capsule ripening. Along the growing season, each sowing date received 2820, 2112, 1816, and 1615 m^3 of water for SW1, SW2, SW3, and SW4, respectively.

Considering the subsequent ripening of primary and secondary racemes and the different sowing dates adopted, two different harvests were carried out manually for all sowing dates. The first harvest was carried out on 10 October 2021 collecting primary and secondary racemes already ripened. The lately ripened secondary racemes were harvested on 20 November 2021. At harvest, racemes were collected and weighted in each plot. After harvest, in each raceme, the capsules were separated from the peduncle, and then the capsules were mechanically treated to separate the seeds from the husk. After the separation, the seeds were weighted to determine the yield.

A sample of raceme from five plants was collected for each plot to measure the length of the raceme, the number of capsules per raceme, the weight of the seed per raceme, and the weight of the empty capsule per raceme. Thus, the collected capsules were manually separated from the husks to obtain clean seeds. The seeds were separated, weighed and ground for laboratory analysis.

3.2.2 Measurements and Determinations

During the growing season, air temperature and rainfall were measured through a weather station connected to a data logger (Delta-T Devices Ltd., WS-GP1 Compact Weather Station, Cambridge, UK). The evapotranspiration (ET0) was measured by means of a Class A evaporation pan. Both equipment were located 150 m apart from the experimental field.

Dates of phenological phases for each genotype were measured twice a week during the growing period. The scoring has been performed as described by Gervasio et al., (2016) [21] Hence, the phases recorded during the trial were emergence = E; main inflorescence appearance = F1; main flowering on the raceme = A1; beginning of fruit set on the main raceme = G1/1; conclusion of the main raceme fruit set = G1/2; maturation of the main raceme considered at the browning of all the present capsules = M.

The ‘germination rate’ was calculated according to the following formula:

$$\text{Germination rate (\%)} = \frac{GN}{SN} \text{ x } 100$$

where *GN* = total number of plantlets emerged; *SN* = total number of seeds used in the experiment [22].

The ‘Germination rate’ was related to the mean temperature of the period sowing (S)-emergence (E) calculated as follows [23]:

$$\text{Mean temperature Sowing} - \text{Emergence} = \sum_{i}^{n} (Tm_i/n)$$

where $i = 1$; Tm_i = mean temperature at day i; n = number of days from Sowing to Emergence. The relation was described by a linear regression calculated using SIGMAPLOT®11.0 software; Systat Software Inc., San Jose, CA, USA.

3.2.3 Oil extraction and determination

The seeds of the primary and secondary racemes were analysed separately to evaluate if the order of racemes influenced the oil yield. GM200 blade mill (Retsch, GmbH, Haan, Germany) was employed to crush seed into a paste (cake). According to AOAC (Association of Official Analytical Chemists) [24], oil extractions were performed as follows. The solvent extractor SER 148/6 (produced by Velp Scientifica Srl, Usmate Velate (MB), Italy) was used, and an evolution of the Randall method, as reported by Lovkis et al. (2018) [25] was utilised to obtain the oil. The method involves the use of 3–10 g of ground seeds and the use of a hot extraction solvent, such as n-hexane, allowing a reduction of times compared to the classical technique. The extractor foresees a three-stage process: immersion, washing, and recovery of the solvent used.

In the current work, the preparation of the samples involved the filling of the crucibles (porous cellulose fibre thimble) with 3 g of the ground seed samples, immersed in 70 mL of boiling solvent (boiling temperature of 130°C) and placed into a Soxhlet extractor. An immersion and washing phase of 60 min each followed to obtain the oil. After the first two stages, most of the solvent used is recovered in the recovery step. At the end of the procedure, the extraction vessels were placed in an oven at 105° for 30′ to ensure the evaporation of any solvent residues. The vessels were cooled in a desiccator and weighed to calculate the total fat percentage.

The oil content of castor seed was calculated by the following formula, as described by Danlami et al. (2015) [26].

$$\text{Oil content (\%)} = \left(\frac{Weight\ of\ the\ extracted\ oil}{weight\ of\ total\ sample}\right) \text{x } 100$$

The oil yield was calculated by multiplying the oil content (%) obtained by the seed yield (t/ha).

3.2.4 Statistical analysis

Data were statistically analysed using a factorial two-way ANOVA. The data were processed using CoStat version 6.003 (CoHort Software), considering the genotype and sowing dates as fixed factors. When "F" ratios were significant, the means of main factors were separated by Tukey's test ($p < 0.05$), while significant interaction in two-way ANOVA was indicated by the LSD test ($p < 0.05$).

3.3 Results

3.3.1 Climatic conditions

During the entire growing season from April to November, the average temperatures were 26.8 °C for the maximum temperature and

16 °C for the minimum temperature and 21.3 °C for the mean temperature (Figure 3.1).

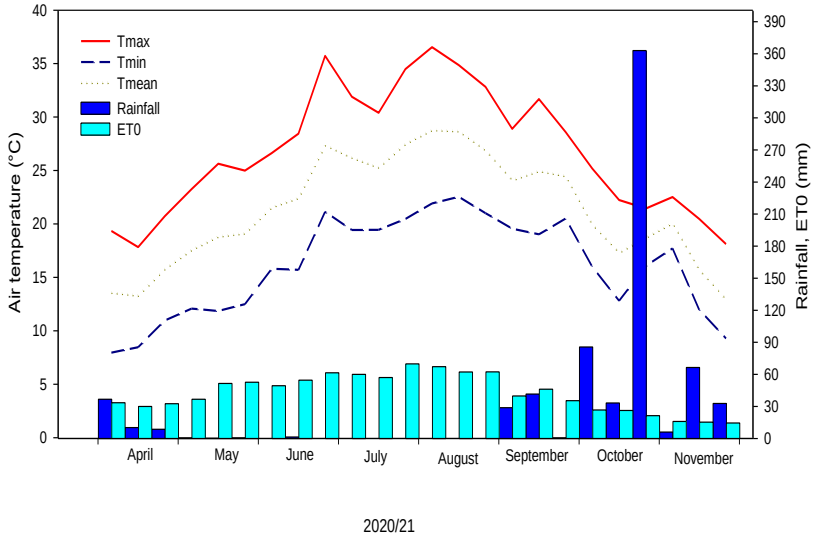

Figure 3.1 - Daily maximum and minimum air temperature, ten-day rainfall, and reference evapotranspiration—ET0) through the growing season at the experimental site (37◦24′31″ N; 15◦ 3′33″ E.; 10 m a.s.l.).

Apart from some rain in April, no rain occurred until the beginning of autumn, in which abundant rainfall was registered from September to November, with an average of

29.9 mm per ten-day period. The evapotranspiration (ET0) raised from April to a maximum in the last ten-day period of July with 69.8 mm; precisely 8.9 and 8.8 mm on 10/07 and 03/08, respectively. The dry period (ET0 > R) ranged from the second ten-day period of April to the second ten-day period of September.

3.3.2 Phenological stages

The length of the main phenological stages for primary racemes

is shown in Figure 3.2. For the average of genotypes, the period "Sowing-E" was 45, 19, 12, and 8 days in SW1, SW2, SW3, and SW4, respectively. The period "E-M" was higher on the first sowing date, decreasing in the subsequent ones (160 and 119 days, 103 and 94 days, respectively, for SW1, SW2, SW3, and SW4).

Moreover, in the average of the genotypes, the ones having the longest period "E-M" were 'C857' and 'Local' (127 and 124 days, respectively), followed by 'C1012' and 'C856' (113 and 112 days, respectively).

The time interval "E-F1", being the stage that had the main impact on the length of the growing cycle, in the average of genotypes, decreased from the first to the last sowing date with 61, 44, 33 and 31 days, respectively, for SW1, SW2, SW3, and SW4. The 'Local' genotype showed the highest value for the "E-F1" period with 52 days.

The time interval "F1-G1/1" presented negligible differences concerning the sowing dates and the genotypes; in the average of the four genotypes, it was between 4 and 6 days. Similar behaviour for the time interval "G1/1-G1/2" (from 4–8 days) and "G1/2-M" (from 8–14 days).

This last stage, corresponding to the beginning of the browning of the capsules, also had a strong impact on the growing cycle, depending on the sowing date. Being higher in the late sowings (SW3 and SW4, 37 and 36 days, respectively) and lower in the early sowing (SW1 and SW3, 28 and 30 days).

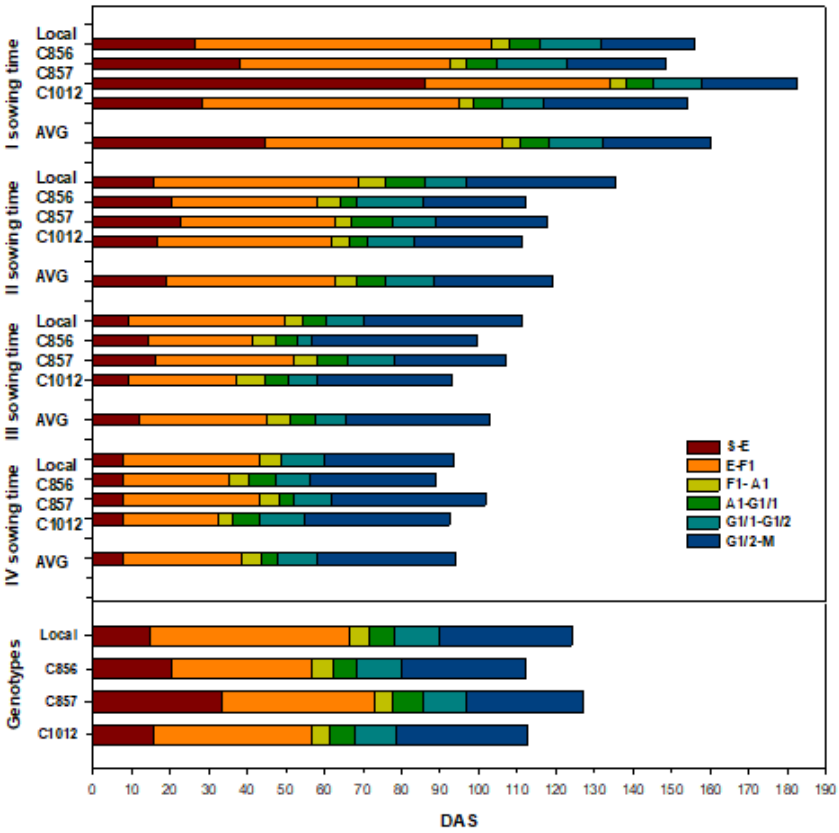

Figure 3.2 - Length of the growing cycle expressed in DAS (Days after Sowing) of phenological stages of primary racemes in castor. Sowing-emergence (S-E), emergence-main inflorescence appearance (E-F1), main inflorescence appearance-main flowering on the raceme (F1/A1), main flowering on the raceme- beginning of fruit set on the main raceme (A1-G1/1), beginning of fruit set on the main raceme-conclusion of the main raceme fruit set (G1-1/G1/2), conclusion of the main raceme-maturation of the main raceme (G1/2-M).

3.3.3 Germination rate and temperature relation

In Figure 3.3, the relation between the percentage of seed germination in the soil and the average temperature of the period "sowing–emergence" is reported for the studied genotypes. Across the studied sowing dates, a positive correlation between the level of temperature and germination rate was found, especially in the dwarf genotypes. Nevertheless, the 'Local' genotype showed a lower influence of temperatures on the germination rate, being 90% in SW1 and 100% in the following sowings.

In-depth, high values of germination rate were obtained in all genotypes with temperatures higher than 22 °C reaching the maximum rate of germination at an average of 26 °C.

Below this temperature, only the 'Local' genotype maintained a high germination rate, while for the other genotypes, the germination rate decreased to almost 40% at 14.1 °C for 'C1012', 14.9 °C for 'C856', and 18.4 °C for 'C857'.

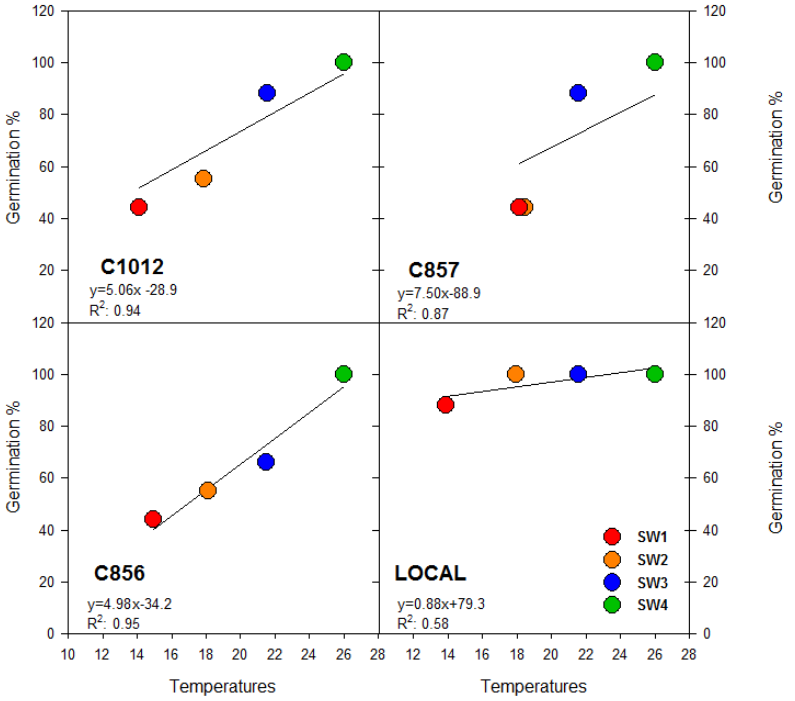

Figure 3.3 - Relation between germination rate (%) and temperature (◦C) of the period 'sowing- emergence' in each sowing date (SW1, SW2, SW3, and SW4) and genotypes (C1012, C857, C856, Local).

3.3.4 Total yield

The ANOVA showed a significant effect of genotype (G) and sowing date (S) on yield, such as the interaction S x G ($p < 0.001$). As shown in Figure 3.4, across the genotypes, 'SW3' was statistically the sowing date with the highest value (1825.7 kg/ha), followed by SW2 (1564.6 kg/ha). The yield significantly decreased in 'SW1' and 'SW4' (1330.9 and 1194.5 kg/ha, respectively), which were not statistically different.

Across the sowing dates, the yield varied significantly with the

genotype ($p < 0.001$), being the highest in 'C856' with a yield of 1806.9 kg/ha followed by C1012 with 1567 kg/ha. The less productive genotypes were 'C857' and 'Local' (1302.8 and 1238.1 kg/ha, respectively).

A clear effect of sowing date on yield can be found in the 'Local' genotype, in which the yield decreased from 1601.1 kg/ha in SW1 to 809.7 kg/ha in SW4, passing through 1467.4 and 1074.1 kg/ha, respectively for 'SW2' and 'SW3'. Regarding the dwarf genotypes, the highest value was found for 'C857' in 'SW3', with a yield of 2311.4 kg/ha, followed by 'C1012', which had the highest value in 'SW3' (1948.2 kg/ha). Both genotypes had a strong reduction in both 'SW1' and 'SW2' due to the strong reduction of plants germinated. In the 'C856' genotype, we did not find statistically different yields among the sowing dates.

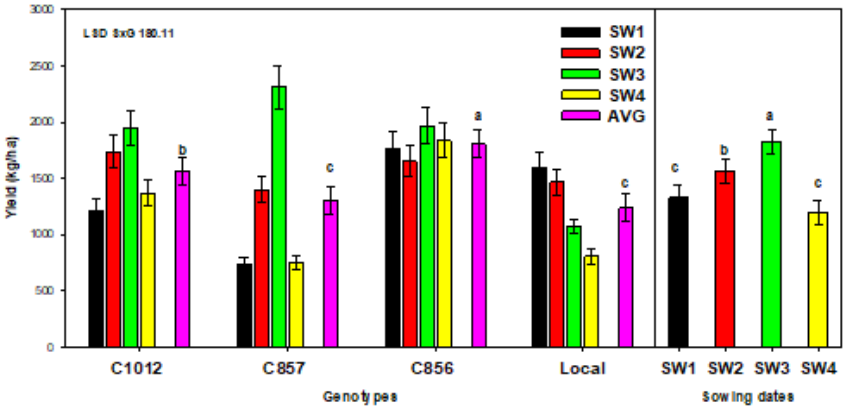

Figure 3.4 - On the left, the total yield (kg/ha) in relation to genotypes (C1012, C857, C856, and Local) and sowing dates (SW1, SW2, SW3, and SW4), and the mean separation of the genotypes, with errors bars indicating the standard errors. On the right, the mean separation per sowing date. Mean values sharing the same letters are not significantly different at $p < 0.05$, according to Tukey's test (HSD). Value for LSD ($p < 0.001$) is reported in the case of a significant interaction.

3.3.5 *Yield incidence of seed weight of primary and secondary racemes*

The yield previously obtained is the result of primary and secondary racemes harvested. As shown in Figure 3.5, the yield incidence of seed weight of primary and secondary racemes was statistically significant for both sowing dates ($p < 0.001$) and genotypes ($p < 0.001$), and so was their interaction SxG ($p < 0.001$).

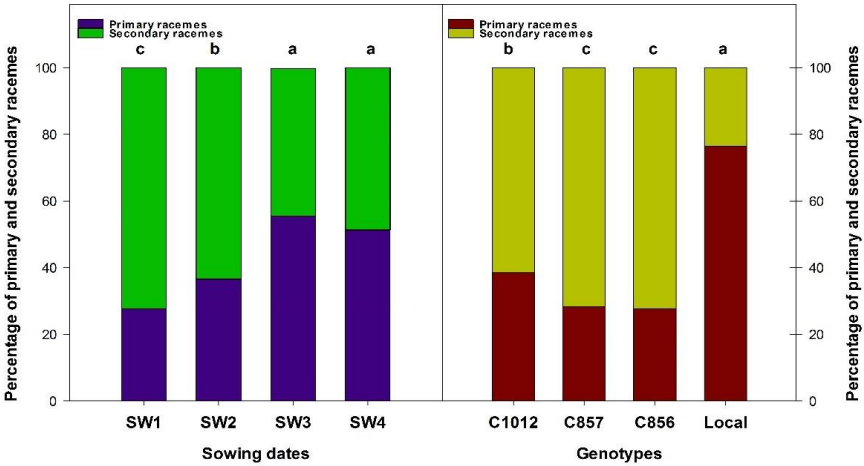

Figure 3.5 - On the left, the incidence of seed weight of primary and secondary racemes (%) in relation to sowing dates. On the right, the incidence of primary and secondary racemes (%) in relation to genotypes. Bars with different letters indicate statistical differences in the incidence of primary raceme according to Tukey's (HSD) test at $p < 0.05$.

By analysing the sowing dates, the incidence of main raceme was significantly the lowest in SW1 and increased in the following sowing dates up to SW3, which was not significantly different from SW4 (55.43 and 51.31%, respectively). This is attributable to the fact that longer cycles, given by earlier sowings, positively affect the production of secondary racemes.

As far as the genotypes are concerned, the genotype with the

highest incidence of primary racemes is the 'Local' (76.4%), statistically different from the dwarf hybrids, which have a lower percentage for primary racemes (38.47, 28.37, 27.59% for C1012, C857, and C856, respectively).

3.3.6 Yield per plant

For the average of genotypes, the sowing dates with higher yield per plant were SW1 (282.7 g) and SW2 (297.8 g), both not significantly different, followed by SW3 (245.0 g) and SW4 (132.7 g), confirming the strong impact of the sowing date on the yield per plant (Table 3.1).

Table 3.1 - Yield per plant (g) in relation to genotypes (C1012, C857, C857, and Local) and sowing dates (SW1, SW2, SW3, and SW4). Mean values sharing the same letters are not significantly dif-ferent at $p < 0.001$, according to Tukey's test (HSD).

Genotype	SW1	SW2	SW3	SW4	Mean
C1012	304.2	347.5	243.5	152.2	261.8 ± 22.1 b
C857	185.0	350.2	288.9	84.3	227.1 ± 29.8 c
C856	441.4	330.5	328.2	204.5	326.2 ± 25.5 a
Local	200.1	163.1	119.3	90.0	143.1 ± 12.6 d
Mean	282.7 ± 30.4 a	297.8 ± 23.7 a	245.0 ± 23.4 b	132.7 ± 14.6 c	
Source of variation	G	S	GxS		
	***	***	***		

*** indicate significance at $p < 0.001$.

Even the genotype had a relevant influence on the yield. In fact, across the sowing date, the genotype that gave better results is 'C856' (326.0 g), followed by 'C1012' (261.0 g), 'C857' (227.0 g), and the 'Local', which resulted in the genotype with the lowest yield per plant with 143.1 g.

The significant S G interaction ($p < 0.001$) is to be ascribed mainly to the behaviour of 'C857', which gave, on the first sowing date, the lowest yield per plant among the genotypes at the same sowing date and by far lower than the yield per plant of SW2 and SW3 in the same genotype.

3.3.7 Yield components

The yield components for the primary raceme, such as the length of the raceme, the seed weight per raceme, the number of capsules per raceme, and the weight of the husks, were significantly affected by the studied factors (Table 3.2). The 100-seed weight was the exception, significantly influenced only by the genotype.

The length for primary racemes was significantly affected by genotype and sowing date. Across the genotype, SW3 and SW4 were not statistically significantly different (36.6 and 37.3 cm, respectively), contrary to SW1 and SW2, in which the difference was statisti- cally significant (21.7 and 32.2 cm, respectively). 'Genotype' also significantly influenced the racemes length (G, $p < 0.001$). Across sowing dates, the longest are 'C1012' and 'C857' (38.6 and 31.9 cm), and 'C856' and the 'Local' genotype, the shortest being not significantly different (28.1 and 29.2 cm, respectively).

The seed weight per raceme was significantly affected by both the studied factors (G; S, $p < 0.001$). Across the sowing date, only the 'Local' genotype was statistically significantly different from the others (Local, 27.6 g). As for the sowing date, for the average of the genotypes, the seed weight per raceme was the highest in SW3 (33.8 g), showing a decrease in respect to this of 41.0% in SW1, 30.7% in SW2 and 37.2% in SW4.

The statistical analysis of the number of capsules showed a significant effect caused by the sowing date and by the genotype. The increase in temperature registered in the SW3 had a greater effect (38.8 number of capsules). The 'Local' genotype confirmed great adaptation, showing a higher number of capsules (32.4).

For the average of genotypes, SW3 and SW4 were statistically significantly different (12.8 and 8.6 g, respectively), whereas SW1 and SW2 did not show any significant difference (both 10 g). Independent of sowing dates, the best value was for the 'Local' genotype (13.2 g),

statistically different from the others (‘C1012’, ‘C857’, and ‘C856’, ranging from 9.8 and 10.0 g). The seed weight ranged between 31.1 and 32.8 g for the average of the sowing dates and between 29.9 and 35.3 g for the average of the genotypes.

Table 3.2 - Length of raceme, the seed weight per raceme, the number of capsules per raceme, the weight of husks, and the 100-seed weight on primary racemes in relation to genotype and sowing date. In the mean columns, the average values of the sowing dates (SW1, SW2, SW3, and SW4) and the average values of the genotypes (C1012, C857, C856, Local). Values with the same letter are not significantly different at $p < 0.05$, or (ns) not significant.

	Genotype	Length of raceme	Seed weight /raceme (g)	N. capsule/ raceme (n)	Husk of raceme (g)	100 - Seed weight (g)
SW1	**C1012**	22.8	19.9	24.3	8.2	31.9
	C857	16.5	7.9	14.5	3.3	30.2
	C856	20.7	22.4	30.0	12.2	30.1
	Local	27.1	28.6	34.3	18.6	37.5
SW2	**C1012**	33.2	20.4	24.2	7.8	32.8
	C857	34.0	21.8	32.0	11.5	29.6
	C856	35.5	21.4	23.5	8.6	32.6
	Local	26.3	30.2	32.6	12.4	36.5
SW3	**C1012**	47.2	32.8	35.8	12.4	31.2
	C857	39.3	42.5	47.7	14.2	31.4
	C856	29.1	34.7	41.3	13.6	28.0
	Local	30.9	25.5	30.9	11.2	34.0
SW4	**C1012**	51.6	26.0	28.6	11.2	35.2
	C857	38.0	19.3	23.5	7.2	29.6
	C856	27.1	13.1	18.6	5.7	29.3
	Local	32.5	26.4	31.5	10.6	33.3
	LSD SxG	4.04	5.75	3.55	1.29	
		***	***	***	***	ns
	SW1	21.7 c	19.7 c	25.8 b	10.5 b	32.4 a
	SW2	32.2 b	23.4 b	28.1 b	10 b	32.8 a
	SW3	36.6 a	33.8 a	38.8 a	12.8 a	31.1 a

SW4	37.3 a	21.2 bc	25.6 b	8.6 c	31.8 a
	***	***	***	***	ns
C1012	38.6 a	24.7 b	28.2 b	9.8 b	32.7 ab
C857	31.9 b	22.8 b	29.5 ab	9 b	30.2 b
C856	28.1 c	22.9 b	28.33 b	10 b	29.9 b
Local	29.2 bc	27.6 a	32.4 a	13.2 a	35.3 a
	***	***	**	***	***

3.3.8 Oil content primary racemes

The oil content showed a significant difference in relation to sowing dates and genotypes (Figure 3.6). Across the genotypes, the oil content for primary racemes exceeded 45.0%, except for SW1 and SW4, in which the oil content was significantly lower than the others. The most productive sowing date appears to be SW3, with the highest value of 48.4%, not significantly different from SW2 (45.4%).

Across the sowing dates, the oil content was significantly different, being the highest in '856' (48.9%) and '1012' (46.0%). Whereas 'C857' and the 'Local' were the genotypes with the lowest values (42.9 and 41.8%, respectively).

In depth, the genotype 'C856' is the one that kept values up to 45% in all the sowing dates, reaching the highest value of 54.5% in SW3. The genotype '857' has shown significantly lower values with respect to SW3 (48.2%), having a decrease of 20.1% in SW1 and 15.4% in SW4.

The local genotype has shown the significantly lowest values in SW2 (40.0%) and SW4 (39.3%).

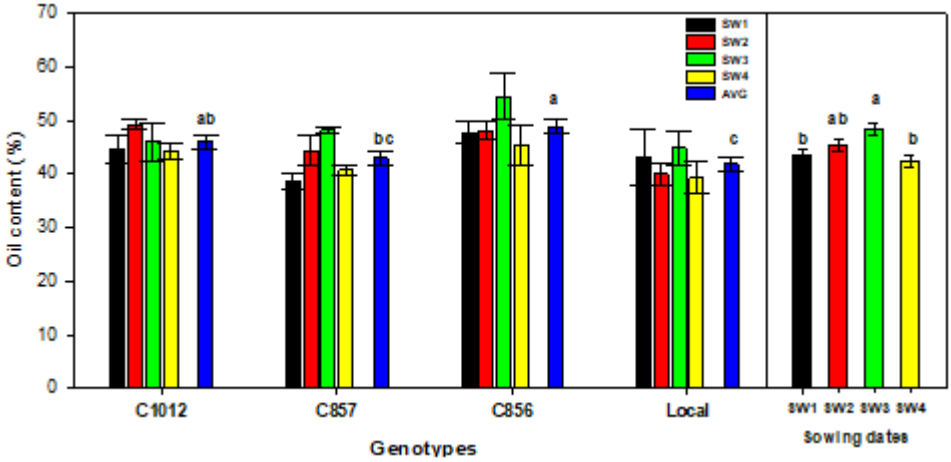

Figure 3.6 - On the left, the oil content for primary racemes (%) in relation to genotypes (C1012, C857, C856, and Local) and sowing dates (SW1, SW2, SW3, and SW4) and the mean separation of the genotypes with errors bars indicating the standard errors. On the right, the mean separation per sowing date. Mean values sharing the same letters are not significantly different at $p < 0.05$, according to Tukey's test.

3.3.9 Oil content secondary racemes

According to ANOVA, the oil content of secondary racemes showed a significant difference in relation to sowing dates but did not show any difference in relation to the genotypes, and their interaction was not significant (Figure 3.7).

Across the genotypes, the highest percentage of oil content was found in SW1 (47.6%), followed by SW2 (45.7%) and SW3 (43.8%), while the lowest oil percentage was obtained in SW4 (41.0%). The highest value in SW1 is mainly due to the 'Local' genotype, which had the best result for the oil content (55.1%). Moreover, even if the 'Local' genotype for the secondary racemes kept the values up to 44%, the SW1 to SW4 had a slight decrease in the oil content (44.9, 44.5, and 44.1% in SW2, SW3, and SW4, respectively).

SW4, on average, is the sowing with the statistically lowest values. This is strongly affected by ‘C857’ and ‘C856’, which had values lower than 39.0%.

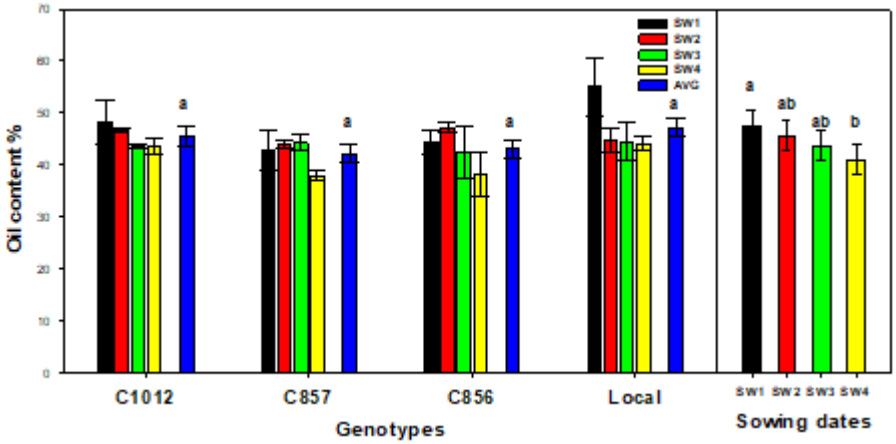

Figure 3.7 - On the left, the oil content for secondary racemes (%) in relation to genotypes (C1012, C857, C856, and Local) and sowing dates (SW1, SW2, SW3, and SW4) and the mean separation of the genotypes with errors bars indicating the standard errors. On the right, the mean separation per sowing date. Mean values sharing the same letters are not significantly different at $p < 0.05$, according to Tukey's test.

3.3.10 Oil yield

The oil yield was significantly affected by the different seed yields obtained in the field experiment. As shown in Figure 3.8, across genotypes, the sowing dates were statistically different from each other, with SW3 being the sowing with the highest value (840.4 kg/ha). SW2 is the second sowing with the highest value (708.0 kg/ha), while SW1 and SW4 had the lowest (612.4 and 491.0 kg/ha, respectively).

Across the sowing dates, ‘C856’ is, on average, the genotype with the best results (814.4 kg/ha), followed by ‘C1012’ with 719.1 kg/ha. A reduction of 29.4% and 33.2% was recorded for the

genotypes ‘C857’ and the ‘Local’, respectively.

The best combination of Sowing date and Genotype were obtained for ‘SW3 x C857’, which had a value of 1073.1 kg/ha, followed by ‘SW3 C856’ and ‘SW3’ ‘C1012’ (932.6 and 874.8 kg/ha, respectively). Whereas the best combination of Sowing date Genotype for the ‘Local’ was obtained in SW1 with 767.1 kg/ha. These results confirm that the dwarf hybrids prefer late sowings, while the ‘Local’ prefers earlier sowings.

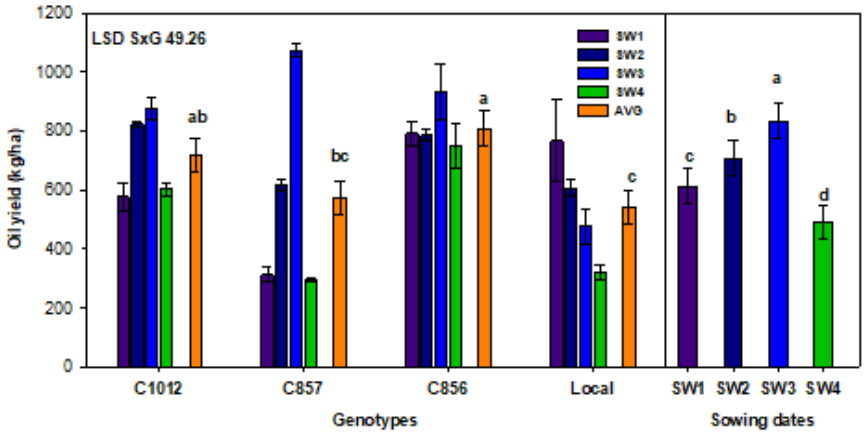

Figure 3.8 - On the left, the total oil yield (kg/ha) for primary racemes in relation to genotypes (C1012, C857, C856, and Local) and sowing dates (SW1, SW2, SW3, and SW4) and the mean separation of the genotypes with errors bars indicating the standard errors. On the right, the mean separation per sowing date. Mean values sharing the same letters are not significantly different at $p < 0.05$, according to Tukey’s test.

3.4 Discussion

The area of cultivation of castor seems to be one of the main aspects to influence the length of the growing season. Different studies confirm that in semi-arid and arid regions, the higher the length of the

growing cycle (up to 180 days), the higher the yield [27,28]. Our findings confirmed this, at least for the 'Local' genotype, where the rate of germination was high. Although, it has also been reported that 100 days may be sufficient in humid climates [29]. As can be expected, the time of sowing strongly affects the length of the growing cycle of the castor. The low temperatures recorded in the first two sowing dates induced a delay in the plant emergence and main inflorescence appearance and consequently extended the crop cycle of the plant. These results highlight, on one side, the advantage of early sowing for a long growing cycle and, on the other side, the great advantage of the possibility of shorter production cycles with late sowings to use the castor crop as a valid candidate as a catch crop, as requested in the Directive 2018/2001/UE (RED—Renewable Energy Directive) [17] of the European Commission for considering a subsidizing production.

Our results confirmed other studies in the Mediterranean area. Indeed, Calcagno et al. (2023) [30] found that, in castor sown late in April, the ripening was reached in just 145 days in a local variety, in line with our 'Local' genotype, which took between 156 and 135 days for the E-M cycle in spring sowings. Whereas, Patanè et al. (2019) [31] found that 99 days are sufficient with sowings in May, matching the time that occurred in the late sowings of our 'Local' genotype, which needed 111 and 94 days (SW3 and SW4, respectively).

As far as dwarf hybrids are concerned, Alexopoulou et al. (2015) [32] reported a growing cycle perfectly in line with ours. In fact, in Greece, genotypes 'C856' and 'C857' sown in May, needed 159 days (from sowing to harvest), whilst the same genotypes, in our research, took 165 days for the same period (30/04), corresponding to the 2nd sowing date. In the present work, low temperatures of the soil have negatively affected the emergence of dwarf seeds. Linear regression was used to describe the relation between the average temperature during the 'Sowing-emergence' and the rate of seed

germination. Low levels of germination were recorded with temperatures below 22°C. Specifically, the dwarf genotypes 'C1012' and 'C856' achieved 44% of germination at around 14°C, while the genotype 'C857' achieved 44% of germination at an average temperature of 18°C. By postponing the sowing date, the increase in temperature positively influenced germination, and levels of 88% were recorded at a temperature higher than 21.5°C in the dwarf genotypes. The germination potential of oilseed crops linearly increases with temperatures increase, even if excessive thermal rises fail crop germination [33]. Moreover, the delay in germination is also strongly associated with the fatty acid composition of the seed [34]. Unsaturated fatty acids keep the fluidity of the membrane, even at low temperatures [35]. Considering that castor is made of 90% of ricinoleic acid, a high level of susceptibility to temperature is found to influence germination, even if ricinoleic acid has a freezing point below most other fatty acids [34]; thus, further studies are requested.

On the contrary, the 'Local' genotype was the most tolerant to the lowering of temperature, achieving 88% of germination at temperature <14°C and reaching the maximum level of germination, in the following sowings, even at 17.9 °C. In this context, the 'Local' genotype, developed in the semi-desert area of Tunisia, probably was more acquainted with the low night temperatures of desert areas. Windauer et al. (2012) [36], by studying *Jatropha curcas* L., a crop belonging to the same family of castor, found and confirmed that increased temperatures shortened the germination time requested for germination, reaching 82% of germination at 25 °C in 8 days, comparable to our results (for the dwarf and 'Local' genotypes).

By evaluating the main factors studied, it emerged that the sowing date and genotype both played a major role in the attainment of the total yield. The three dwarf hybrids examined in this study ('C1012', 'C857' and 'C856') showed a strong reduction of yield due to a reduction in the number of plants caused by the low germination

rate in the first two sowing dates. In the 3rd sowing, the hybrids supplied considerably higher rates of total yield compared to the 'Local' genotype (more than 50% lower than the hybrids). Dwarf hybrids were also studied by Alexopoulou et al. (2015) [32], which obtained a yield production of 1.9 Mg ha−1 for genotype 'C856', when sown late in May in 2012, whilst the sowing of April 2014 attained 3.1 Mg ha^{-1}. This confirmed our result for 'C856', sown on the 1st of June (1.9 Mg ha^{-1}), while our sowing on the 1st and 30th of April yielded an average of 1.8 Mg ha^{-1} because of the low seed germination rate, which reduced the number of plants per square meter. Moreover, Alexopoulou et al. (2015) [32] with the genotype 'C857' attained a yield of 2.5 Mg ha^{-1} when sown late in May 2014, similar to our 'C857', which achieved 2.3 Mg ha^{-1} in the sowing on the 1st of June (3rd sowing date).

Whilst Zanetti et al. (2017) [37] found a seed yield of almost 2 Mg ha^{-1} for 'C856' when sown on the 25th of April and 14th of May in Aliartos and Bologna, similar to our results on the same dates.

The rate of germination was also found to influence the yield per plant. The dwarf hybrids had considerably higher yield per plant than the 'Local' genotype, particularly in the 3rd and 4th sowing dates. It is probable that the low germination rate did not allow the dwarf genotypes to enhance their potential productivity, which could have been much more elevated. On the contrary, the 'Local' genotype behaves better in the earlier sowing dates.

Earliest sowing (SW1 and SW2) promoted the development of secondary racemes by the extension of the growing cycle. In contrast, the shortest cycle and probably higher temperatures encountered in the latest sowings induced a slight delay in the development of secondary racemes in SW3 and SW4, although a strong influence was also given by the genotype factor. Overall, the 'Local' genotype had a major yield contribution by the production of primary racemes. Whereas the dwarf genotypes had a major secondary racemes production, attributable to

the higher contribution of racemes per plant on the dwarf genotypes, as further reported by Alexopoulou et al. (2015) [32], who, by comparing three annual field trials, found that a longer growing period gave almost double the number of raceme (4.5 racemes per plant vs 8 racemes per plant, in 2012 and 2014, respectively).

Severino and Auld (2013) [38] by reporting that the response of yield components is dependent on several factors, focused on the difficulties of using this information to improve yield, pointing out how the selection has to focus on all the parameters, that only combined can positively influence the production.

Furthermore, our findings highlighted how the various yield components were influenced by both sowing date and genotype in different ways, the number of plants per square meter being the main factor. The data showed that, for the results of the seed weight per raceme, the number of capsules, and the husks, higher values were obtained in the 'Local' genotype than in the dwarf hybrids. However, this seems to have slightly influenced the final yields.

The importance given to castor is strongly related to the oil produced from its seeds [14]. The oil content reached the maximum rate of 54% in primary racemes and ranged from 38.5 in 'C857' (SW1) to 54.5% in 'C856' (SW3). The oil content variation in primary and secondary racemes varied according to both sowing date and genotype. Higher results were reported for secondary racemes, which reached a range from 38.0 'C856' (SW4) to 55.1% (SW1) in the 'Local' castor. Thus, negligible differences were found among the dwarf genotypes, and only our 'Local' genotype showed a major influence depending on the order of the raceme. Zanetti et al. (2017) [37], by evaluating dwarf hybrids in Italy and Greece, found differences due to the environment of cultivation. Particularly, 'C856' cultivated in Italy reached an oil content (%) value lower than in Greece (around 50% against 55%, respectively). These results confirmed what was found for our 'C856', which averaged,

independently of the sowing date, about 50%. Nevertheless, our results are perfectly in line with those reported in the literature for the oil content, ranging from 49.0–53.0% in dwarf hybrids [39] from 30.4 to 50.6% (in 'Local' genotype) [30], from 40.1 and 57.4% [40], and from 24.2 to 50.0% in dwarf and normal castor [41].

Finally, the oil yield measured in the 'Local' genotype was lower compared to those of the dwarf genotypes (542.6 kg/ha vs 702.6 kg/ha) mainly due to the total yield obtained in the dwarf genotypes more than to the oil content (%). Anastasi et al. (2014) [42] found that oil yield in local castor was dependent on the genotype by reporting 420 kg/ha of oil yield in the Sicilian area of Ragusa for a 'Local' variety against 710 kg/ha of a 'Brazilian' genotype.

3.5 Conclusions

Castor can be a suitable crop to produce oil when established in the Mediterranean basin, pointing out that dwarf hybrids prefer warmer temperatures, and the 'Local' genotype could be a valid candidate for early sowings. Briefly, castor is a promising low-maintenance oil crop suitable for marginal land and to satisfy industrial and chemical requests. This study aimed to highlight the potential of using castor, on the one hand, the advantage of early sowing for a long growing cycle, and on the other, the possibility of shorter production cycles with late sowings to use the castor crop as a valid candidate as a catch crop, as requested in the Directive 2018/2001/UE (RED—Renewable Energy Directive) of the European Commission for considering a subsidising production. In this last case, the dwarf genotypes can play an important role considering their short growing season and high yield potential.

Overall, the present study highlighted the 'Local' castor as the best genotype for performing early sowing in Spring, whereas the dwarf hybrids prefer warmer temperatures given by performing late sowings.

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4 Germination response of different castor bean genotypes to temperature for early and late sowing adaptation in the Mediterranean regions

This chapter is based on the published version of the article:

Cafaro et al., (2023) Germination response of different castor bean genotypes to temperature for early and late sowing adaptation in the Mediterranean regions
Agriculture, 2023, 13(8), 1569.
https://doi.org/10.3390/agriculture13081569

Germination response of different castor bean genotypes to temperature for early and late sowing adaptation in the Mediterranean regions

Valeria Cafaro [1*], Efthymia Alexopoulou[2], Salvatore Luciano Cosentino [1] and Cristina Patanè [3]

[1] Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli Studi di Catania, via Valdisavoia 5, 95123 Catania, Italy
[2] Centre for Renewable Energy Sources and Saving, 19th km Marathonos Avenue, 19009, Pikermi, Greece
[3] CNR-Istituto per la BioEconomia (IBE), Sede secondaria di Catania, Via P. Gaifami 18, 95126 Catania, Italy

***** Correspondence: valeria.cafaro@phd.unict.it

Abstract

Germination of castor seeds of seven dwarf hybrid genotypes, compared to a 'Local' genotype, selected from a Tunisian population by the University of Catania well adapted to the Mediterranean environment, were studied at six different temperatures (8, 12, 16, 25, 32, and 40 °C). The results indicate that the optimal temperature (25 °C) and near-optimal temperature (32 °C) are the best temperatures for ensuring castor germination (final germination percentage (FGP) ≥ 82.81%). Furthermore, these temperatures positively influenced the vigour index (VI) and the radicle elongation. At a temperature of 8 °C, no germination occurred, while temperatures of 12 and 40 °C negatively affected the seed germination, which, in some genotypes, was null or negligible (<21.25%). A temperature of 16°C allowed good results to be reached for the FGP and the other considered parameters. Overall, the dwarf hybrids performed better at high temperatures than at low temperatures, thus, making them suitable for late sowings, with the exception of the genotype 'C1020', which resulted the best performance at 16 and 40°C, being suitable for both early and late sowings. On the other hand, the 'Local' castor genotype, being the best-performing genotype at 12 and 16°C, and the most tolerant to low temperature (base temperature (*T*b) 12.1 °C), could be used in the early sowing in spring.

Keywords: cardinal temperatures; dwarf hybrids; seed germination; seed vigour; synchrony

4.1 Introduction

Germination is a key stage in the establishment of plants. Germination consists of the activation of the metabolic mechanisms of the seed, which leads to the birth of a new seedling. To obtain the germination of a mature seed, three conditions should be satisfied: (1) the seed must be vital; (2) seed environmental requirements must be appropriate; and (3) any form of primary dormancy must be overcome.

The germination process begins with the absorption of water by the dry seed and ends with the elongation of the embryo [1]. During the imbibition, the seed is not uniformly moistened and this stage can be divided into two steps: the first one comprehends the hydration of the outer part of the seed, while the second includes the hydration of the inner part, the activation of metabolic processes, and, ultimately, the radicle extrusion through the structures surrounding the embryo [2]. Environmental factors such as soil moisture, temperature, oxygen, light, and pH influence seed germination in wild and cultivated plants [3]. In particular, optimal rate of seed germination and, consequently, plant establishment, are the first conditions required to achieve adequate levels of crop productivity, even under harsh weather conditions [4]. Therefore, it is important to analyse the influence of biotic and abiotic conditions that can maximize or limit seed germination in the field and the following crop establishment [5].

Castor bean (*Ricinus communis* L.) is a plant belonging to the Euphorbiaceae family. It is a non-edible vegetable oil crop with lots of applications in the industrial and medical sectors [6]. Different from other oilseed crops, the interest toward this plant has increased exponentially in recent years due to its high seed oil content (around 35–65%) and a high percentage of ricinoleic acid (85–90%). The latter, in particular, has lately been re-evaluated for its wide range of industrial applications [7].

The chance to use marginal lands for its cultivation, and

therefore avoid competition with food crops in the use of more valuable lands, may facilitate castor's exploitation [8,9]. Despite the multiple purposes that castor oil can satisfy, its main use remains the production of fuels such as biodiesel, to conform with the agricultural policies of the Agenda 2030 to produce green energy, replacing fossil sources [10].

As a crop native to Africa, castor is well adapted to tropical and subtropical climates. In the Mediterranean environment, its adaptability expression and cultivation requirements are easily satisfied. Studies confirm that castor needs a range of temperatures between 25 and 30 °C during germination [11] and an amount of water of 400 L per kg of seeds produced [12].

In the semiarid regions of the Mediterranean Basin, the assessment of seed germination response to temperature is important in castor, to predict the seed performance under different thermal conditions that the plant may experience during the initial stages of the growing season. This is particularly true when early sowings are required, to make better use of the water stored in the soil during the rainy period. Therefore, it is also important to identify the thermal thresholds in the seed germination of castor, which may limit the adoption of early or late sowing. On the other hand, the identification of the maximum temperature for germination in castor is also important, to fix the time threshold for late sowing, when castor follows a winter crop within cropping systems. Therefore, this study aimed to evaluate and compare the seed germination characteristics of different genotypes of castor under different thermal conditions. The main purpose of this research was to define the limits in the adoption of early or late sowing in these genotypes, with the final goal of better exploiting resources (water, soil, etc.) from the perspective of environmental sustainability.

4.2 Materials and Methods

The experiment was conducted in a laboratory of the University of Catania studying two experimental factors: genotype and temperature. There were eight genotypes: seven dwarf hybrids of castor bean (*Ricinus communis* L.), provided by Kaiima Company (Campinas—São Paulo, Brasil), compared to a 'Local' genotype of castor, mass-selected for adaptation to the Mediterranean environment from a wild Tunisian population at the Department of Agriculture, Food and Environment (Di3A) of the University of Catania, used as control.

The seeds of the genotypes were assessed for germination traits at six constant temperatures: 8, 12, 16, 25, 32, and 40 °C, with 25 °C considered as the control, being reported as the optimal temperature for seed germination in castor according to ISTA (International Seed Testing Association) [13].

4.2.1 Seed Material

The seeds were open field produced between April and November 2022 at the experimental farm of the University of Catania (37°24′31″ N; 15°3′33″ E, Italy).

The experiment was conducted on seeds harvested in November 2022, stored in paper bags, and kept at room temperature (10–20 °C) until use. Seeds were surface-sterilized with 1% sodium hypochlorite solution (NaClO) (Sigma-Aldrich s.r.l., Milano, Italy) for 3 min, rinsed in distilled water, and then dried at room temperature for 24 h, before germination tests. Before the tests, the seed weight (g) was measured in all genotypes, in eight replicates of 100 seeds each. Seed moisture contents ranged between 3.5 and 4%.

4.2.2 Germination tests

For each genotype and temperature, four replicates of 20 seeds each were placed in Petri dishes (Ø 120 mm) containing two filter

papers—one above and one below the seeds—to allow complete seed hydration. Each filter was moistened with 5 mL of distilled water. Petri dishes were hermetically sealed with Parafilm to avoid evaporation and randomly placed in a thermostatically controlled (1 °C) incubator, in dark conditions, at one of the above mentioned temperatures. Seeds were considered germinated when the testa broke near the caruncle and the radicle emerged from the seed (at least 2 mm long). The Petri dishes were checked daily for germination. Germination was scored until no further germination occurred for at least one week. At the lowest temperatures (8, 12, and 16 °C), the maximum period of observation was extended to 30 days.

The germination tests were conducted between the end of 2022 and the beginning of 2023.

4.2.3 Radicle length measurements

The length of the radicle was measured in seeds of all genotypes at all temperatures. For this purpose, five seeds per replicate were randomly chosen from those first germinated, on the fourth day from the recorded start of germination (i.e., first radicle appearance) (Figure 4.1). In the case of germination being lower than 5 seeds, only those germinated were measured. Seeds were then photographed to document the radicle length at different temperatures.

Figure 4.1 - Germination of castor seeds, photographed on the fourth day from the recorded start of germination

Photos were taken using an iPhone X smartphone. Scanned images were then analysed using ImageJ software, a Java image open-source program used for processing digital images, developed at the National Institutes of Health (NIH) [14], because the program requires a minimum input of information, it is necessary to indicate the correct metric in the desired image. This can be conducted by including an object whose dimensions are known in the picture. In the current work, a ruler was considered for the calibration. After setting the reference measurement, the radicle length in each seed was measured simply using the cursor.

4.2.4 Calculations and Data Analysis

At the end of the germination tests, the parameters reported in

Table 4.1 were calculated.

Parameters	Symbol	Unit	Formula	Explanation	Reference
Final germination percentage	FGP	%	FGP = GN/SN * 100	GN= total number of seeds germinated; SN=total number of seeds tested	[154] [155]
Mean germination time	MGT	Day	MGT= $\sum(\frac{ni \times di}{n})$	ni= number of seeds germinated on day i; d is the incubation period in days; n the total number of germinated seeds.	[154]
Vigor index	VI	-	VI= FGP * radicle length	-	[156]
Synchrony of germination	Z	-	$Z = \sum_{i=1}^{n} Cn_{i;2}$ /N	Cni; 2 = ni(ni-1)/2 and N = $\sum n_i$ ($\sum n_i$-1)/2, where Cni; 2 is the combination of seeds germinated at the ith time, two together, and ni is the number of seeds germinated at time i.	[157][158]

Table 4.1 Germination parameters calculated at the end of the tests.

Data of the 100 seed weight were analysed using one-way ANOVA. Data of final germination percentage (FGP; previously arcsine transformed), mean germination time (MGT), radicle length, vigour index (VI), and data at 16, 25, and 32 °C for synchrony of germination (Z) were checked for normality and homogeneity of variances and statistically analysed via factorial two-way ANOVA. The data were processed using CoStat version 6.003 (CoHort Software), considering the genotype and the germination temperature as fixed factors. When "F" ratios were significant, means were separated using Tukey's test ($p < 0.05$). Data of germination occurred at 8 °C and are not shown, because no germination occurred at this temperature, as for data of Z at 12 and 40 °C, because low levels of

synchronization were reported for some replicates.

The time course of the cumulative values of seed germination was described using a nonlinear iterative regression method (SIGMAPLOT® 9.0 software, Systat Software Inc., San Jose, CA, USA) using a sigmoidal model with three parameters.

$$y = \frac{a}{1 + \left(\frac{x}{x_b}\right) b}$$

where *a* is the maximal value of y (maximum germination), *x* is the time (days) after seed imbibition, x_0 is the time (days) to reach 50% of maximal germination, and *b* is a fitting parameter of the curve. The *x* value on the curve corresponds to 50% germination (*y* value of the curve) and was assumed as theoretical time to 50% germination or t_{50} (days).

The data set of germination rates of 50% germination fraction ($1/t_{50}$ or GR_{50}) of the seed population analysed at 16, 25, and 32° C, resulting from the germination time course, was plotted against *T*, separately for each cultivars; due to the low level of germination data at 12 and 40 °C, these were not considered in the *T*b calculation. The theoretical minimum or base temperature (*T*b) was calculated by the linear regression of GR_{50} vs. *T*, at which seed germination of each cultivar is reduced to 50%. The slope b of the regression line is the germination rate with a decreasing temperature (the higher the b, the faster the germination with the temperature increase). The abscissa intercept is an estimate of the theoretical minimum temperature of germination (*T*b) [20, 21]. The thermal time (θ_T) needed to achieve 50% germination ($\theta_{T\ (50)}$) at the temperatures evaluated was calculated according to the following equation:

$$(\theta_{T\ (50)}) = (T - T\text{b}) \times t_{50}$$

where $\theta_{T(50)}$ = thermal time needed for 50% germination (°Cd), T = actual germination temperature (°C, constant in controlled environment), Tb = base germination temperature, t*50* = the time to 50% germination (median response time) [20,21].

To compare both methods of $\theta_{T(50)}$ calculation, thermal time was also calculated as the inverse of the slope b of the regression line used for *T*b estimation.

4.3 Results

4.3.1 100-seed weight

The values of the 100-seed weight measured for the eight genotypes are reported in Table 4.2. According to the ANOVA, the weight was significantly affected by the genotype, ranging from 29.88 g (in 'C1008′) to 52.05 g (in 'C1013'). Seeds of the 'Local' castor exhibited a seed weight (33.88 g) lower than the average values of all genotypes.

Table 4.2 - 100-seed weight (mean ± SE) of 8 genotypes of castor. Values with different letters are significantly different at p<0.05, according to Tukey's test

Genotype	100 – seed weight (g)
Local	33.88±0.99 de
857	35.60±0.37 bd
1008	29.88±0.15 fh
1012	37.02±0.07 b
1013	52.05±0.07 a
1018	35.90±1.20 cd
1019	35.78±0.03 bc
1020	36.01±0.07 bd
Average	37.01±2.92

4.3.2 Cumulative Germination Time Course

Because no germination occurred at 8 ˚C, this temperature

was not shown for all parameters. The cumulative seed germination time course of the eight genotypes of castor under different temperatures (12, 16, 25, 32, and 40 °C) is illustrated in Figure 4.2. The course is effectively described ($R^2 > 0.90$ Table 4.3) using a three-parameter sigmoidal function, whose trend reveals a short initial phase of low germination at optimal or near- optimal temperature (25 and 32 °C), followed by a rapid increase in germination, up to a maximum (*a* parameter of the curve). After that, germination remained constantly stable. In some genotypes, the initial phase of slow germination became negligible at 32 °C. At a high temperature (40 °C), seed germination started later than at an optimal temperature (25 °C) and proceeded poorly and slowly. Only in 'C1013′ and 'C1019′ did germination at 40 °C start earlier than at 25 °C, although germination at this temperature, in these two genotypes, was maintained lower than 50%. At the suboptimal temperature (12 °C), the start of germination was delayed to a great extent, and very low levels of germination were scored. At 16 °C, all the genotypes germinated, slower than at other temperatures, and only the 'Local' genotype approached 100%. The supraoptimal temperature (40 °C) enhanced the germination percentage and rate. Only in the 'Local' castor were the seeds able to germinate at 12 °C, approaching a maximum value (*a* parameter) that was even higher than that at 40 °C.

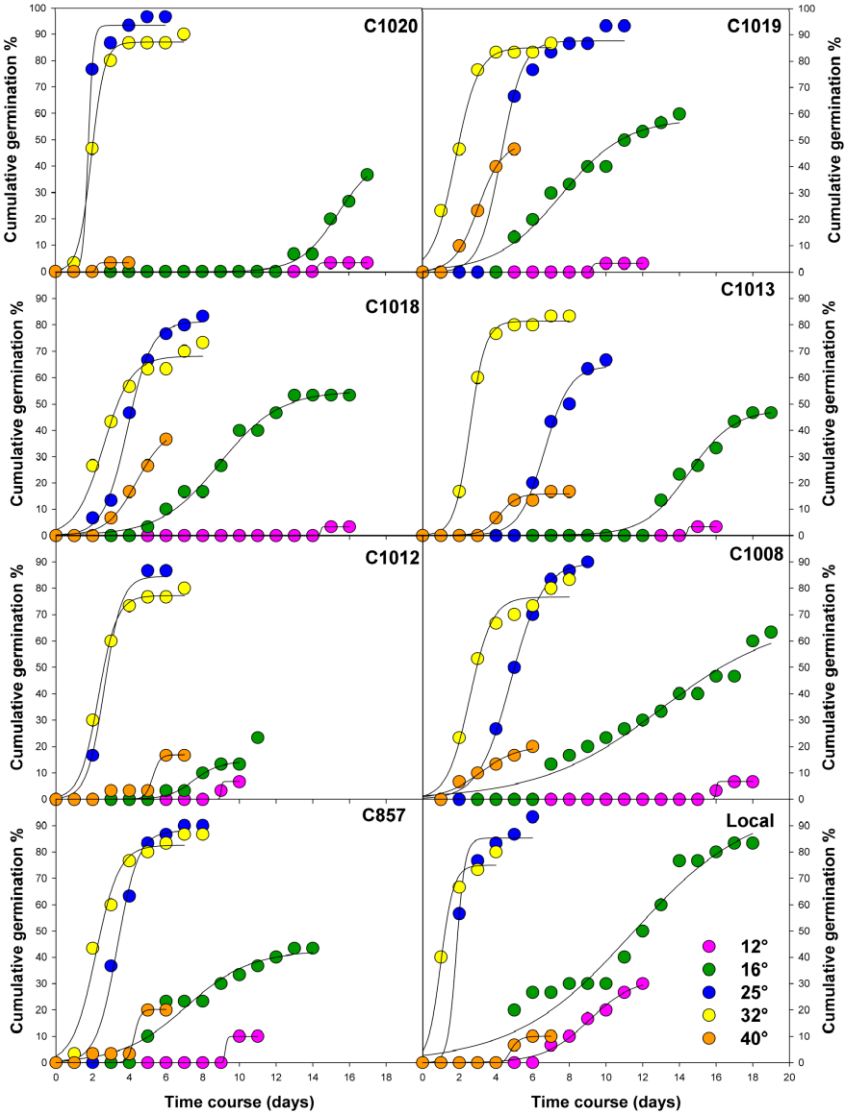

Figure 4.2 - Cumulative germination time courses (solid curves) at different temperatures in 8 genotypes of castor. Symbols represent the observed daily percentages at 12, 16, 25, 32, and 40 °C vs. time.

4.3.3 Seed germination under controlled temperatures

At the optimal temperature (25°C), the final germination percentage (FGP) of seed germination reached 87.97% across the genotypes (Table 4.4). An FGP of 82.81% was also recorded at 32 °C, which was not significantly different. The FGP started to significantly decrease at 16 °C in the average for all the genotypes (63.72%) and significantly decreased down to 21.25% at 40 °C and 8.75% at 12 °C. The FGP, across temperatures, also changed with the genotype, although within a narrow range, being the highest in 'C1019′ (with a 64.75% FGP) and the lowest in 'C1013′ (43.25%). Good results were also shown for the 'Local' genotype, which was lower than the best with only a 5.50% difference.

Table 4.3. Values of parameters of the sigmoidal logistic function interpolating data of seed germination of 8 genotypes of castor at 12, 16, 25, 32, and 40°C.

Genotype	T (°C)	a	R2
Local	12	19.20	0.98
	16	32.20	0.99
	25	75.80	0.99
	32	75.00	0.98
	40	11.00	1.00
C857	12	11.00	1.00
	16	48.70	0.99
	25	88.00	0.98
	32	82.60	0.98
	40	20.00	0.95
C1008	12	6.60	1.00
	16	13.80	0.99
	25	89.80	0.99
	32	76.70	0.98
	40	20.30	0.97
C1012	12	6.66	1.00
	16	14.9	0.99
	25	84.50	0.99
	32	77.20	0.99
	40	1.68	0.93

	12	3.33	1.00
	16	49.80	0.99
C1013	25	64.40	0.98
	32	80.70	0.99
	40	15.00	0.98
	12	3.59	1.00
	16	59.30	0.99
C1018	25	81.20	0.99
	32	68.10	0.97
	40	41.10	0.99
	12	3.33	1.00
	16	67.60	0.99
C1019	25	74.70	0.98
	32	85.10	0.99
	40	49.00	0.99
	12	3.59	1.00
	16	48.80	0.99
C1020	25	92.20	0.99
	32	87.00	0.99
	40	3.33	1.00

However, the significant TxG interaction indicated a different FGP response to temperature, depending on the genotype. Generally, the FGP at 32 °C matched that at 25 °C, in all genotypes (excluding 'C1013′, which was 66.25 and 83.75% at 25 and 32 °C, respectively) (Table 4.5). The genetic differences in the FGP became evident at extreme temperatures (12 and 40 °C). In particular, germination was significantly depressed at both 12 and 40 °C in all genotypes, and the FGP was reduced down to levels that, in most cases, were not different between the two temperatures. Only in the 'Local' genotype were the values of the FGP lower at 40 °C than at 12 °C (10.00 and 30.00%, respectively). In general, the genotypes that germinated at their best under optimal thermal conditions were 'C1019′ and 'C1020', with final germination that approached 100%. Under the same conditions, 'C1013′ exhibited the lowest germination (66.25%). At 40 °C, the highest FGP (47.50%) corresponded to genotype 'C1019', which was

also the genotype that had the highest germination at 16 °C (90.00%).

Table 4.4. Mean effect and standard error (SE) of temperatures (T) and genotypes (G), and their interaction (TxG) on the final seed germination percentage (FGP), mean germination time (MGT), synchrony of germination (Z), radicle length, and vigour index (VI). Data of Z at 12 and 40 °C were excluded from the statistical analysis (see Section 2).

	FGP (%)	MGT (day)	Synchrony of germination (Z)	Radicle length (cm)	VI
Temperature (T)					
12°C	8.75± 1.67d	23.33 ± 2.21a	-	0.60± 0.12d	10.98 ±2.68d
16°C	63.72± 3.77b	12.05± 05.57 b	0.12 ± 0.03c	1.50± 0.11c	90.85 ± 8.20c
25°C	87.97± 2.31a	4.65± 0.27c	0.32 ± 0.03a	1.96 ± 0.15b	177.39± 14.92b
32°C	82.81± 1.50a	3.14± 0.17d	0.25± 0.02b	4.54± 0.27a	381. 21± 26.75a
40°C	21.25± 2.60c	4.76± 0.31cd	-	0.83± 0.04d	16.47± 1.85d
Genotype (G)					
C857	52.00 ± 7.73ac	8.88± 1.61bc	0.20 ±0.03b	1.77 ±0.18c	108.92 ±22.84ce
C1008	53.25± 7.56ac	11.06± 2.31a	0.15± 0.02b	1.77± 0.33c	125.24±31.61bd
C1012	48.00 ±7.66bc	8.25± 1.11c	0.23 ±0.05b	2.36± 0.38ab	159.33± 36.01ac
C1013	43.25 ±6.69c	11.78± 1.50a	0.19 ± 0.03b	1.13± 0.24d	71.30± 21.27e
C1018	50.75± 6.74ac	10.34± 1.31ab	0.14± 0.02b	1.22± 0.18d	81.73± 15.70de
C1019	64.75 ±8.01a	9.20± 1.25bc	0.19± 0.02b	1.88± 0.40bc	162.33± 36.30ab
C1020	51.95± 9.60ac	11.20± 1.81ab	0.47± 0.06a	2.61± 0.51a	207.10± 55.49a
Local	59.25± 7.48ab	5.99± 0.85d	0.29± 0.02ab	2.37± 0.37ab	167.07± 37.01ab
Significance					
T	***	***	***	***	***
G	**	***	***	***	***
T x G	*	***	ns	***	***

Values with the same letter are not significantly different at $p < 0.05$ level (n= 4)

*, ** and *** indicate significance at $p < 0.05$, 0.01 and 0.001, respectively.

Table 4.5. Final germination percentage (FGP, %) in eight genotypes of castor at five temperatures (0, 16, 25, 32, 40°C) (n=4).

Temperature	Genotype							
(T)	C857	C1008	C1012	C1013	C1018	C1019	C1020	Local
12°C	10.00 c B	10.00 b B	5.00 c B	3.75 c B	3.75 c B	3.75 c B	3.75 b B	30.00 b A
16°C	53.75 ab	62.50 a	50.00 ab	46.25 b	56.25 ab	90.00 a	67.25 a	83.75 a
25°C	90.00 a	90.00 a	86.25 a	66.25 a	83.75 a	97.50 a	97.50 a	92.50 a
32°C	86.25 a	83.75 a	80.00 a	83.75 a	73.75 ab	85.00 a	90.00 a	80.00 a
40°C	20.00 bc A-C	20.00 b A-C	18.75 bc BC	16.25 c BC	36.25 bc AB	47.50 b A	1.25 b C	10.00 c BC

4.3.4 Mean Germination Time under controlled temperatures

The germination speed was significantly affected by temperature ($p < 0.001$), and at 32 °C, the seeds germinated faster (MGT 3.14 d) than those at the optimal temperature (4.65 d at 25 °C), similar to the extreme temperature of 40 °C (MGT 4.76). A longer time was needed to germinate at 16 °C (12.05 d). Nevertheless, the longest time was needed at the suboptimal temperature (12 °C), which needed 23.33 days to reach germination (Table 4.4).

The germination time also significantly varied with the genotype ($p < 0.001$). Seeds of the 'Local' castor were significantly the fastest in germination (5.99 d) contrary to the dwarf hybrids that on average had an MGT of 10.10 d. The most delayed in germination was 'C1013′, which, across temperatures, took more than 11.78 days to complete germination. The significant TxG interaction ($p < 0.001$) on MGT indicated a different genotype response to temperature for this trait. At optimal (25 °C) or near-optimal (32 °C) temperatures, the dwarf genotypes took much more time to germinate (MGT up to 7.34 in 'C1013′ and 4.75 d in 'C857′, at 25 °C and 32 °C, respectively) (Table 4.6). This pattern was more evident at the optimal temperature, whereas, at 16 °C, 'C1020′ was the longest with 16.93 d.

Table 4.6. Mean Germination Time (MGT, days) in eight genotypes of castor at five temperatures (12, 16, 25, 32, 40°C) (n= 4).

Temperature (T)	Genotype							
	C857	C1008	C1012	C1013	C1018	C1019	C1020	Local
12°C	23.00 a B	30.67 a A	16.00 a C	28.00 a AB	28.00 a AB	23.00 a B	28.00 a AB	10.00 a C
16°C	7.97 b C	11.79 b A-C	13.37 a A-C	15.03 b AB	9.93 b BC	11.57 b A-C	16.93 b A	9.90 a BC
25°C	3.97 c CD	5.46 c BC	3.30 b D	7.34 c A	5.60 c B	5.43 c BC	3.37 c D	2.77 b D
32°C	4.75 c A	3.53 c AB	3.10 b AB	3.20 d AB	3.60 c AB	2.47 c B	2.70 c B	1.77 b B
40°C	4.75 c	3.83 c	5.50 b	5.33 cd	4.60 c	3.53 c	5.00 c	5.53 b

Within each column, values with different lower cases, when present, indicate significant difference at $p<0.05$ (Tukey's test)

Within each row, values with different upper cases, when present, indicate significant difference at $p<0.05$ (Tukey's test)

4.3.5 Synchrony of germination

The synchrony of germination (Z) indicates the level of synchronization of seed germination. Synchrony, which was quite low in all genotypes, was significantly higher at 25 °C (Z = 0.32, against 0.25 at 32 °C and 0.12 at 16 °C). It also changed with the genotype (p <0.001), and the greatest Z (0.47) was calculated in genotype 'C1020′. This value was statistically different from the rest of the dwarf genotypes, while the 'Local' one was not statistically different from the highest or the rest of the dwarf genotypes. In turn, the lowest Z (0.14) was calculated in 'C1018′, whose Z did not differ from the rest (Table 4.4). At the optimal temperature (25 °C), the highest value of 0.63 of synchrony was obtained in 'C1020′; contrarily, a low value of 0.03 was reached at 16 °C for 'C1012′, 'C1013′, and 'C1018′. At 32 °C, the highest (0.30) was achieved by the 'Local' genotype (Table 4.7).

Table 4.7. Synchrony of germination (Z) in eight genotypes of castor at three temperatures (16, 25, 32°C) (n= 4).

Temperature	Genotype							
(T)	C857	C1008	C1012	C1013	C1018	C1019	C1020	Local
16°C	0.07 b B	0.07 B	0.03 b B	0.03 a B	0.03 b B	0.10 a AB	0.53 a A	0.17 c AB
25°C	0.33 a AB	0.13 B	0.50 a AB	0.27 a AB	0.13 ab AB	0.20 a AB	0.63 a A	0.40 a AB
32°C	0.20 ab	0.27	0.17 ab	0.27 a	0.27 a	0.27 a	0.27 a	0.30 b

Within each column, values with different lower cases, when present, indicate significant difference at $p<0.05$ (Tukey's test)
Within each row, values with different upper cases, when present, indicate significant difference at $p<0.05$ (Tukey's test)

4.3.6 Base temperature and Thermal time

A linear model was used to estimate the critical germination temperature, based on the germination rate GR50 (i.e., 1/t50). As above mentioned, data at 12 and 40 °C were excluded from the calculation because of the low level of germination, as well as 'C1012′ and 'C1020′, because a germination lower than 50% occurred at 16 °C. The average minimum or base temperature allowing germination of the genotypes was 12.5 °C (Table 4.8). The base temperature varied with the genotype, from 12.1 °C ('Local') to 12.8 °C ('C1008′). It is interesting to notice that all genotypes exhibited a similar Tb (>12 °C), with just a slight variation among them. Nonetheless, they presented different germination rates (i.e., b slope of linear regression of GR50 vs. T), revealing a faster or slower response and sensitivity to increasing (or decreasing) temperatures. In depth, the 'Local' genotype, being the most tolerant to the lowering of temperature (83.75% germination at 16 °C), had the lowest Tb (12.1 °C). The genotype with the highest Tb value was 'C1008′, having 90% of germination at 25 °C, with a 30.55% decrease (62.50%) at 16 °C. In this regard, it resulted as the most sensitive to the lowering of

temperature.

Table 4.8 Values of base temperature (Tb), estimated (from the model ±σ) and calculated (inverse of the slope b) thermal time θT in six genotypes of castor. Data of 'C1012' and 'C1020' were excluded since levels of germination (<50%) were reached at 16°C.

Genotype	Tb (°C)	θT (°Cd) (From model)	θT (°Cd) (1/b)
Local	12.1	33.9±2.49	32.8
C857	12.6	46.3±1.98	46.7
C1008	12.8	58.8±6.44	59.5
C1012	-	-	-
C1013	12.7	77.3±2.88	76.3
C1018	12.3	52.4±5.47	57.4
C1019	12.4	46.6±6.95	47.3
C1020	-	-	-
Average (±σ)	12.5±1.0	52.6±4.3	53.4±4.3

4.3.7 Radicle length

The mean effects of the two experimental factors (temperature and genotype) on the radicle length of castor are summarized in Table 4.4. At the optimal temperature (25 °C), seeds developed a radicle that, by the fourth day, across genotypes, was 1.96 cm long. Interestingly, at a higher temperature (32 °C), seed radicles reached significantly longer lengths (T, $p < 0.001$) than those measured at 25 °C (4.54 cm). However, a further thermal increase to 40 °C slowed the radicle growth, and at this temperature, the mean length was

0.83 cm. Lower temperature negatively affected the radicle length, which reached 0.60 and 1.50 cm at 12 and 16 °C, respectively.

'Genotype' also significantly influenced the radicle length (G, $p < 0.001$). Across temperature, the fastest radicle elongation was measured in 'C1020′ and the 'Local' castor, along with 'C1012′,

whose seeds developed radicles that, by the fourth day from the appearance of the first radicle, were 2.36 cm. Much smaller radicles (1.88 cm) were developed by the remaining genotypes. Among the remaining genotypes, the lowest value (1.13 cm) corresponded to 'C1013'.

The significant TxG ($p < 0.001$) on the length of radicles revealed that the radicle growth changed among the different genotypes with temperature. At the optimal temperature (25 °C), the longest radicles were measured in 'C1012' and 'C1020' (3.07 and 2.67 cm, respectively) and the smallest developed in 'C1013' (0.67 cm) (Table 4.9). The increase in temperature to 32 °C boosted the radicle growth in all genotypes, with more remarkable effects in 'C1008', whose seeds developed a radicle that was threefold longer (>4.60 cm) than that measured at 25 °C. In this genotype, both a thermal decrease and increase to 12 and 16 °C and 40 °C, respectively, strongly impacted the seedling growth, and the final radicle was 0.67 cm at 12 °C, 1.27 cm at 16 °C, and 0.70 cm at 40 °C.

Table 4.9. Radicle length (cm) in eight genotypes of castor at five temperatures (12, 16, 25, 32, 40°C) (n= 4).

Temperature	Genotype							
(T)	C857	C1008	C1012	C1013	C1018	C1019	C1020	Local
12°C	1.40 A	0.67 b A-C	0.40 d BC	0.17 d C	0.23 c BC	0.13 d C	0.33 d BC	1.53 bc AB
16°C	1.80	1.27 b	2.13 bc	0.63 c	0.97 b	1.77 bc	1.87 bc	1.60 bc
25°C	2.43 AB	1.63 b AB	3.07 b A	0.67 bc B	1.47 b AB	1.97 b AB	2.67 b A	1.83 b AB
32°C	2.43 D	4.60 a C	5.20 a BC	3.20 a D	2.60 a D	5.10 a BC	7.30 a A	5.90 a B
40°C	0.80 AB	0.70 b AB	1.00 cd A	1.00 b A	0.87 bc AB	0.43 cd B	0.90 cd AB	1.00 c A

Within each column, values with different lower cases, when present, indicate significant difference at $p<0.05$ (Tukey's test)
Within each row, values with different upper cases, when present, indicate significant difference at $p<0.05$ (Tukey's test)

4.3.8 Vigor index (VI)

As mentioned in Table 4.1, the vigour index (VI) is calculated by multiplying the germination percentage (%) and the radicle length (cm). The mean values obtained are shown inTable 4.4. Statistically, VI showed a significant difference in relation to temperature, genotype, and their interaction ($p < 0.001$).

At suboptimal (12 °C) and supraoptimal (40 °C) temperatures, VI had the lowest values (10.98 and 16.47, respectively), whereas 16 °C had higher values in comparison with these last two temperatures but lower than at 25 and 32 °C, reaching a VI of (90.85). Interestingly, VI was maximized at 32 °C, with a value (381.21) that was significantly greater than that calculated at the optimal temperature (25 °C, VI = 177.39). VI also varied with genotype, being the highest in 'C1020′ (207.10) and 'Local' (167.07) and the lowest in 'C1013′ (71.30) and 'C1018′ (81.73). The significant TxG interaction revealed different VIs calculated for the genotypes of castor, depending on temperature. Specifically, at 25 °C, wide variability was noticed within the dwarf genotypes, whose VIs significantly ranged between 43.70 ('C1013′) and 267.57 ('C1012′) (Table 4.10). This did not occur at 32 °C, where the dwarf genotypes widely differed in VI (from 192.50 in 'C1018′ to 654.75 in 'C1020′). At this temperature, the highest VI was found for 'C1020′. Overall, the greater VI at 25 °C in some genotypes did not correspond to a greater VI at 32 °C for the same genotypes. The 'Local' castor showed the same trend as the dwarf genotypes, having a greater VI at 32 °C (473.60), decreasing to a minimum of 9.87 at 40 °C. Contrarily, having a comparison with the dwarf genotypes, the 'Local' genotype had higher values for the lowest temperature (46.10 at 12 °C and 132.20 at 16 °C), showing greater tolerance to low temperatures.

Significant differences were highlighted among the genotypes studied at 32 °C for the VI. In particular, the highest values occurred

for ‘C1020′ (VI, 654.75) and ‘Local’ (VI, 473.60), followed by ‘C1019′ (439.40), while the lowest value was obtained for ‘C1018′, with 192.50.

Table 4.10. Vigor index (VI) in eight genotypes of castor at five temperatures (12, 16, 25, 32, 40°C) (n= 4).

Temperature (T)	Genotype							
	C857	C1008	C1012	C1013	C1018	C1019	C1020	Local
12°C	18.07 b B	6.90 c B	7.93 c B	1.67 d B	2.33 c B	1.47 d B	3.33 c B	46.10 c A
16°C	68.97 ab A-C	76.99 bc A-C	88.13 c A-C	28.90 bc C	59.73 bc BC	160.18 bc A	111.69 bc A-C	132.20 b AB
25°C	212.03 a AB	148.78 b AB	267.57 b A	43.70 b B	122.04 b AB	191.66 b AB	259.76 b A	173.60 b AB
32°C	226.37 a DE	379.42 a B-E	417.20 a B-D	266.41 a C-E	192.50 a E	439.40 a BC	654.75 a A	473.60 a AB
40°C	19.17 b	14.10 c	15.83 c	15.83 cd	32.07 c	18.93 cd	5.95 c	9.87 c

Within each column, values with different lower cases, when present, indicate significant difference at $p<0.05$ (Tukey’s test)
Within each row, values with different upper cases, when present, indicate significant difference at $p<0.05$ (Tukey’s test)

4.4 Discussion

Slight genetic variability was observed in the 100 seed weight, with an average of 37.01 g. This value is higher than that reported by Wang et al. 2010 [22], who evaluated 1033 accessions of castor seeds with a mean of 28.3 g. This is also similar to what was reported by Santiago et al. 2023 [23], who, by evaluating sixteen Mexican varieties of castor, found a higher seed weight (49.31 g per seed), instead of 31.45 g in some commercial varieties. Negligible differences were highlighted concerning the genotype, indicating a minor influence on this trait.

In this study, the course of germination with time was effectively described using a two-parameter sigmoidal curve. Zhu et al. 2019 [24] adopted a similar function to describe the seed

germination time course in sweet sorghum seeds under salinity stress. After an initial phase of low germination, in which water and oxygen are absorbed through the seed coat [25], seed germination speeds up, until its maximum (which differs with the genotype and temperature) is reached. The same trend of germination was observed at the optimal (25 °C) and near-optimal temperature (32 °C), and, in some cases, the curves for the two temperatures overlapped. This result indicates that in some genotypes, germination at 25° and 32 °C started and proceeded simultaneously. On this basis, we may state that the optimal range of temperature for germination in castor may be extended over that (25 °C) reported in the literature [11].

On the whole, a temperature of 16 °C ensured optimal levels of germination, even if it started later than at 25 °C. The supraoptimal temperature (40 °C) and suboptimal temperature (12 °C) negatively affected germination in all genotypes. Indeed, as confirmed by Severino et al., 2012 [8], inauspicious climatic conditions (i.e., low soil temperature) are considered the most influential factors for the slowing down and inhibition of castor seeds germination.

As confirmed by Marcos Filho, 2015 [26], the importance given to seed germination is related to the establishment of crops and consequently to the yield obtainable. Overall, seed germination at 25 °C was slightly lower in the dwarf genotypes (mean, 87.32%, against 92.50% in the 'Local'). This is similar to what was reported at 16 °C (60.85 and 83.75% for the dwarfs and 'Local' genotype, respectively). However, this did not occur at 32 °C, where the dwarf genotypes had slightly higher values than the 'Local' castor (83.21 against 80.00%, respectively). These results seem to indicate the strong influence of the genotype factor on the FGP, matching what was reported by Amorim Neto et al. 2001 [27] who accomplished germination ranging from 84.00 to 86.70% in dwarf castor. Moreover, considering that seed germination on average achieved a level 82.81% at 32 °C, the optimum for germination in castor may be extended to this

temperature, as validated by Severino et al. 2012 [8] who reported that a temperature of 31 °C is the optimum for germination in castor. In addition, in this context, the dwarf genotypes, selected for arid and semiarid environments, are more adapted and tolerant to higher temperatures, as also confirmed at 40 °C, in which the dwarf genotypes, being more acquainted with higher temperatures, achieved 22.86% of germination against 10.00% of the 'Local'. Nonetheless, Falasca et al. 2012 [28] reported that temperatures > 30 °C represent a limit for castor cultivation, because seeds of castor may fail to germinate.

The 'Local' genotype, which achieved similar results at both 25 and 32 °C (FGP > 80%), was also the only genotype that reached 30.00% germination at 12 °C and reached 83.75% at 16 °C, indicating a greater tolerance of this genotype to suboptimal and lower temperatures, attributable to the Tunisian environment from which it was selected, where low night temperatures characterise the desert area.

In this experiment, no germination occurred at 8, and at 12 °C, most of the genotypes failed to germinate (level lower than 5% of germination). These results indicate that the thermal requirements for castor germination, at least for the dwarf hybrids examined, are above the minimum (15 °C) reported by Severino et al. 2012 [8], and that the base temperature is below that suggested (15–16 °C) by Amorim Neto et al., 2001 [27] and Moosavi et al. 2022 [29], who found that castor may germinate even at temperatures ranging between 5 and 15 °C.

In this regard, seeds present a critical temperature for germination, meaning that seeds, which have a base temperature (Tb) above T, may fail in germination [21]. The necessity of studying the base temperature is related to the acknowledgment of the thermal stress that causes the seeds to reach out, affecting their establishment. The lower the Tb, the greater the tolerance to stress given by a lowering of temperature. Our findings revealed that castor seeds had a

different behaviour when exposed to low temperatures, in terms of germination rate and speed. Indeed, the 'Local' castor resulted as the best genotype for early sowings, having the lower Tb, indicating a greater tolerance to thermal stress. This result matched those of the FGP, with the 'Local' genotype, the only one among the dwarf hybrids, achieving 30.00% germination at 12 °C.

Several studies confirmed that the seed radicle requires a lapse of time to break the testa, and the length of this period depends on the soil temperature [1]. Koutroubas et al., 1999 [30], working on castor grown in two different sites, noticed that in the warmer location, seeds took 2 to 19 days less than in the cooler site to germinate. In the present study, at 25 °C, seeds germinated in 2.77 ('Local') to 7.34 ('C1013′) days, and at 32 °C, germination occurred even 1.51 days earlier than at 25 °C.

Kittock et al. 1967 [31] and Weiss, 2000 [32] reported a germination time of 25 days and 10–23 days, respectively, for the germination of castor in a range of soil temperatures between 10 and 19 °C as confirmed by our results, in which, at 16 °C, seeds took from 7 to 15 days to germinate. In our study, seeds took 10 to 30 days to germinate at 12 °C, but at this temperature, germination was low in most cases. This could be explained by the fact that germination falls sharply when temperatures are reduced from the optimal, because low temperatures slow down metabolic and enzyme activities, affecting the whole germination process [33]. According to Patanè et al. 2009 [34] low temperatures prolong the initial phase (lag phase) of germination, extending the germination time. Seeds of 'Local' castor germinated slightly faster than the dwarf genotypes, indicating that the germination speed may depend on the genotype.

The indication of synchrony is useful for predicting the degree of success of a crop in terms of plant establishment [18]. Z = 1, which is obtained when all the seeds germinate on the same day, indicates simultaneous germination, while values closer to 0 indicate a low level

of synchronization. In this research, the highest temperature (32 °C) showed a lower level of synchrony compared to 25 °C. It seems that high temperatures affect the synchrony of germination in castor. Patanè et al. 2021 [21] obtained a better result at 25 °C, confirming our results. The values obtained by Windauer et al. 2012 [33] in *Jatropha curcas* L. are comparable to our data for synchrony at 25 °C.

Another parameter considered is radicle growth. As reported by Wolny et al. 2018 [35], the beginning of radicle growth starts when germination finishes. Temperature influences the radicle growth process, and in this study, any thermal increase or decrease from the optimum temperature (25 °C) had evident effects on this trait. Indeed, an increase in temperature from 25 to 32 °C accelerated the growth of radicles that, 4 days after the first visible radicle appeared, were 4.54 cm long, on average, among all genotypes, against 1.96 cm measured at 25 °C. According to Patanè et al. 2009 [34] really low and low radicle growth that occurs at 12, 16, and 40 °C may be ascribed to alterations in the metabolic processes that are strictly temperature dependent. The same restrictive effects on radicle growth were reported in seeds of *Brassica napus* L. [36].

Through the analysis of the vigour index, it is possible to evaluate the differential expressions in the crops' response to different external factors (i.e., temperature) [37]. The vigour index resulted highest at 32°C followed by the VI at 25°C; lower values were obtained at 12, 16, and 40 °C. Sharma et al. 2022 [38], in wheat, noted differences in the VI, determined by temperature, reporting a greater VI at 25 °C than at 32 °C. This could be explained by the fact that wheat is a micro-thermal crop, contrary to castor, which is a warm season crop and thus has a higher vigour index when temperatures increase.

4.5 Conclusions

As a warm season crop, castor was found to germinate well at

temperatures ranging from 25 to 32 °C, where seed germination exceeded 82.00% at both temperatures. Decreases and increases in these temperatures negatively affected the seed germination, which was reduced by 90.05% at 12 °C, by 27.56% at 16 °C, and by 75.84% at 40 °C, indicating that seeds of castor may effectively germinate within a rather restricted range of temperatures. The impact of the genotype strongly influenced all the germination parameters. Indeed, the 'Local' castor, selected for good adaptation to the Mediterranean environment, had greater tolerance to low temperature than the dwarf genotypes, which prefer higher temperatures and are better adapted to late sowings. With the 'Local' castor being the most tolerant to the lowering of temperature, it is suggested for early sowings in spring.

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5 Assessment of Germination Response to Salinity Stress in castor through the Hydrotime model

This chapter is based on an article accepted by Journal of Agronomy

Assessment of Germination Response to Salinity Stress in castor through the Hydrotime Model

Valeria Cafaro [1*], Efthymia Alexopoulou[2], Salvatore Luciano Cosentino [1] and Cristina Patanè [3]

[1] Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli Studi di Catania, via Valdisavoia 5, 95123 Catania, Italy
[2] Centre for Renewable Energy Sources and Saving, 19th km Marathonos Avenue, 19009, Pikermi, Greece
[3] CNR-Istituto per la BioEconomia (IBE), Sede secondaria di Catania, Via P. Gaifami 18, 95126 Catania, Italy

Abstract

Germination of castor seeds of five dwarf hybrid genotypes, compared to a 'Local' genotype, selected from a Tunisian population by the University of Catania well adapted to the Mediterranean environment, were studied at five different salt levels (0, -0.3, -0.6, -0.9 and -1.2 MPa) in or-der to assess the seed germination performance under stress conditions. The results confirm that optimum moisture (0 MPa) ensure 100% of germination, contrary salt intensification negatively influence the Final Germination Percentage (FGP), and the radicle elongation, causing severe consequences for the plant establishments. At a level of -1.2 MPa no germination occurred, while level of -0.3 MPa slightly affected the seed germination of the dwarf genotypes, which achieved 77.3%, contrary to the 'Local', in which germination was kept stable. Higher level of salt (-0.6 and -0.9 MPa) caused a linear decrease in the FGP and the radicle elongation. Overall, the dwarf hybrid 'C1019' performed better at higher salt imposition, as long as 'C857', confirming these genotypes as the most tolerant within the dwarf hybrids. Whereas, 'C1013' resulted as the most susceptible genotype, followed by 'C1008'. On the other hand, the 'Local' castor genotype resulted as the best-performing genotype at -0.3 MPa and the most tolerant genotype in response to salt intensification and germination time, which were accurately predicted by the hydrotime model, validating it as a valid analysis to assess the germination response of castor seeds in response to Ψ.

Keywords: dwarf hybrids, marginal land, radicle length, *Ricinus communis* L.

5.1 Introduction

The world is currently experiencing one of the greatest crises of modern history, characterized by great climate change and a severe drop in agricultural production [1]. Salinity, drought, high or low temperature are the main abiotic stresses that threat world-wide agriculture [2]. In depth, salinization affects about 3 million hectares of the land in Europe, and 30% of the irrigated land of the world [3], representing an extreme obstacle for food security. Given the reduction of the total arable land, research is pushing towards the selection of tolerant crops or the development of salt-tolerant plants by breeding [4].

This scenario broadens up the possibilities of castor to be globally exploited. Castor (*Ricinus communis* L.) is an oilseed crop belonging to the Euphorbiaceae family and originated from Asia or, most likely, Africa [5].

Chemical and pharmaceutical industries have positively re-evaluated this crop be-cause of the wide range of uses, mainly as an oil's producer (35-65% oil content) [6,7]. The extensive applications of castor [8] which include biofuel, bio lubricant productions to be used as antimicrobial or antioxidant in varnishes and painting, raw material for the production of sebacic acid, nylon and other resins, and also as feed for animals and as ornamental plant [8, 9], make the reason for a growing interest towards this crop. Furthermore, castor is a rustic, low-key maintenance crop because of its low nutrients requirements and great land and climate adaptation [8]. Thus, the chance of using marginal areas for its cultivation, avoiding the competition with those used for food production, and the necessity of reducing fossil fuels according to the Agenda 2030 [10], make castor the ideal plant to be exploited in the decades to come.

Marginal lands, which are increasing at an annual rate of 1-2%,

are often characterized by high saline levels [11]. As seed germination and seedling emergence and development are the most crucial and sensitive stages during the plant life cycle [12], soil salinization is a severe obstacle to a proper plant growth [13]. Salt stress, induced by numerous causes such intense evaporation, rising sea level, and incorrect irrigation practices, induces a reduction in the water absorption (osmotic stress), and an excessive accumulation of Na+ and Cl- ions (toxic effects) [14]. These stresses adversely affect the overall metabolisms, the seed germination and following plant establishment and development [15], being also responsible for the production of reactive oxygen species (ROS), accountable for cell membrane damage [16].

Therefore, the assessment of seed germination response of castor to salinity stress is important, to predict the seed performance under different soil salinity conditions that the plant may experience during the first stages of its growing season. In this view, a study was conducted to evaluate the relationship between salt stress conditions and seed germination characteristics of castor through the hydrotime analysis, and compare the germination response in different genotypes on the basis of their stress tolerance.

5.2 Materials and Methods

5.2.1 Seed material

Seeds of castor (*Ricinus communis* L.) were used for a laboratory experiment carried out at the University of Catania (Italy), studying two experimental factors: genotype and salt level. Six genotypes were assessed: five dwarf hybrids selected and bred by (Kaiima Company, Campinas – São Paulo, Brasil for commercial purposes), and a local genotype, used as control, mass selected from a wild Tunisian population for adaptation to the Mediterranean basin, at the Di3A of the University of Catania.

Seeds used for the experiment were produced in open-field at the experimental farm of the University of Catania (37°24'31''N; 15°3'33''E, Italy) using the above said genotypes. Seeds were harvested in September 2022, stored in paper bags, and kept at room temperature (10 – 20°C) until used for this study in October 2022 use. Seeds were surface-sterilized before germination tests with 1% sodium hypochlorite solution (NaClO) (Sigma-Aldrich s.r.l., Milano, Italy) for 3 min, rinsed in distilled water, and then dried at room temperature for 24 h. Seed moisture contents ranged between 3.5 and 4%.

5.2.2 Salt solutions

Five salt solutions were used for germination tests, prepared dissolving NaCl in deionized water, to generate five water potentials (ψ): 0 (control), -0.3, -0.6, -0.9 and -1.2 MPa. These solutions had electrical conductivity (EC) values of 0, 8.2, 13.7, 16.7 and 11.9 dS/m, respectively. The osmotic potential was verified using an automatic cryoscopic osmometer (Gonotec Osmomat 030 model, Berlin, Germany). Electric conductivity (EC) was measured in a portable conductivity meter (Mod. CyberScan CON 400/410, Eutech Instruments, Pte Ltd).

5.2.3 Germination tests

Beforehand the germination was tested and resulted as high as 100% of germination. Seeds were germinated at constant temperature (T) of 25°C, considered the optimum for seed germination of castor [12, 17], maintained in a thermostatically controlled incubator (±1 °C), in dark conditions. For each genotype and salt level, four replicates of 20 seeds each were placed in Petri dishes (Ø 120 mm), containing two filter papers – one above and one below the seeds – to allow a complete seed hydration. Each paper filter was moistened with 5 ml of one of the NaCl solutions. Petri dishes were hermetically sealed

with parafilm to prevent evaporation, then randomized in the incubator.

The seeds were kept in the dishes to assess germination. This was scored when the radicle emerged from the seed (at least 2 mm long). Data were collected daily until no additional germination occurred for one week.

5.2.4 Radicle length measurement

Radicle length was measured for each genotype and salt level. To this purpose, five seeds were randomly chosen, within those germinated first from each Petri dish, on the fourth day from the recorded start of germination (i.e., first radicle appearance). Only 5 seeds were considered in order to possibly evaluate the same number for all the treatments. Seeds were photographed, to document the radicle length at the different salt levels.

Photos were taken using an iPhone X smartphone. Scanned images were then analyzed using ImageJ software, a Java image open-source program used for processing digital images, developed at the National Institutes of Health (NIH) [18]. The radicle elongation was measured just by using the cursor. To this end, the program required the setting of the correct metric in the image, easily done by calibration through a ruler.

5.2.5 Calculations and data analysis

Data of the final germination percentage (FGP, %), previously arcsine transformed, and those of root length, were checked for normality and homogeneity of variances and statistically analyzed by a completely randomized two-way ANOVA. The data were processed using CoStat version 6.003 (CoHort Software). ANOVA was conducted considering osmotic potential and genotype as fixed factors. When 'F' ratios were significant, means were separated by the Tukey's test ($p < 0.05$)

The course of cumulative germination with time, separately for genotype and salt level, was described by a sigmoidal equation with three parameters (SIGMAPLOT 9.0 software, Systat Software Inc., San Jose‘, California, USA):

$$y = \frac{a}{1+(\frac{x}{x_0})^b}$$

where a is the maximum value of germination, x is the time after seed imbibition (hours), x_0 is the time (hours) to 50 % of seed germination, b is a fitting parameter of the curve. The x values on the curve, which corresponded to specific y values on the curve (g fractions of seed germination), i.e., 10, 30 and 50 %, this last when achieved, were assumed as theoretical time (tg) to 10, 30 and 50 % germination.

To estimate the base water potential (ψb) to attain germination of g fraction at each salt level, the reciprocal of germination time (1/tg or *GR*g) was regressed vs. ψ. As the abscissa intercept on the temperature axis is assumed as an estimate of theoretical minimum temperature for germination [19] [20], likewise, the intercept on ψ axis (that of abscissa) may be assumed as an estimate of theoretical minimum water potential for germination of g [$\psi_{b(g)}$] [20],[21].

To estimate $\psi_{b(g)}$ of all the observed germination percentages at each t_g, the equation which indicates the hydrotime model [22]:

$$\theta_H = [\psi - \psi_{b(g)}]\, t_g$$

where θ_H is the hydrotime (MPa h) needed for g seed fraction to germinate, ψ is the actual water potential, $\psi_{b(g)}$ is the base water potential ψ for germination of g, t_g is the time to germination of g, was modified as follows [23]:

$$\psi_{b(g)} = \psi - \left(\frac{\theta_H}{t_g}\right)$$

where θ_H is the mean hydrotime of g fractions (10, 30 and 50 %) calculated as the reciprocal of the slope (*b* coefficient) of the regression line of *GRg* (1/tg vs. ψ) [24].

The time courses of the observed cumulative germination percentages were linearized on a probability scale and regressed vs. $\Psi - \left(\frac{\theta_H}{t_g}\right)$ which corresponds to $\psi_{b(50)}$, according to the following equation [23]:

$$\text{probit (g)} = [\psi - \left(\frac{\theta_H}{t_g}\right) - \psi_{b(50)}] / \ \sigma\psi_b]$$

which models the germination time course at different water potentials.

Different values for θ_H were used in repeated probit regressions and the value which best fit to all data was assumed as the optimal one. The reciprocal of the slope (*b* coefficient) of the linear regression corresponded to the standard deviation $\sigma\psi_b$ of seed lot. The $\psi_{b(50)}$ (i.e., $\psi_{b(50)}$ for 50 % germination) corresponded to the value on the regression line at which probit is equal to zero. The estimated values for θ_H , $\psi_{b(50)}$ and $\sigma\psi_b$ were assumed as the hydrotime parameters.

5.3 Results

5.3.1 Cumulative Germination Time Course

Since no germination occurred at -1.2 MPa, this salt level was not considered for all the following traits analyzed.

The cumulative seed germination time course of the six genotypes of castor is illustrated in Figure 5.1. The course is well described (R2 > 0.99) by the three-parameter sigmoidal function (Eq.

1), whose trend reveals a short initial phase of low germination, negligible at 0 MPa, followed by a sharp increase in germination, up to a maximum for all genotypes. The fastest in germination at low levels of salt stress (in distilled water and at -0.3 MPA) was the 'Local' castor that reached maximum germination (> 90%) after 48-96 hours. Similarly, genotypes 'C1019' and 'C1008', were fast in germination at -0.3 MPa, reaching 50% germination after 96 hours.

Overall, further increases in salt concentration slowed down and depressed germination. At -0.6 MPa, genotype 'C1019' reached 50% germination after 168 hours (7 days), and genotypes 'C857' and the 'Local' germinated for 50% in 192 hours (8 days). At maximum level of salt stress at which seed germinated (-0.9 MPa), 'C1019' and 'C857', exhibited the highest tolerance, approaching 50% germination in approximately 200 hours (less than 10 days).

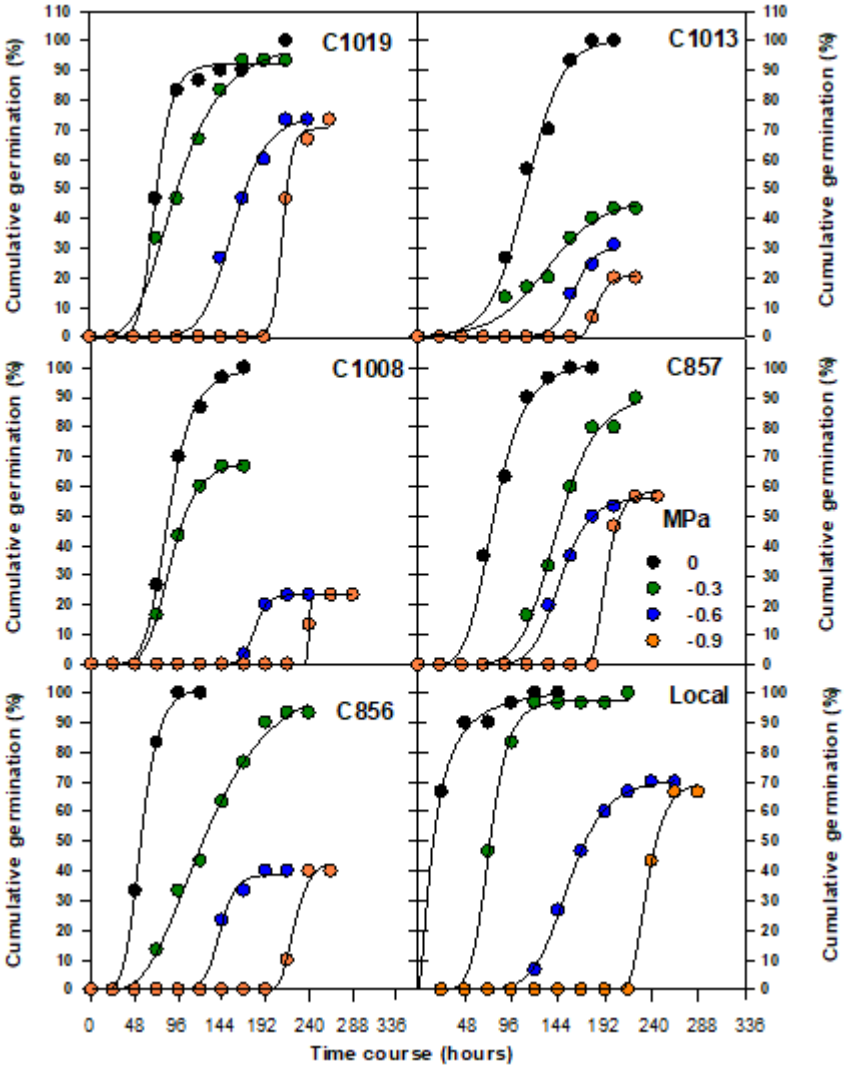

Figure 5.1 - Cumulative germination time courses (solid curves) at different temperatures in 6 genotypes of castor. Symbols represent the observed percentages at 0, -0.3, -0.6, -0.9 MPa vs time.

5.3.2 *Final seed germination percentage (FGP) under salt stress*

Final seed germination percentage (FGP) was full (100%) under optimal conditions (0 MPa, control), in all the genotypes (Figure 5.2). FGP was slightly reduced to 81% at -0.3 MPa, but at lower ψ (-0.6 and -0.9 MPa), the germination decrease was evident (on average to 47 and 46%, respectively).

Across salt concentrations, FGP significantly differed with genotype (G, p <0.001), being the highest in 'Local' and 'C1019' (84.3 and 85%). Good levels of germination were also scored in 'C857' and 'C856' (75.8 and 68.3%, respectively). The lowest germination occurred in 'C1013' (44.3%) and in 'C1008' (53.3%), not significantly different.

Anyway, significant SxG interaction (p <0.001) indicated a different FGP response to the salt levels, depending on genotype. The 'Local' castor was the only to germinate at the maximum level (100%) at both 0 and -0.3 MPa, indicating a tolerance to low levels of salt stress. Under the same stressing conditions, seed germination was also high in 'C856' and 'C1019' (93.3% in both), and 'C857' (90%). The genetic differences in FGP became extremely evident when higher levels of salt stress were imposed (-0.6 and -0.9 MPa). This result was clearer at -0.9 MPa, under which conditions 'C1008' and 'C1013' germinated by 23.2 and 16.8%, respectively, confirming their scarce tolerance to salt stress.

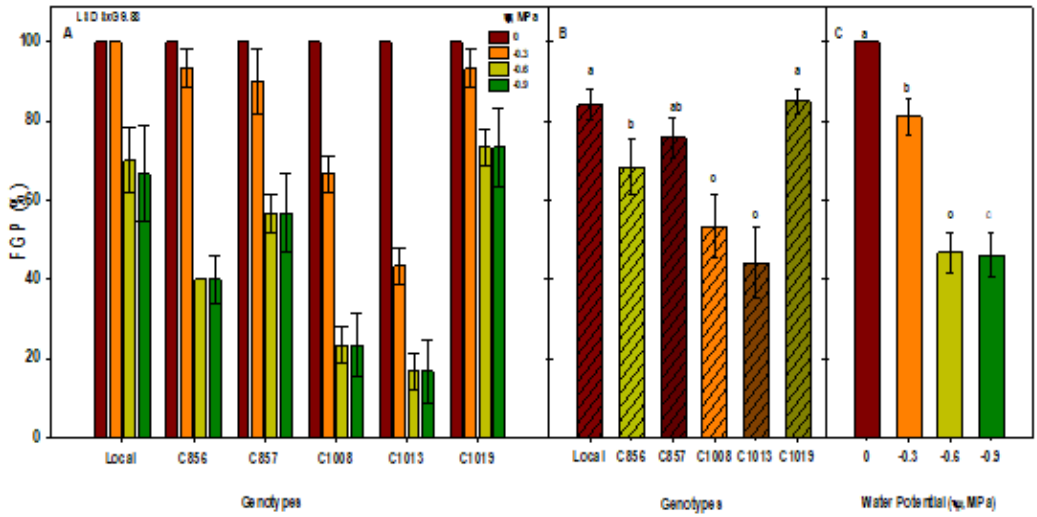

Figure 5.2 - Final germination percentage (FGP, %) in six genotypes of castor at four different salt levels (0, -0.3, -0.6, -0.9 MPa) (n=4) (A). The average value of genotypes (B) and water potentials (C) is reported. Values with the same letter are not significantly different at $p < 0.05$ level. LSD value for SxG ($p < 0.05$) is reported.

5.3.3 Radicle length measurements

No stress conditions (control, 0 MPa) allowed a regular development of the radicle that, at the 4th day, across genotypes, reached 1.72 cm length (Figure 5.3). Interestingly, a low level of salt stress (-0.3 MPa) induced a slight but not significant decrease in radicle length (1.53 cm) respect to the control. The length decrease became significant at -0.6 MPa (1.20 cm), and at -0.9 MPa radicle length was reduced to 0.95 cm (i.e., 45% lower than control at 0 MPa).

'Genotype' also significantly influenced the radicle length (G, $p < 0.05$). Across salt concentrations, the longest radicles (≥1.52 cm) were measured in 'C1019' and 'C856'. The smallest radicle (0.95 cm)

was measured in ‘C1013’.

5.3.4 Hydrotime model and estimated parameters

The seed germination response to salt stress of each genotype of castor was assessed by the hydrotime analysis (Figure 5.4). The analysis evidenced a wide genetic variability for $\psi_{b(50)}$, estimated (from the model) (Table 5.1). The lowest the ψb, the highest the tolerance to salinity stress. In particular, the results highlighted that ‘C1019’ was the most tolerant, having a -1.39 MPa $\psi_{b(50)}$, rather close to that calculated by x-axis intercept of GR_{50} vs. ψ (-1.19 MPa). High salt tolerance was also found in ‘C857’, having a -1.23 MPa ψb(50) as estimated by the model (-1.07 MPa as calculated). The most susceptible to salt stress during germination was ‘C856’, with a -0.75 MPa ψb(50) (as estimated). A -0.91 MPa $\psi_{b(50)}$, was estimated by the hydrotime model in the ‘Local’ castor (very close to a calculated -0.92 MPa) that, therefore, exhibited a moderate tolerance to salt stress.

The analysis highlighted that θ_H as estimated by the model, was maximized in ‘C1013’ (147.5 MPa h). This genotype also exhibited a low $\psi_{b(50)}$, both estimated and calculated (-0.88 and -0.53 MPa, respectively). Similar high θ_H (< 100 MPa h) was also estimated in ‘C1019’ and ‘C857’, i.e. those genotypes that, in turn exhibited the highest tolerance to salt stress in terms of $\psi_{b(50)}$. A quite low θ_H was estimated (20.0 MPa h) and calculated (30.6 MPa h) in ‘Local’ castor.

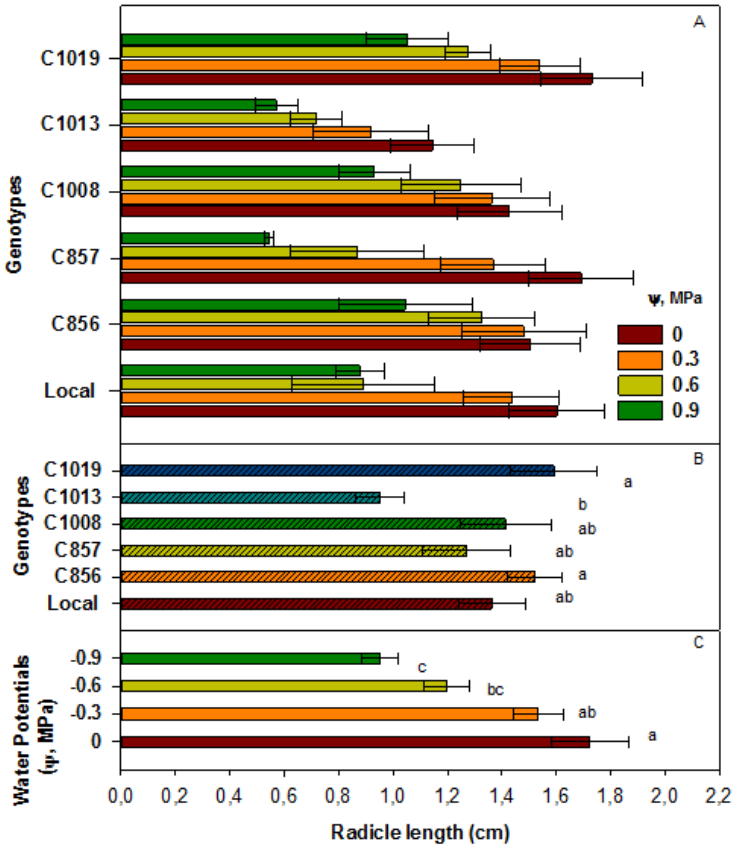

Figure 5.3 - Radicle length (cm) in six genotypes of castor at four different salt levels (0, -0.3, -0.6, -0.9 MPa) (n=4) (A). The average value of genotypes (B) and water potentials (C) is reported. Values with the same letter are not significantly different at p < 0.05 level. LSD value for SxG (*p* < 0.05) is reported

Figure 5.5 showed how ψb of each seed lot varied following a Gaussian distribution. The uniformity of germination is described by σψb calculated as the inverse of the slope of the fitted line (probit analysis). A wide σψb was calculate for genotypes 'C1013' (0.72) and

'C1019' (0.55), both also exhibiting the highest ψb (the least negative).

Table 5.1 - Calculated (from x-axis intercept of GR_{50} vs ψ) and estimated (from hydrotime model) values of base water potential for 50% germination [$\psi_{b(50)}$], and calculated (from the inverse of the slope b of x-axis intercept of GR_{50} vs ψ) and estimated (from model) values of hydrotime (θ_H) in six genotypes of castor.

Genotype	$\Psi_{b(50)}$ (MPa)	$\Psi_{b(50)}$ (MPa)	$\sigma\Psi_b$ (MPa)	R2	θ_H (MPa h)	θ_H (MPa h)
	calculated	estimated			1/b±σ	From model
Local	-0.92	-0.91	0.35	0.71	30.6±0.09	20.0
C856	-0.55	-0.75	0.22	0.91	38.1±0.40	51.0
C857	-1.07	-1.23	0.42	0.74	90.5±0.66	110.0
C1008	-0.53	-0.82	0.28	0.91	45.9±0.18	60.0
C1013	-0.53	-0.88	0.72	0.83	87.7±0.07	147.5
C1019	-1.19	-1.39	0.55	0.87	88.6±0.33	104.5

The standard deviation ($\sigma\psi_b$) is the estimated parameter of the hydrotime model, obtained by the reciprocal of the slope of the regression of probit germination against ψb. Coefficient of determination (R^2) is also reported

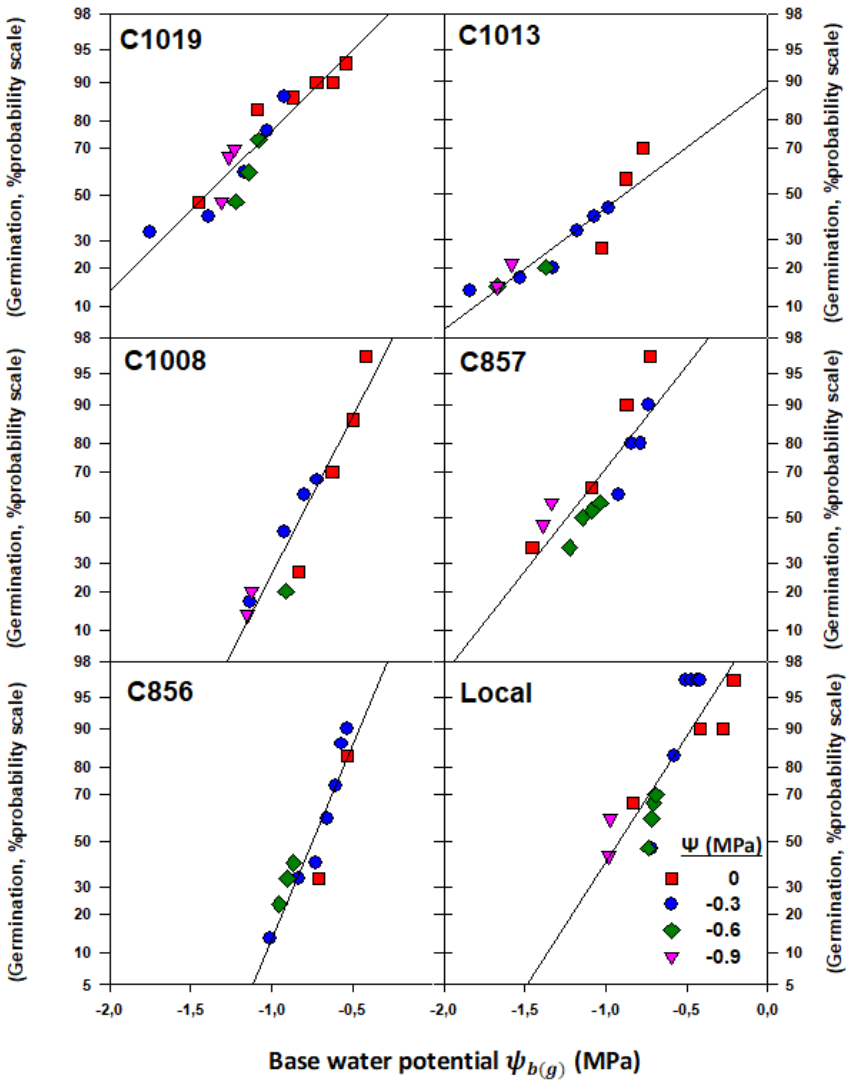

Figure 5.4 - Probit analysis of germination at different water potential (ψ) for dwarf and Local seeds of castor. Seeds were germinated at 25°C, testing four different salt levels (0, -0.3, -0.6, -0.9 MPa). Percentages (*symbols*) from germination time courses of Figure 5.1, were plotted as a function of ψ – (θ_H/t_g) which is equivalent to $\psi_{b(g)}$. The parameters values of the fitted line are reported in Table 5.1.

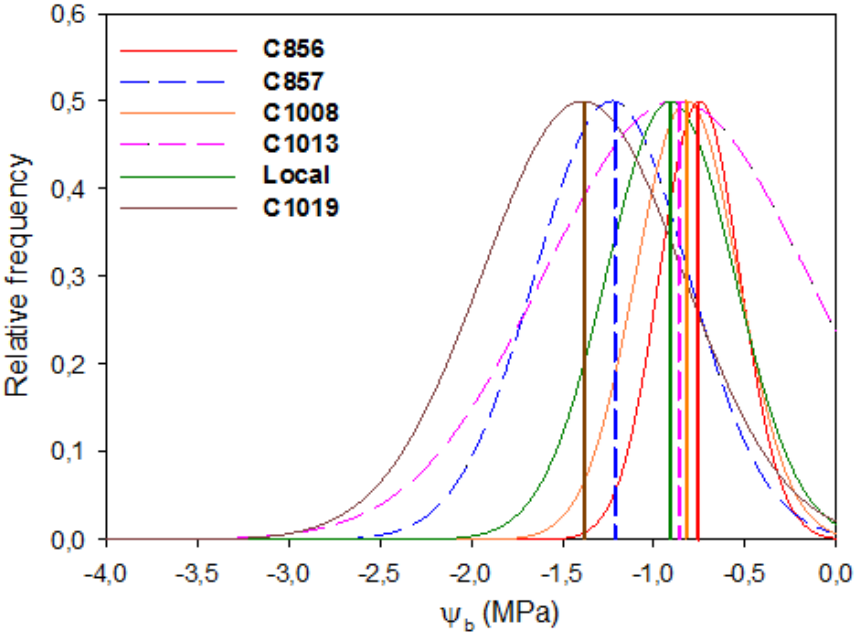

Figure 5.5 - Normal distribution of relative frequencies of $\psi_{b(g)}$ values for different genotypes of castor. Vertical lines indicate $\psi_{b(50)}$ values in x-axis for castor seeds as estimated by the hydrotime model.

5.4 Discussion

The impact of abiotic stresses, such as salinity, on seed germination and following seedling growth, has been widely studied [25, 26, 27], but still remains a considerable issue in agricultural crops.

Salt (NaCl) stress inhibits the water uptake of seeds due to an increase in the osmosis activity, which negatively influences either the rate of germination, thus de-laying seed germination, and subsequent plant establishment and growth and, ultimately, final yield [28].

Numerous studies highlighted how germination and radicle growth are the most critical stages in crops life [26, 29, 30], including castor [12]. Despite some studies documented castor as a salt-tolerant crop [31, 32], the main research always focused merely on agronomic traits, such as seed productivity and oil yield, content and quality [33][34]. Little is known about the effects of salt stress on the very early stages of its growing season, i.e. germination and seedling emergence. In the present research, the seed germination performance under salt stress conditions was assessed in different genotypes of castor. In this study, in most genotypes a slight stress (-0.3 MPa) resulted in a small delay in the start of seed germination, while final germination approached the same levels as under no stressing conditions. A similar slightly extension of the germination beginning but same final germination as compared to no salt stress was recorded at -0.5 MPa water potential in seeds of several genotypes of carob [35]. How-ever, higher levels of salt stress (up to -0.9 MPa) in our study prolonged the initial phase of low germination and significantly depressed the germination in all genotypes of castor, indicating a great susceptibility of castor to salinity, and at -1.2 MPa, germination was totally suppressed. Similarly, Han et al. (2022) [36], working on castor seeds germinated under different levels of alkalinity, reported a low tolerance to osmotic stress in terms of germination, which was reduced by 65% even at 150 mM NaHCO3.

The mechanism of plants water uptake from the soil when their water potential is lower than that of the soil is extensively known [37]. Salt limits the plant growth due to an osmotic impact and a toxic effect. Indeed, excess of soluble salts reduce the water potential of the soil around the roots by limiting water absorption, causing either an osmotic stress or a water deficit [13].

Tolerance to osmotic stress manifests itself with a decrease in cellular expansion of the roots and leaves, decreasing the stomatal conductance to save water [38]. Moreover, according to literature, the delay and decrease in seed germination due to increasing levels of salt stress are strictly attributable to Na+ accumulation. Secondly, the capacity of Na+ accumulated by seeds, to compete with K+ for binding sites, lead to a severe ionic stress, and in an interference in essential cellular activities [13 , 39].

According to Farrooq et al. 2017 [40] who worked on grain legumes, higher salt concentration in the soil inhibits seed germination by creating a low osmotic potential around the seed which limits the water uptake, also impacting the seed metabolic processes. Similarly, Ullah et al. [41] by evaluating seed germination in barley, found a proportional decrease in final seed germination as the water potential is reduced, confirmed that stress correlated to Ψ influences seed germination.

Overall, across all levels of salt concentrations, all the genotypes studied showed the same radicle growth rate, as measured at the 4th day after the start of germination, except ‘C1013’, whose radicle growth was rather low. A similar slow radicle growth was recorded in this genotype in a previous work by Cafaro et al. [12].

Increasing salt stress levels in this study, adversely affected also radicle growth, potentially preventing a regular plant establishment. Similar adverse effects on radicle growth where reported by Li et al. [42], who working with seeds of sunflower (*Helianthus annus* L.), found that soil salinization strongly affected seed germination and

seedling growth and development.

The hydrotime model provides useful information on seed germination characteristics under stress conditions, defining the tolerance or sensitivity of a variety to salt stress [43]. The parameters are hydro time requirements (θH), which defines the speed in germination and base water potential (Ψb), which indicates the minimum potential (Ψ) of a seed lot reach germination. Therefore, these parameters greatly influence the subsequent plant establishment (plant population), and the potential productivity in unfavourable environment, such as those in marginal.

The model highlights that genotypes having similar ψb can have different θH to germinate or, on the contrary, different ψb but similar θH. In this study, the local genotype and 'C1013' of castor had a ψb of -0.91 and -0.88 MPa, respectively, but θH was quite different (20 and 147.5 MPa d, respectively). Differently, genotypes 'C857' and 'C1019' had different ψb (-1.23 and -1.39 MPa, respectively) but similar θH (110 and 104.5 MPa d, respectively).

The $\sigma\Psi_b$ is another important parameter of the hydrotime model. It indicates the germination distribution of a seed lot depending on ψb of each g fraction, i.e, the susceptibility of a given genotype to salt stress during germination. A high value of $\sigma\Psi_b$ indicates a higher chance to germinate at low (more negative) ψ value. In this regard, genotypes 'C856' and 'C1008' had the smallest $\sigma\Psi_b$, therefore, their chance to germinate at low ψ is restricted. On the other hand, 'C1019' and 'C1013', having wide $\sigma\Psi_b$, may better germinate under increasing salt stressing conditions.

The variability found for salinity tolerance during germination allows us to select, within the studied genotypes, the most tolerant ones. Likewise, a wide genetic variability has been found for the tolerance to low temperatures during germination in a previous work by Cafaro et al. [12]. Indeed, the 'Local' castor was described as the

most tolerant genotype to the lowering of temperatures. Similarly, in this study the 'Local' resulted as the genotype with the lowest θH requirements, thus being appointed as the best candidate genotype to be cultivated in soil affected by salt and in early sowings [6] as long as the soil ψb does not drop below levels of -0.9 MPa.

Contrary, the dwarf genotype 'C1019' was slower in germination due to a high value for θH. However, in the previous work showed a great tolerance to high temperature [12], which speed up the germination and water uptake processes of the seeds [20], make it the best candidate to be used in late sowings and in very low ψb conditions, which can easily be found in marginal land.

5.5 Conclusion

Castor, as for all the crops, suffers by salinity stress, and the main phenological stages affected are the earliest one, i.e. germination and the consequential seedling growth. Being salinization one of the leading obstacle to the exploitation of marginal lands, which could be enhanced by non-food cultivation, evaluating the assessment and tolerance capacity is fundamental.

The study highlighted that genotypes 'C856', 'C1013' and 'C1008' are more susceptible to salt stress compared to genotypes 'C1019' and 'C857' for the different agronomic traits studied, confirming these last genotypes as the most tolerant among the dwarf hybrids. The 'Local' castor, selected for the Mediterranean environment, had greater tolerance to salinity stress, in respect to some dwarf genotypes, as demonstrated by the hydrotime model, which accurately predicted the germination and the germination time, thus being a valid candidate for marginal land exploitation.

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6 General discussion and conclusion

Today's panorama is issued by a growing climate crisis, due to various factors, which include greenhouse gas emissions (GHG), agricultural intensification, the use of pesticides, herbicides and a continuous demographic increase, which leads to demand and overexploitation of natural resources [1][2]. The current policies, including RED (Renewable Energy Directive) and the Agenda 2030 seek to limit the use of fossil fuels and to achieve energy neutrality by 2050 (Chapter 1) [3] [4]. To meet the goals settled at the European level, the use of renewable energies could be a valid alternative, which can both limit the use of already scarce resources and enhance the exploitation of crops not used for food purposes.

Among these crops, castor (*Ricinus communis* L.), an oil-seed crop belonging to the Euphorbiaceae family, fulfills the needs set by the growing climate changes [5]. Indeed, castor being an alternative crop, easy to grow and rustic, and adapt to different environments, could not only be used for the production of biofuels as alternative energies but could also be cultivated in marginal lands, to valorise unused soil at an agricultural level, which does not compete with lands used for food production [6] [7].

In the Mediterranean basin, characterized by dry climate in the summer and mild in the winter, with rainfall concentrated from autumn to spring, castor grow spontaneously as a wild crop. However, despite its great adaptive capacity, selecting a genotype that can survive the winter, and consequently make the most of the productivity capacity of castor is challenging. Therefore, the selection of a castor genotype, perfectly adapted to the pedo-climatic conditions of the Mediterranean, and the study of the the best agricultural practices to be performed, as well as the best harvesting mechanical methods [8][9] are necessary in the scientific panorama.

In this context, my goals aimed on the selection of a genotype

and on the enhancement of its potential, focusing also on which agricultural practices are the best to enhance its production.

In particular, my research has focused on the improvement of morphological and qualitative-quantitative traits, concerning the seed yield and the oil content, also focusing on better growth development of the plant, which could be more suitable to the current Mediterranean conditions (Chapter 2). The research involved the use of mass selection techniques for agronomical traits and the study of genetic characteristics to verify the presence of genetic variability within the studied population, aimed at the selection of the best genotype to be used in the Mediterranean environment. Indeed, a promising genotype was selected, responding to the adaptation requirements of the Mediterranean basin, having a high seed yield production with a final high oil content, also tied to a shorter production cycle which allows the use of castor as a catch crop. In addition, chapter 3 focused on the identification of the best period of sowing, to avoid extreme thermal conditions, which can negatively influence germination, and on how the length of the growth cycle can influence the seed yield and the consequential oil content [14]. The results confirmed that the local genotype prefers early sowings, being more tolerant to lower temperatures. The choice of early sowings allows the use of the water stored in the soil during the rainy period, thus respecting the levels of sustainability required and the conservation of resources. On the other hand, the dwarf genotypes prefer late sowings characterized by higher temperatures, which allow a shortening in the growth cycle, still ensuring the achievement of high germination levels, great plant establishment, and high seed yield. Therefore, the choice of the best sowing date is highly related to the genotype used and on the environment in which the cultivation will be carried out. In addition, the identification of the sowing periods is closely linked to the temperatures that favors the cultivation of castor, and ensuring germination is the first step to think about when a cultivation has to

start and to obtain a satisfactory production. To identify the optimum range of temperature, different temperatures and the response of various castor genotypes were evaluated. The local genotype was compared to dwarf genotypes (Chapter 4), and the results confirmed what was already found in the literature [10], indicating that temperatures of 25-30° guarantee an excellent level of germination, perfectly fitting the temperatures of the Mediterranean basin. Temperature decreases, which may be related to temperature variations that occur in tropical areas, cause reductions in seed germination, as well as excessive increases in temperature. In addition, the choice of the genotype plays an important role in the germination parameters evaluated. The local genotype had a greater tolerance to the lowering of temperatures, compared to the dwarf genotypes, which instead preferred warmer temperatures. As well, as seed germination, radicle elongation needs to be studied, to ensure a good plant establishment.

Finally, the last chapter (chapter 5), studied the response to salt stress. Considering the intensification of marginal areas in the Mediterranean basin [11][12], evaluating the response to salinity and predicting germination performance, through the hydrotime model[13], under different salt stress concentrations is essential to guarantee high yield. My research showed that regardless of the genotype, castor has a good tolerance to low saline levels; while, the intensification of the water potential, given by a higher salt concentration, causes strong germination decreases. Furthermore, my research has underlined that the local genotype effectively had a greater tolerance to stress conditions, compared to the dwarf genotypes, which instead were more susceptible to salt stress, proving its greater suitability to the Mediterranean basin.

Overall, the findings of the present Ph.D. Thesis provides useful information to be included in the growing scientific knowledge concerning castor, and in particular its agronomic management, pedo-

climatic requirements and the best genotypes that could be used.

Nevertheless, further studies are requested in the near future to better investigate the yield response of castor to abiotic stresses, such as (i) the yield response and the consequential oil content and plant growth under different salt concentrations; (ii) the yield response and the consequential oil content to different thermal conditions applied; (iii) the germination response of castor, its seed yield and oil content in contaminated soils, which can easily be found in marginal land; and (iv) the optimization of mechanical harvesting on dwarf and local genotype.

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7 Annexes: Scientific Curriculum

7.1 Research and Professional Experience

- **2020-2023**: Ph.D course in Agricultural, Food and Environmental Science, University of Catania.
  Research project: "Selection of a *Ricinus communis* L. genotype and improvement of the agronomic management in semi-arid Mediterranean environment". Under the supervision of Prof. Salvatore Luciano Cosentino, Prof. Danilo Scordia and Prof. Angela Roberta Lo Piero.
- **May 2021-September 2021:** Fixed term assignment for the 50th Conference of the Italian Society of Agronomy "Evolution of agronomic systems in response to global challenges". Udine, Italy
- **June 2019-February 2020**: Undergraduate Internship at CREA-ACM, Corso Savoia, Acireale (CT)
- **July 2018-October 2018**: Undergraduate Internship at CREA-ACM, Corso Savoia, Acireale (CT)

7.2 Education and Professional qualifications:

- **2022**: Qualification to the profession of 'Agronomist'

- **2020**: Qualification to the profession of 'Biologist'

- **2018-2020**: Master's degree on "Biotecnologie Agrarie" (LM-7) - University of Catania, Catania, Italy. Thesis: "Tobacco biofactory plants with prolongues juvenility and increase biomass". Under the supervision

of Prof. Angela Roberta Lo Piero and Dr. Marta Vazquez Vilar.

- **03/2020-07/2020**: Erasmus plus (+) at Universitat Politecnica de Valencia (UPV), Instituto de Biologia Molecular y Celular de Plantas (IMBCP), Valencia, Spain

- **2015-2018**: Bachelor's degree on "Science e Tecnologie Agrarie", (L-25) - University of Catania, Catania, Italy. Thesis: "Le nuove Biotecnologie Sostenibili (NBT) per il miglioramento genetico: potenzialità per l'agricoltura italiana". Under de supervision of Prof. Alessandra Gentile

- **2015**: High School Diploma: Istituto de Nicola, San Giovanni la Punta, Catania, Italy

7.3 Memberships and Ids

- ORCID ID: https://orcid.org/0009-0006-9552-2769

- SCOPUS ID: 57980557700
https://www.scopus.com/authid/detail.uri?authorId=57980557700

- Research gate:
https://www.researchgate.net/profile/Valeria-Cafaro

7.4 Scientific contributions

7.4.1 Published articles

- Cafaro et al., (2023) Effects of sowing dates and genotypes of castor (*Ricinus communis* L.) on seed yield and oil content in the South Mediterranean basin. Agronomy 2023, 13(8), 2167 https://doi.org/10.3390/agronomy13082167

- Cafaro et al., (2023) Germination response of different castor bean genotypes to temperature for early and late sowing adaptation in the Mediterranean regions. Agriculture, 2023, 13(8), 1569. https://doi.org/10.3390/agriculture13081569

- Arlotta et al., (2020) Disease resistant citrus breeding using newly developed high resolution melting and CAPS protocols for Alternaria Brown Sport marker assisted selection. Agronomy 2020, 10(9) 1368 https://doi.org/10.3390/agronomy10091368

7.4.2 Conference proceedings

- Alexopoulou E., Cafaro V., Iordanoglou I., Cosentino S.L.'Screening Trials among high yielding castor bean hybrids in Greece'. 34th Annual Meeting of Association for the advancement of industrial crops (AAIC), 27-30th August 2023, Oregon State University, Oregon, USA

- Testa, G., Scandurra A., Caruso P., Cafaro V., Patanè C., Cosentino S.L., Corinzia S.A., "Leaf gas exchange of Sicilian wheat landraces under contrasting soil water

content". 52th Convegno Nazionale Società Italiana di Agronomia (SIA), 25-27th September 2023

- Ciaramella B.R., G., Piccitto A., Scandurra A., Cafaro V., Calcagno S., Cosentino S.L., Testa G., "Tolerance of african fodder cane to the cultivation in heavy metal polluted soil". 52th Convegno Nazionale Società Italiana di Agronomia (SIA), 25-27th September 2023

- Piccitto, A., Calcagno, S., Corinzia, S.A., Scordia, D., Cosentino, S.L., Castiglione, R., Cafaro, V., Testa, G. "Biomethane Potentials from castor Capsule Shells Pretreated with White-Rot Fungi" ISBN: 978-88-89407-22-6". European Biomass Conference & Exhibition Proceedings 30 th , Paper DOI: 10.5071/30thEUBCE2022-4CV.3.9

- Calcagno, S., Copani, V., Castiglione, R., Buscemi, L., Piccitto, A., Scordia, D., Testa, G., Cafaro, V., Cosentino, S.L. "The best castor hybrids sowing date in order to escape adverse weather during start and final phases of growth cycle". European Biomass Conference & Exhibition Proceedings 30 th , ISBN: 978-88-89407-22-6; Paper DOI:10.5071/30thEUBCE2022-1AV.4.3

7.5 Training in national or international universities and/or research institutions

I visited as a Ph.D. guest two important institution within my

Ph.D. research.

I have been abroad from June to September 2020 at 'CRES' (Centre for Renewable Energy Sources and Energy Saving) in Pikermi, Greece, under the supervision of the researcher Efthymia Alexopoulou. I performed analysis on castor dwarf samples, carrying out experiments on ashes and biomass. By using proximate analysis, I analyzed the percentage of the permanent carbon left in the samples, the humidity and the percentage of the ashes. The latter are then used for the analysis of the melting point, which measure the maximum temperature reachable until the ash melts. Furthermore, other analysis have been carried out on the biomass. After a grinding process in which leaves and stems are grinded, a sample of castor is obtained and used for the calorimeter's analysis. This process is used for measuring the heat released from combusting a specific amount of biomass sample. It is necessary to use a bomb calorimeter, in which one gram of sample is ground and condensed to fit in a capsule for combustion in the bomb. Through release of heat, the combustion happens, and it will be possible to calculate the gross heat of fuel.

My second training abroad was at the University and Research of Wageningen 'WUR' (Netherlands) from September 2021 to March 2022, under the under the supervision of the Professor Luisa Trindade. I took part in two different projects regarding experiments on Vicia Faba and *Miscanthus sinensis*. The experiment on Fava bean (*Vicia faba*) regards the GWAS approach. The latter is a genome association study used to identify the genes related to the off-flavors in Fava. I took part in the first step of the project, in, which was necessary to collect phenotypical data and genotyping. While, the second project in which I participated was related to *Miscanthus sinensis* and it was an expressional study to analyze the expression of some genes in different pathway.

7.6 Attended Congresses/Workshops/Meetings

- Oral presentation at 31st EUBCE 'European Biomass Conference and Exhibition', Bologna, Italy. 5th June 2023-9th June 2023

- 4th Joint Meeting of Agriculture-oriented Ph.D. Programs UniCT, UniFG and UniUd, 3-7 October 2022, Paluzza, Udine (Italy)
- Online Oral presentation at 30th EUBCE 'European Biomass Conference and Exhibition', Marseille, France. 24th June 2022-27th June 2022

- 3th Joint Meeting of Agriculture-oriented Ph.D. Programs UniCT, UniFG and UniUd,

- "eLabJournal Workshop" – University of Wageningen

- Plant Insect Interaction Workshops 2021 – "Making the invisible visible". 21st October at the University of Wageningen

- EPS Symposium "Genome Biology". 17th January at the University of Wageningen

- "Miscuglio evolutivo di frumento per l'adattamento ai cambiamenti climatici" Presentazione del progetto Mixwheat – University of Catania _ 18th March

- "La canapa: coltura innovativa multiuso per

l'agricoltura siciliana" – University of Catania_ 11th March

- Seminar "Sapere tradizionale e genomica: un connubio possibile? Suggestioni dall'Etiopia" by Enrico Pè

- Italian member of SIA (Società Italiana di Agronomia) and official assignment for bibliographic research and data organization for "50th National Convention of SIA" held in Udine

7.6.1 Attended Courses

- 'Scientific publishing in the peer review era', held by Prof. Antonio Biondi and Michele Ricupero. 23th June 2023-27th June 2023

- 'ICT for participatory methods, citizen science and disseminating research', held by Prof. Teresa Graziano. 10th July 2023-12th July 2023

- 'Writing a scientific review article' held by Prof. Daniela Spina and Gaetano Chinnici. 6th June 2023 and 9th June 2023

- "Corso di Analisi Statistica di Base" at the Univerity of Catania 19 - 23 Settembre 2022

- "Corso di Metodologia Statistica per le Scienze Agrarie «Dario Sacco»: I modelli lineari generali e generalizzati" held by SIA (Società Italiana di Agronomia)

- "Advanced B2+/C1 course" organised by CLA, held by Professor Suzanne Vickery, University of Catania

- Course at JM English School for achieving Cambridge C1 English level

- "CAD, GIS, and participatory mapping Course," Prof. Teresa Graziano and Prof. Francesca Valenti, 8-18 February 2021 (45 hrs). Main topics: CAD Course and ICT for Participatory Mapping & Disseminating. Department of Agriculture, Food and Environment, University of Catania, Italy

- 'Corso di Metodologia Statistica per le Scienze Agrarie «Dario Sacco»: I modelli lineari generali e generalizzati' organized by Società Italiana di Agronomia (SIA), 2nd – 11th febbraio 2021

7.6.2 Teaching activities

- Laboratory training in classrooms of the Section of 'Agronomia generale e coltivazioni erbacee' (Di3A, Università di Catania, Italy)

7.6.3 Thesis mentoring

- Vanessa Pappalardo, MS thesis on –going