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Strategies to overcome drought stress in ornamental plants in Mediterranean climate

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Research highlights

- Drought stress severely limits plant growth.
- The Mediterranean climate is characterized by long, hot, and dry summer.
- The availability of good quality water is decreasing in urban areas.
- Water scarcity increases the difficulty in maintaining urban green areas.
- Particular attention should be paid to improving the sustainability of ornamental plant maintenance.
- Various strategies can be adopted to overcome drought stress.
- Some green infrastructure, such as green roofs, exacerbates drought stress problems.
- Identifying genotypes capable of tolerating drought stress is of considerable importance.
- Since there are both herbaceous and woody ornamental plants, it is appropriate to analyze the response of both groups of plants.
- To identify these genotypes, it is essential to analyze morphobiological and functional responses.
- Non-destructive measurements, such as chlorophyll *a* fluorescence analysis, can accurately characterize plant responses.
- The effects of drought stress primarily affect the photosynthetic system.
- The use of biostimulants is a sustainable way to mitigate the effects of drought stress.
- The response to biostimulants is species-specific and depends on product dose and the extraction method.

Abstract

Drought stress, a consequence of climate change, poses a significant challenge to plant growth and, at times, survival. This issue is especially pronounced in the Mediterranean region, a climate hotspot where the impacts of climate change are particularly severe. This challenge is critical for ornamental plants, especially when used in urban green spaces. On one hand, water availability is often inadequate or entirely absent; on the other, the plants in green infrastructure provide essential ecosystem services that improve the quality of life and well-being in cities. Several solutions exist to alleviate the challenges associated with water scarcity. Selecting the appropriate genotype is undoubtedly a crucial strategy; however, ornamental plants exhibit vast biological diversity, with over 90,000 species used for ornamental purposes. This diversity necessitates a wide range of choices and repeated testing of numerous potentially viable genotypes. In this context, it is essential to identify the response mechanisms of both herbaceous and woody plants to facilitate the efficient and rapid screening of potentially useful genotypes. Additionally, technical solutions, such as biostimulants, may help mitigate the adverse effects of drought stress. In this context, the doctoral thesis examined several preliminary aspects of the issue through two reviews. Specifically, it focused on (i) strategies to enhance the resilience of shrubby plants to abiotic stress and (ii) the selection of plants suitable for green roofs—an infrastructure where the effects of drought stress are particularly pronounced. The potential contribution of certain native shrubs to the sustainability of Mediterranean green spaces was analyzed, along with the morphological, physiological, and biochemical responses of various herbaceous plants subjected to drought stress and subsequent sheltering. The final two trials investigated the application of

biostimulants, assessing species-specific effects as well as the influence of treatment timing, dosage, and extraction methods.

Keywords: biodiversity; biostimulants; chlorophyll *a* fluorescence; climate change; extensive green roofs; gas exchange; green areas; native plants, plant species selection; Relative Water Content; SPAD index; sustainable landscape; urban environment.

Riassunto

Anche a seguito del cambiamento climatico, lo stress da siccità rappresenta uno dei principali ostacoli per la crescita e talvolta per la sopravvivenza delle piante. Il problema è particolarmente avvertito nell'area mediterranea che rappresenta un hotspot climatico, in cui gli effetti dei cambiamenti climatici sono particolarmente intensi. Il problema è fortemente avvertito per le piante ornamentali soprattutto quando impiegate negli spazi a verde urbani. Da una parte, infatti, la disponibilità idrica è spesso subottimale se non assente, dall'altra sono proprio le piante presenti nelle infrastrutture verdi a fornire quei servizi ecosistemici in grado di migliorare la qualità della vita e il benessere in città.

Diverse sono le soluzioni per lenire i problemi connessi con la carenza idrica. La scelta del genotipo adatto è sicuramente una strategia importante; il problema è che le piante ornamentali si caratterizzano per un patrimonio biologico molto ampio – si stima che le piante utilizzate come ornamentali siano superiori a 90.000 – e questo presuppone che, accanto all'ampia possibilità di scelta, occorra fare i conti con la necessità di reiterare le prove per i numerosi genotipi potenzialmente impiegabili. In questo contesto, diventa importante individuare i meccanismi di risposta delle piante – siano esse erbacee che legnose – in grado di consentire un adeguato e rapido screening fra i genotipi potenzialmente utilizzabili. Anche alcuni mezzi tecnici, quali i biostimolanti, potrebbero consentire di ridurre gli effetti negativi dello stress da siccità.

In quest'ambito la tesi di dottorato ha affrontato, con due review, alcuni aspetti preliminari della questione e, in particolare, (i) le strategie per incrementare la risposta delle piante arbustive agli stress abiotici e (ii) quali piante è possibile usare nei tetti verdi, un'infrastruttura in cui gli effetti dello stress da siccità sono

particolarmente intensi. È stato poi analizzato il possibile contributo che alcuni arbusti autoctoni possono assicurare per rendere più sostenibile il verde mediterraneo e la risposta morfologica, fisiologia e biochimica di alcune piante erbacee sottoposte a stress da siccità e a successivo ricovero. Le ultime due prove hanno riguardato l'analisi dell'impiego di biostimolanti, analizzandone sia l'effetto specie-specifico che l'influenza del momento in cui si effettua il trattamento, della dose e della modalità di estrazione del biostimolante stesso.

Parole chiave: ambiente urbano; aree verdi; biodiversità; biostimolanti; cambiamento climatico; fluorescenza della clorofilla *a*; indice SPAD; piante autoctone; Relative Water Content; scambi gassosi; selezione di specie vegetali; sostenibilità spazi a verde; tetti verdi estensivi.

Preface

Drought stress is particularly severe in the Mediterranean region because of its unique climatic conditions. Historically, irrigation has been employed to mitigate abiotic stress, particularly in ornamental plants. However, growing ecological awareness increasingly advocates for sustainable solutions that align with the principles of Agenda 2030.

The relationship between ornamental plants and stress is further enhanced by the recognition that these plants, beyond their striking and aesthetically appealing characteristics, serve as valuable tools for the redevelopment of degraded areas, often resulting from human activity, such as urban or post-industrial environments. In these settings, drought stress is prevalent and can significantly jeopardize plant survival.

The relationship between ornamental species and stress involves several specific aspects. The first and most immediate consideration is that damage assessment cannot be based on plant growth alone. In some cases, smaller or slower-growing plants may be regarded positively. Instead, it is essential to evaluate the aesthetic value of plants. Damage to foliage, such as necrotic areas on leaves, can significantly impair the primary function of ornamental plants.

Another aspect of the issue is the significant potential, owing to the wide variability characterizing species suitable for landscaping in the Mediterranean environment, to identify genotypes best equipped to tolerate and/or resist stressful conditions. This extensive genetic heritage of plants available for landscaping is advantageous because it enables the identification of plants capable of withstanding the specific environmental stresses of a given area. However, this vast diversity can paradoxically pose a limitation, as it requires costly

evaluation of various species and/or cultivars to determine the most resilient genotypes.

However, the response of the same genotype to stress varies depending on factors such as age, phenological stage of the plant, intensity and duration of stress, and interactions with environmental factors and other stressors. There is also a need to develop experimental protocols suitable for analyzing stressors, often in laboratory settings and/or under controlled conditions. Although this approach allows for the isolation of individual stressors, it can also result in scenarios that are not fully representative of what occurs under operational conditions. Therefore, it is essential to identify the most effective measurements for quickly and accurately assessing the effects of stress.

Recently, attention has been directed toward the potential role of certain substances known as biostimulants in alleviating the detrimental effects of drought stress. Some of these substances can be directly extracted from plants using methods such as maceration or ethanol extraction, which facilitates the identification of cost-effective and sustainable solutions. This PhD thesis focuses on these themes.

1. General introduction

Over the years, human activities related to urbanisation have generated enormous pressure on natural environments. Recently, green infrastructures (GIs) have been widely used in various cities worldwide to counteract the negative impacts of urbanisation (Nowak et al. 1998, 2006; Yang et al. 2005a, 2005b). The presence of GIs, namely trees, shrubs, lawns, roofs, and green walls, has been shown to be effective in reducing heat island and harmful emissions of air pollutants within cities (Akbari et al. 2001; Tzoulas et al. 2007; Baró et al. 2014). GIs offer important benefits to urban communities in terms of social, health, environmental, and economic factors (Benedict and McMahon 2002; Mansor et al. 2012).

In the Mediterranean region, the difficulty in establishing GIs is mainly related to water scarcity. Drought stress severely limits plant growth. The climate of the Mediterranean region, as is well known, is characterised by a marked seasonality, with long, hot, dry summers and cool, wet winters. Forecasts for the future, due to global change that will further reduce water availability, especially in arid and semi-arid environments such as the Mediterranean, are not positive. The availability of high-quality water will decrease especially in large cities (WWAP 2014; Paz et al. 2016). This will lead to difficulties in maintaining green areas because competition for access to water becomes more intense.

For these reasons, much attention has recently been paid to water use and management to improve the sustainability of ornamental plant maintenance in semi-arid environments, such as the Mediterranean basin. Water scarcity has led to the spread of water-saving landscaping techniques (xeriscaping), favouring the use of water stress-tolerant species, which are native species such as the carob tree, which is highly tolerant of high temperatures and low soil water efficiency

(Ouzounidou et al. 2012). This focus on water saving is also related to the fact that, although water in the urban environment is widely used for purposes other than irrigation (e.g., for industrial and residential uses), a green space, due to its 'visual exposure', is an immediate indicator of water consumption (Thayer 1976).

Water savings can be maximised by using various strategies, such as the appropriate choice of species, identifying those that exhibit high tolerance to water scarcity without compromising their ornamental value, and/or adopting innovative cultivation methods to reduce the effects of water stress (Toscano et al. 2019).

It must be remembered that ornamental plants are increasingly seen not only as species and/or cultivars that offer aesthetic pleasure to our eyes but also as effective tools for improving the environment and quality of our lives (Savé 1999). Therefore, ornamental plants are used to restore degraded landscapes, control erosion processes, reduce energy inputs for climate control, and improve the aesthetic quality of urban, peri-urban, and rural landscapes. The number of plant species used for ornamental purposes is very high. The wide biodiversity of ornamental or potential ornamental species increases the chances of identifying genotypes that can cope with drought stress and be used for landscaping.

The choise of plants for creating green spaces, can be based on many species from numerous geographical areas that are capable of performing different functions (Savé 1999). In contrast to agricultural activity, the performance of an ornamental green space is not assessed by 'quantifiable yield', but by the fulfilment of the expectations of the user or individual/entity that has borne the cost of planting and/or maintaining the space. These expectations often include aesthetics and/or the possibility of providing shady spaces, ground cover, and opportunities for enjoyment (Kjelgren et al. 2000). In the restoration of degraded green areas, plant survival is often the sole purpose of cultivation. Furthermore, for ornamental plants used in the

landscaping of ornamental green areas, rapid growth is not always desirable because excessive plant vigour leads to frequent pruning and thus higher management costs. To maintain a compact growth habit, frequent pruning or the use of growth regulators is required (Cameron et al. 2004). A reduced amount of water can have positive benefits on growth control, and thus moderate water scarcity can be a useful tool to provide plants with a compact habitus and slower growth, allowing for easier maintenance of green spaces (Niu et al. 2007).
However, the effects of water stress on plants are difficult to study because the sensitivity and reaction time to water scarcity vary between species and are related to the intensity and duration of the water stress itself. Plant responses to water scarcity involve the activation of numerous physiological and biochemical mechanisms (Rafi et al. 2019a; Giordano et al. 2021) that can be exploited as markers for the identification of stress-tolerant species (Álvarez et al. 2018).

1.1 State of the art

1.1.1. Response of ornamental plants to drought stress

Drought stress severely limits plant growth in the Mediterranean climate, which is characterised by pronounced seasonality, with hot, dry summers and cool, wet winters (Paz et al. 2016). The main aspect that influences plant characteristics and vegetation in this environment is the prolonged dry season. Therefore, the growth and survival of plants are threatened by long periods of water shortage and high temperatures in summer, which lead to more or less intense stress conditions (Medrano et al. 2009). Global climate changes, which are likely to occur in the future, will worsen water availability, especially in arid and semi-arid environments. The availability of fresh, high-quality water will decrease especially in large cities (WWAP 2014; Paz et al. 2016). This will make it difficult to maintain green areas because competition for water will become a particularly critical issue.

For these reasons, much attention has recently been placed on water use and management to improve the sustainability of maintaining green spaces in semi-arid environments, such as the Mediterranean basin (Pantaloni et al. 2022; Pumo et al. 2023; Sharma et al. 2023; Silva 2023). Water saving can be maximised by using different solutions that can be traced back to two main strategies: i) an appropriate choice of ornamental species, identifying those that can able to exhibit a high tolerance to drought stress without compromising their ornamental value; ii) the reduction of the effects of drought stress through innovative cultivation methods.
As mentioned in the introduction, ornamental plants are numerous, which increases the possibility of identifying genotypes that can cope with water stress and can therefore be used for green spaces. The response of plants to such stress involves numerous morpho-anatomical and functional changes (Jafari et al. 2019; Tribulato et al. 2019; Zulfiqar et al. 2020; Toscano e Romano 2021; Wang et al. 2024).

Growth and morpho-anatomical modifications
Plant responses to drought are different and interconnected. The ability to adapt to water stress varies among genera, species (Sánchez-Blanco et al. 2002; Torrecillas et al. 2003), and cultivars. The main morphological changes under drought conditions are the reduction of the epigeal portion and leaf growth, which negatively affect the ornamental value and visual appearance of the plants, particularly important factors (from an aesthetic point of view) that must be prioritised when assessing the tolerance of genotypes to this stress (Farieri et al. 2016).
The reduction of leaf area is a typical response observed in plants subject to drought stress, as highlighted by Toscano et al. (2018a), often as a consequence of a reduction in the leaf number (Álvarez et al. 2013) or as a result of a smaller leaf size (unit leaf area) (Toscano

et al. 2014). This is a typical avoidance mechanism, whereby plants counteract the limited availability of water by reducing the transpiring surface area.
When a plant is subjected to water stress, in addition to a reduction in leaf area, changes in leaf unit size and cuticle thickness are also observed.
The reduction of Specific Leaf Area (SLA) could be a strategy to improve Water Use Efficiency (WUE); thicker leaves usually have higher concentrations of chlorophyll and protein per unit leaf area and therefore exhibit higher photosynthetic capacities per unit leaf area than thinner leaves (Liu and Stützel 2004).
Leaf anatomical changes are specific features: for example, in Polygala, anatomical changes in leaves have been linked to spongy tissue, whereas in Viburnum, to palisade tissue (Toscano et al. 2019).
In plants subjected to drought conditions, an increase in the root/shoot ratio has frequently been observed, which is useful in reducing water consumption (Wu et al. 2008) and increasing water uptake (Smirnoff 1998). Although destructive, this parameter, could also be suggested as a screening factor for classifying species according to their different tolerance to the stress in question.

Physiological parameters
As far as physiological parameters are concerned, the main consequences of drought stress in plants are stomatal closure, reduced gas exchange, slowed photosynthetic activity and, as a final consequence, plant death (Campbell et al. 2010; Hu et al. 2010). Drought stress conditions mainly affect the photosynthetic system; in particular, they impair elements involved in the process, such as electron transport to the thylakoids, the carbon cycle, and stomatal control of CO_2 supply. The reduction in photosynthetic activity, in particular, is related to stomatal conductance mechanisms (Del Blanco et al. 2000; Samarah et al. 2000; Ahmadi and Baker 2001; Anjum et

al. 2011; Sánchez-Blanco et al. 2019). The first response of plants to water stress is stomatal closure and the consequent reduction in carbon assimilation required for photosynthetic activity. As a consequence of stomatal closure, there is not only reduced water loss but also reduced nutrient uptake, resulting in altered metabolic pathways (Xiong and Zhu 2002).

The reduction in leaf water potential is also related to a reduction in plant growth.

Under water stress conditions, high ratios of net photosynthesis (A_N) and stomatal conductance (gs), also expressed as WUE, indicate that leaves (particularly chloroplasts), even if immediate stomatal closure is observed, try to maintain high photosynthetic performance. As reported by Álvarez et al. (2018), the reduction of gs in lentisk (*Pistacia lentiscus* L.), subjected to drought stress limited water losses by controlling transpiration.

To estimate water stress tolerance in plants, it is essential to determine the transpiration rate. Indeed, it has been observed that species that can retain more water and therefore lose less water through their stomata are those that are more drought tolerant (Riaz et al. 2013). Galmes et al. (2007) reported that shrubs have a better ability to regulate transpiration than herbaceous plants.

Under drought stress conditions, one of the parameters commonly used to identify the presence of photosynthetic damage in plants is the measurement of chlorophyll *a* fluorescence. This parameter is useful for analysing the influence of environmental factors on the efficiency of the photosynthetic apparatus (Lichtenthaler and Rinderle 1988). The decline in photosystem II (PSII) activity causes an imbalance between electron generation and utilisation, leading to changes in quantum yield (Reddy et al. 2004). The Fv/Fm ratio (i.e., the maximum primary photochemical efficiency of PSII in a sample of dark-adapted leaves) allows the evaluation of PSII photosystem efficiency, indirectly measuring the physiological state of the plant

(Maxwell and Johnson 2000). In a study conducted by Álvarez et al. (2011) on Callistemon plants maintained under different levels of water stress, the Fv/Fm values remained constant at 0.80. Drought stress with this value did not compromise PSII; therefore, Callistemon can be considered a drought-resistant species.

Effects of drought stress on the ornamental value of plants

Plants under water stress modify their morphology and physiology to survive in arid environments. These changes can also directly affect the visual appearance and ornamental value of plants (Rafi et al. 2019b; Toscano et al. 2019).

Water scarcity has a direct impact on plant habitus and ornamental quality, especially at the leaf level. Leaves may fall due to ethylene production, change colour, or show necrosis. The duration and turnover of flowers are often compromised. The presence of flowers on plants greatly influences their visual appearance; therefore, tolerant plants should be able to produce a high number of flowers with a longer life, because in water deficit, flower turnover is reduced (Rafi et al. 2019b). Flower turnover or the production of new flowers depends, on the energy availability of the plants, and in the case of prolonged drought stress, reduced photosynthesis results in fewer carbohydrates available to support the flowering process.

However, reducing growth can have a positive effect on ornamental plants used in urban green spaces because it reduces the frequency of pruning required, thereby management costs. This is particularly important for ornamental plants used in topiary; slower growth helps preserve the desired shape for longer.

1.1.2. Use of various tools to mitigate drought-induced damage

One solution to overcome the problems associated with drought stress is the appropriate choice of species. The response to drought varies greatly among plants used in green spaces. In regions where drought stress is frequent, the choice of ornamental plants may favour those growing in desert areas (such as xerophytes or succulents), which are particularly well-equipped to survive in conditions of water shortage (Leotta et al. 2023).

Symbiosis with arbuscular mycorrhizal (AM) fungi can increase host resistance to drought stress, although the effect is not always predictable (Chen et al. 2020; Rydlova and Püschel 2020). Because drought stress is frequent in drought-prone soils, the influence of AM on the water-deficiency response of plants may partially result from the influence of AM on salt stress. With this aim in mind, Cho et al. (2006) investigated whether AM-induced effects on water shortage responses were more pronounced when plants of similar size were exposed to drought in saline soils than when they were subjected to water shortage alone. In the experiment, conducted in two greenhouses, different characteristics of water relations were measured in sorghum plants with roots colonised by *Glomus mosseae*, *Gigaspora margarita* or a mixture of AM species, during a long period of drought followed or not by NaCl-supplemented water to simulate salt stress. The results confirmed that AM fungi can modify plant responses to drought but do not contribute to salt resistance. The beneficial effects of AM are related to the improved ability of the roots to absorb water by increasing the active root surface area. The increased root adsorption capacity is also due to the production of gibberellins and cytokinins by AM (Barea and Azcón-Aguilar 1982).

Plant growth-promoting bacteria (PGPB) have been found to play a direct and indirect positive role under stress conditions (Wong et al. 2015). The positive effects of PGPB have been validated by their activation of the enzyme 1-aminocyclopropane-1-carboxylate

deaminase, which reduces ethylene production and increases auxin concentration at the root level (Glick 2014).

In recent years, there has been a considerable increase in the use of biostimulants in agriculture to improve the abiotic stress tolerance of crops (du Jardin 2015). Abiotic stress reduction is the most frequently cited benefit of biostimulant formulations (Yakhin et al. 2017). Biostimulants are derived from organic substances through various industrial processes and can be composed of microorganisms such as fungi or bacteria (du Jardin 2015). They help improve plant adaptation to sub-optimal conditions by acting on plant physiology and biochemistry (Toscano et al. 2018b). The contribution of cytokinin-producing bacteria under drought conditions is of great interest (Calvo et al. 2014). Some microbial inocula, known to have a positive effect on plant development, may also help plants overcome or tolerate abiotic stress conditions.

Even in ornamental plants, production can be improved by the application of biostimulants. For example, Hibiscus plants treated with commercial biostimulants showed an increase in gas exchange (Massa et al. 2018). In a pot experiment with bedding plants, algae extract (*Ascophyllum nodosum*) had positive effects on the growth and development of petunia, pansy and cosmos subjected to water shortage (Battacharyya et al. 2015). Positive effects of biostimulants on flowering have also been found due to earlier flower induction, increased number of flowers, and increased biomass accumulation (Vernieri et al. 2006).

Plant growth promoting rhizobacteria (PGPR) have also been adopted to stimulate plant growth under resource-limiting conditions by enhancing tolerance to abiotic stress and increasing nutrient availability, uptake, and assimilation. Plants of *Petunia* × *hybrida* (Hook.) Regel, *Impatiens walleriana* Hook.f., and *Viola* × *wittrockiana* Gams treated with two bacteria strains of *Pseudomonas* had greater shoot biomass than untreated control plants when grown

under low-nutrient conditions and after recovery from drought stress (Nordstedt et al. 2020).

The effects of exogenous application of salicylic acid (SA) on morpho-physiological and molecular characteristics of *Impatiens walleriana* Hook.f. plants grown under water deficit stress were investigated (Safari et al. 2022). The application of 2 mM SA increased growth and physiological indices in drought-stressed plants, mitigating the detrimental effects of water deficit in this important ornamental species.

With the aim of assessing differences in the mechanisms involved in the resistance of ornamental species to drought stress resulting from regular suspension and recovery of water supply, Toscano et al. (2014) subjected the plants of five ornamental shrubs [*Callistemon citrinus* (Curtis) Skeels, *Laurus nobilis* L., *Pittosporum tobira* (Thunb.) W.T.Aiton, *Thunbergia erecta* (Benth.) T.Anderson, and *Viburnum tinus* L. 'Lucidum'] to two consecutive cycles of suspension/recovery (S/R) in comparison with plants grown under constant water supply conditions (C). The five species exhibited different responses to drought stress. At the end of the experimental period, the S-R treatment had no effect on the dry weight of any species, except for *P. tobira*. In the latter species, drought stress reduced the total plant biomass by 19%. Drought stress induced negative effects in the plants, including a decrease in dry matter of the epigeal portion and an increase in the root/area ratio, especially in *C. citrinus* and *P. tobira*. All species adapted to water shortage using physiological mechanisms [Relative Water Content (RWC) and regulation of water potential, stomatal closure and reduced photosynthesis]. After irrigation, all species recovered completely; therefore, deficit irrigation can be considered a suitable strategy for landscaping in a Mediterranean environment. It must be emphasised that this is a species-specific response, as *L. nobilis* and *T. erecta* appeared to be the least sensitive

species to water shortage stress compared to the other genotypes studied.

Drought stress can be used to control the growth of potted plants. Davies et al. (2016) used deficit irrigation compared to conventional irrigation on two crops with different canopy characteristics (*Cornus alba* L. and *Lonicera periclymenum* L.). In a subsequent experiment, *Forsythia* × *intermedia* Zabel was grown in two substrates with different amounts of peat (60 and 100 %). Deficit irrigation was found to be particularly effective in controlling vegetative growth when applied with overhead irrigation, with results similar to those of drip irrigation (Davies et al. 2016). This comparable response suggests that deficit irrigation can be applied in the absence of precision drip irrigation.

1.2. Aim of the thesis

The drought tolerance of ornamental plants varies widely between genotypes and in response to different environmental conditions and soil or substrate characteristics. Species used in ornamental green spaces exhibit drought tolerance mechanisms similar to those of agricultural crops; however, the assessment of such tolerance for these plants should be based primarily on aesthetic value rather than on growth effects. Because of the very large number of species that can potentially be used for ornamental purposes, the identification of genotypes suitable for arid conditions should be easy, although screening must be performed by considering a large number of species and/or cultivars.

The main problems that arise when dealing with the response of ornamental plants to water stress are mainly related to the following:

I. the need to experimentally analyse a wide range of genotypes to identify those best suited to such stress conditions;
II. interest in identifying parameters useful in discriminating water scarcity stress tolerance;

III. development of irrigation methods or plant management strategies suitable for enhancing the tolerance of species to water stress is needed.

The study of plant response mechanisms to water stress, and in particular, the physiological mechanisms of tolerance to water shortage, could allow the identification, on a more certain basis, of plants capable of surviving the stress in question, which is very frequent in the Mediterranean environment.

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2. New strategies to increase the abiotic stress tolerance in woody ornamental plants in Mediterranean climate

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New strategies to increase the abiotic stress tolerance in woody ornamental plants in Mediterranean climate

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Abstract

The native flora of different Mediterranean countries, often woody species, was widely recognized for its ornamental potential. The shrubs, in particular, are a typology of plants very widespread in the Mediterranean environment and constituent the 'Macchia', the typical vegetation of this ecosystem. The use of native shrubs for the realization of ornamental green areas has been recently examined for their adaptability to abiotic stress. Abiotic stresses, in fact, are the major limiting growth factor in urban and peri-urban areas. The identification and use of tolerant ornamental species allow the reduction of management costs and preserve the aesthetical value of

green areas. Tolerance to drought stress, for instance, in the Mediterranean climate can improve the ecosystem services of these plants in the urban environment. In this review, the possibility to early individuate different plant species' mechanisms to tolerate or avoid the stresses is analysed, as well as the possibility to increase abiotic stress tolerance through genetic and agronomic strategies. The exploration of wild or spontaneous species can be a good source for selecting tolerant plants to be used as ornamental plants in urban areas. Among agronomic strategies, biostimulants, mulching, and plant combination can provide a useful solution to counteract abiotic stress in the urban environment.

Keywords: urban environment; shrubs; green areas; landscape; abiotic stress; pollution; plant choice; biostimulants

2.1. Introduction

Woody plants, trees or shrubs, represent the most common plants in many natural and semi-natural environments (Götmark et al. 2016). Almost all these plants have characteristics, such as being perennial or the same structure of ramifications, that allow their use for ornamental purposes (Read and Bavougian 2014). All 'woody ornamental plants' can be used in gardens and landscaping, thanks to the presence of flowers, more or less showy, of different colours, the colour and morphology of leaves, and the shape of the plant (height, shape, and width) (Read and Bavougian 2014). The species of woody ornamental plants belong to numerous botanical families and genera; within a single species, there are numerous cultivars, expressing enormous variability (Van Laere et al. 2018). An indication of the wide biological diversity of woody plants used for horticultural purposes is the "List of Names of Woody Plants" by Naktuinbouw, which contains the 'preferred botanical names and common synonyms and trade names of almost 45,000 woody nursery plants' (Lists of Names

of Woody Plants and Perennials, 2023). The new edition (2021–2025) contains more than 8,200 new names of woody plants. Shrubs, in particular, which are the plant typology on which our attention is focused in this review, are a category of woody plants widespread above all in the Mediterranean Basin, where they form the so-called in Italian 'Macchia', i.e., the vegetation typical of this environment.

The diffusion of this plant typology in the Mediterranean regions is justified by its adaptability to stressful climatic conditions, characterized by hot summers and low rainfall, which determine a long and droughty summer (Paz et al. 2016). Climatic conditions of the Mediterranean environment are also found in four other regions of the Earth (California, Chile, South Africa, and some areas of Australia). The frequency of abiotic stresses, and in particular water and saline stresses due to the poor quality of the water, has selected the plants of all these environments, leading to the convergence of the morphophysiological traits of the various species, determining that all the plant communities of the Mediterranean climate are dominated by sclerophyllous evergreen shrubs. In the Mediterranean regions, water stress and poor water quality (high salt content) are among the main problems hindering the use of ornamental plants. Global climate change will certainly accentuate problems associated with water deficits and salt levels, especially in urban areas (WWAP 2014). The possibility of the use of native Mediterranean shrub species can be a solution for drought (Munné-Bosh and Peñuelas 2004) and saline stress (Cassaniti et al. 2013). It is of interest to increase the sustainability of the landscape. Native Mediterranean shrubs are able to adapt to conditions of accentuated drought, which is one of the most important factors influencing plant survival and species distribution (Filella et al. 1998). Many woody ornamental plants and native Mediterranean shrubs are particularly suitable to use in landscape planning. These plants, in addition to their high aesthetic value, are characterized by wide biodiversity. Indeed, the Mediterranean Basin

is one of the Earth's areas with the greatest biodiversity, as it hosts 10% of the world's higher plants in an area that is just 1.6% of the Earth's surface (Médail and Quézel 1997). Of the approximately 25,000 species recorded in the Mediterranean area, half are endemic to the region (Heywood and Skoula 1999). Hotspots represent about 22% of the total area of the Mediterranean Basin and host about 5,500 restricted endemic species (Médail and Quézel 1999).

Thanks to their particular morpho-physiological characteristics, woody plants are very suitable to be used for ornamental purposes. Since shrubs are characterised, as Givnish (1984) recalled, by a high number of active meristems, which are potential sites for stem regeneration, they can tolerate abiotic stresses more than trees. It is no coincidence, in fact, that shrubs are associated with degraded environments, where abiotic stresses are very frequent, for these reasons. They are plant species suitable for low maintenance green infrastructures. Shrubs, perennial plants with numerous branches that branch out at or near the ground (Götmark et al. 2016), are widespread in the different biomes of the Earth; their tolerance to numerous stresses allows them to ensure numerous ecosystem services (Francini et al. 2022). These plant species are able to control temperatures, stabilize the soil, and ensure the water balance of the ecosystem, absorption, and carbon storage. The capacity to assure ecosystem services is crucial in the choice of ornamental plants for sustainable green infrastructures. From an ornamental point of view, shrubs are characterised by different features (high number of twigs, which influences their growth pattern; pulvinate shapes, which reduces their transpiration and improves the visual qualities of the green infrastructures; leaf characteristics; different blooming periods; and colours of flowers) that add interest and variety to the landscape (Romano and Scariot 2021).

2.2. Methodology and literature research

According to the objective of this review, i.e., to analyse the strategy and improve the tolerance of ornamental shrubs to abiotic stresses that are commonly frequent in urban areas, the research has been searched in the most important scientific databases such as Scopus and Web of Science. The published research papers were selected considering their impact and keywords that matched with the aim of our review. Recent works with robust statistical data analysis were considered, especially in the last 10 years. The main criterions were the ornamental value of the plants adopted and the role of human intervention in man-designed green infrastructures (gardens and urban parks). Certainly, it was taken into consideration plants that have essential ecological functions, but attention, and therefore the exclusion of some articles, was focused on agronomic and horticultural aspects, including the choice of species, which can improve tolerance to stress and therefore maximize the ecosystem services of the plants introduced by man into the city for ornamental and fruition purposes.

2.3. Ornamental shrubby plants in the urban environment

Urbanisation significantly modifies the physical environment, biological components, and ecosystem processes of cities (McDonald et al. 2007). Woody plants, trees, and shrubs become essential components of urban green infrastructures for their numerous ecosystem services and, in particular, for the reduction of pollution (Francini et al. 2022). Another aspect linked to urban environment quality is heat island reduction, determined by green areas and vegetation, particularly important in relation to increasing global warming. Plants, thanks to their shade (Massetti et al. 2019) and transpiration, are able to improve urban conditions for the inhabitants but also for tourists who favour areas with green infrastructure to spend their time outdoors (Lopes et al. 2022a, 2022b). It is wellknown that urban areas have temperatures 5–7 °C higher than rural areas.

Mitigation of the high temperatures is obtained by the transpiration of plants, and the efficiency depends on water availability. Ornamental shrubs are able to maintain environmental quality and offer pleasant landscape effects, even if urbanisation, with its environmental changes, exerts negative effects on plants, which mainly affect the characteristics of the leaves (Ilyas et al. 2021). At the same time, the variations that, due to the stressful effects of urbanisation, are observed in plants, such as leaf thickness, unit leaf area, specific leaf area, etc., can be used as indicators of the urban environment quality (Ilyas et al. 2021).

2.4. Mechanism of tolerance and/or resistance of ornamental shrubs to abiotic stress

Abiotic stresses are the major limiting growth factor in urban and peri-urban areas. The identification and use of tolerant ornamental species allow the reduction of management costs and preserve the aesthetical value of green areas. Urban environments can be subjected to more stressful conditions than rural areas (Francini et al. 2022).

Drought tolerance is most important for agricultural production, as most of the plants are obtained in Mediterranean, semi-arid, and tropical regions (Tanveer et al. 2018). Soil water limitation during drought affects evaporation, evapotranspiration, and ultimately, precipitation (Iqbal et al. 2020). Plants have developed various adaptive strategies to cope with drought stress (Hussain et al. 2018). Plants adapt to drought in several ways, such as drought escape, tolerance, and avoidance mechanisms (Levitt 1980). Perennials especially rely on drought tolerance (Kozlowski and Pallardy 2002), which can be achieved through morphological adaptations of roots, stems, and leaves. Tolerant plants have a high-water potential with higher water uptake or physiological adaptations through the reduction of transpiration (Vinod 2012). Plant adaptation varies among plants depending on species, genotype, phenological development, or organ

type (leaves) (Chen et al. 2009). Optimisation of carbon assimilation with minimisation of water loss, i.e., improvement of intrinsic water use efficiency, has been described as an adaptive trait for plants that are exposed to severe drought, like, for example, the Mediterranean woody plant species (Medrano et al. 2009; Álvarez et al. 2018).

Woody species are particularly important in this context, due to their longevity and the possibility of studying long-term adaptation mechanisms. To understand the tolerance level, many physiological traits, such as the measurement of leaf water potential before sunrise and at midday, photosynthetic rate, stomatal conductance, transpiration rate, and intercellular carbon dioxide concentration, were analysed. Biochemical characteristics, such as ascorbic acid, glutathione, chlorophyll content, tocopherols, amino acids, carotenoids, and soluble sugar, have also been used to control the tolerance level of plants to drought stress (Toscano et al. 2019). It has been observed that species that can retain a greater quantity of water and therefore lose less through the stomata are more tolerant to drought (Riaz et al. 2013). As reported by Galmés et al. (2007), shrubs have a better ability to regulate transpiration than herbaceous plants (Toscano et al. 2019).

Many of the favourable characteristics for resisting drought are present in shrubs; it is not a coincidence that in semi-arid environments, most of the plants are sclerophyllous evergreen shrubs, or deciduous or seasonally dimorphic shrubs, which possess the main adaptive approaches of perennial species to drought stress (Toscano et al. 2018a). The main role of shrubs in semi-arid ecosystems lies in the fact that these plants can grow under conditions of environmental stress where trees cannot survive (Wilson 1995; Iqbal et al. 2020). Some perennial species, such as *Euphorbia dendroides* L., a Mediterranean shrub, keep their leaves during the winter and/or spring and drop them with the onset of the hot season.

The use of Mediterranean shrubs for revegetation in semi-arid areas has increased due to their ability to adapt to severe drought conditions, which is considered one of the most important factors influencing plant survival and species distribution (Vilagrosa et al. 2014). In the case of Mediterranean evergreens, leaves undergo several drought events that can further hinder photosynthetic capacity (Niinemets and Kenan 2014; Nadal et al. 2020). One of their most distinctive characteristics is a higher water use efficiency (WUE) at the leaf level, due to the reduced stomatal conductance but higher carboxylation capacity of Rubisco compared to evergreens of other biomes (Flexas et al. 2014). Hence, stomatal and mesophyll diffusion constraints are the most important factors limiting photosynthesis in evergreens (Nadal and Flexas 2019). However, Mediterranean sclerophylls are able to sustain positive CO_2 assimilation rates at relatively low leaf water potentials compared to Mediterranean deciduous species (Flexas et al. 2014). This increased drought tolerance has been partly attributed to the robustness of sclerophyll leaves (De Micco and Aronne 2012), which tend to sustain shrinkage and collapse, thus preventing negative effects on photosynthesis and water transport (Flexas et al. 2014; Scoffoni et al. 2014). Beyond the response mechanisms to drought stress, ornamental plants used in landscaping must ensure an aesthetic value that can be influenced by a reduction in the number of flowers, an excessive decrease in plant growth, and a worsening of foliage quality (Toscano et al. 2019). The analysis of the mechanisms adopted by different species to overcome drought stress and reduce water loss could allow the identification of the most tolerant species to be used in arid and semi-arid environments, thus increasing the sustainability of ornamental green infrastructures (Table 2.1).

Table 2.1. Effect of drought on ornamental plant quality and traits associated to tolerance.

Target organs	Stress effects	Tolerance or adaptation response	References
Roots	Increase of root biomass	Increase the functional roots and architectures	Toscano et al. 2019
Stem	Decrease the growth, elongation, diameter and biomass	Increase the lignification process	Geng et al. 2019; Jafari et al. 2019; Toscano et al. 2019
Leaves	Reduction of size and leaf number	Increase the wax or thickness, and trichome number	Chen et al. 2009
Flowers	Reduction of flower production and longevity	Increase the flower longevity and turnover	Cai et al. 2012

Salt stress is another important abiotic stress that ornamental plants and shrubs in landscaping can be exposed to. There are not numerous studies on the effects of saline stress (Toscano et al. 2020). Salinity can affect the growth of ornamental shrubs by reducing leaf growth and expansion due to osmotic effects or by toxicity due to the high concentration of Na^+ and Cl^- in saline water (USEPA 1992). In ornamental plants, the aesthetic value can be compromised by salinity inducing leaf necrosis or abscission (Percival 2005; Cassaniti et al. 2013). In many ornamental species, salinity usually induces dry shoot biomass and leaf surface. Morphological adaptations such as resinous buds, and waxy leaves and stems in tolerant species allow woody plants to cope with salinity stress. The salt exclusion mechanisms are represented by smooth twigs, sunken buds, and low surface area to volume ratios (as occurs, for example, in pine needles) (Appleton et al. 1999; Tribulato et al. 2019).

Exposure to salt can affect plant metabolism through an osmotic effect, causing water deficit, or through a specific ion effect, causing excessive ion accumulation (Azza Mazher et al. 2007). Under saline conditions, plants must activate various physiological and biochemical mechanisms to cope with saline stress, which include water relationships, photosynthesis rate, hormonal profiles, toxic ion distribution, antioxidant metabolism, and soil response (Acosta-Motos et al. 2017). In particular, the changes in leaf tissue cell walls and factors limiting photosynthesis under these conditions and their possible interactions with leaf tissue damage are not well understood (Álvarez et al. 2018). Plants that have some degree of tolerance to salinity may show quality reductions when exposed to this stress, and this is an important factor in the selection of ornamental plants for use in gardens and landscaping (Cameron et al. 1999).

The ionic composition of irrigation water can influence the response of shrubs and trees to salt stress. Chloride salts appear to be more harmful than SO_4^{2-} salts, and Mg^{2+} associated with Cl^- is more harmful than Na^+ with Cl^- (Devitt and Morris 2005). Among many salinity tolerance mechanisms (Munns and Tester 2008), the ability to limit the entry of saline ions through the roots and to limit the transport of Na^+ and/or Cl^- to the aerial parts, retaining these ions in the root and in the lower part of the stem, is one of the most important characteristics associated with salt tolerance (Colmer et al. 2005). Species that maintain acceptable growth rates under saline conditions have effective mechanisms for excluding Na^+ and Cl^- from roots or leaves, thus maintaining good aesthetics and are ideal for landscaping. The low reduction and absence of symptoms of salt damage in *Eugenia myrtifolia* L. was associated not only with the root storage of Na^+ and Cl^-, but also with their limited uptake with increasing salinity (Boursier and Läuchli 1990). An important aspect of salt tolerance is related to a plant's ability to compartmentalise toxic ions, such as Na^+

and Cl^-, in roots or stems (Ferguson and Grattan 2005; Cassaniti et al. 2009).

The response of plants to salinity depends not only on the intensity of the salt treatment but also on the time of exposure to the salt treatment (Álvarez et al. 2015). These aspects are of primary importance, especially in the Mediterranean area when saline water is used for irrigation of perennial species, such as woody plants, as the interaction between intensity and duration of exposure to salt will determine physiological and molecular changes. At nursery level, the selection of plants tolerant to salinity stress can be carried out by the evaluation of plants' responses to salinity treatments (Table 2.2).

Table 2.2. Effect of salinity on ornamental plant quality and traits associated to tolerance.

Target Organs	Stress Effects	Tolerance or Adaptation Response	Reference
Roots	Increase of roots biomass	Increase the water uptake and exclusion of some toxic ions such as Na^+ or Cl^-	Sánchez-Blanco et al. 2014
Stem	Decrease the growth and biomass	Increase the extrusion or storage	Álvarez et al. 2012
Leaves	Reduction of size, necrosis, or abscission	Increase the storage of ions in vacuole	Cassaniti et al. 2012
Flowers	Reduction of flowers production and longevity	Increase the flower longevity and turnover	Fornes et al. 2007

In urban areas, hypoxia is an abiotic stress that ornamental plants can be often exposed to in compacted soil. Compaction is determined by physical degradation, which reduces the volume of a given mass of soil and decreases porosity. This promotes the formation of urban flooding (Yang and Zhang 2015). An excess of water is usually

considered to be deleterious to plant health and growth, and total submergence rapidly kills most plant species. Hypoxia/anoxia conditions restrict processes such as plant respiration and water and nutrient absorption (Jackson 1997; Kozlowski 1997). The most common symptoms in the aerial part of a plant under hypoxia/anoxia conditions include leaf curling (epinasty) and stem twisting, leaf chlorosis and wilting, marginal browning of the leaf and shedding/defoliation, as well as fruit drop. The physiological consequences of hypoxia are a decrease in stomatal conductance (Folzer et al. 2006) and a reduction of water potential (Else et al. 2001). In woody plants, waterlogging tolerance responses are associated with hypertrophied lenticels, new adventitious roots, and aerenchyma development (Kreuzwieser and Rennenberg 2014). These morphological and anatomical changes depend on the intensity, duration, and timing of the flooding cycle (Kozlowski 1997). The presence of hypertrophied lenticels is a common anatomical change observed in many woody species (Yamamoto et al. 1995). The development of hypertrophied lenticels is supposed to simplify the downward diffusion of O_2 as well as the potential discharge of compounds produced in the roots as by-products of anaerobic metabolism (Parent et al. 2008).

Oxygen depletion is one of the most important events during flooding. The diminishing in gas diffusion to the root environment as a result of the presence of excessive water in the soil or deprived aeration in soilless cultures, accompanied by reduction of available oxygen by aerobic processes (i.e., root and microbial respiration), will deprive the rhizosphere of available O_2.

A flood-tolerant plant can overcome the adverse effects of flooding through numerous morphological modifications, such as hyponasty (upward bending of leaves), improved shoot extension, aerenchyma formation, the development of barriers against radial O_2 loss (ROL) in roots, the development of adventitious roots, leaf anatomical changes,

and the formation of a gas film on leaf surfaces (Sauter 2013; Voesenek and Bailey-Serres 2015). The formation of adventitious roots improves the plant's adaptation to flooding stress, effectively transports atmospheric O_2 into the roots, and may support or replace the primary root system (Sauter 2013). Furthermore, aerenchyma occurs in adventitious roots and acts as an O_2 transportation pathway (Table 2.3).

Table 2.3. Effect of hypoxia or anoxia on ornamental plant quality and traits associated to tolerance.

Target Organs	Stress Effects	Tolerance or Adaptation Response	References
Roots	Increase the ethylene production	Cell death and in some herbaceous plants the aerenchyma formation	Cameron et al. 2010; Yang et al. 2020
Stem	Decrease the growth and biomass	Increase the ethylene biosynthesis	Cameron et al. 2010
Leaves	Leaf yellowing, abscission	Increase the storage of ions in vacuole	Cameron et al. 2010
Flowers	Reduction of flower production and longevity	Increase the flower longevity	Yang et al. 2020

Urban environment pollution is also a source of stress in ornamental plants. Urban areas can be highly polluted by human activities. Pollution can be represented by heavy metals derived from heating systems, vehicular traffic, and industrial emissions (Asgari Lajayer et al. 2019). Combustion of engines and tire emissions can represent a mobile pollutant source, while industries and heating systems represent fixed sources of pollution (Francini et al. 2022). Around pollution sites, the concentration of heavy metals increases. Ornamental plants can have different degrees of pollution tolerance or

ability to uptake and degrade them if they are organic pollutants. The use of suitable plant species can recover the visual appearance of polluted areas. The success of green area establishment depends on the tolerance of ornamental plants to the pollutant concentrations. Heavy metals are represented by different elements such as aluminium (Al), arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), mercury (Hg), and zinc (Zn). The tolerance of ornamental plants to heavy metals is strictly connected with the ability of these species to exclude the toxic heavy metals from the uptake or the ability of plants to uptake and translocate heavy metals to organs that can be released, such as older leaves or even fruits (Khan et al. 2021).

The identification of tolerant ornamental plants at nursery level can be performed by exposing the interested species to increasing concentrations of heavy metals. The biochemical and physiological response of plants allows their classification to different levels of tolerance. The specific markers for the evaluation of tolerance to heavy metals can be the production and accumulation of some protection molecules such as phytochelatins. These proteins can protect the plants by removing metals from the active cell metabolism by chelation, and their biosynthesis is induced by heavy metals accumulation. The phytochelatin biosynthesis is mediated by phytochelatin synthase and starts from glutathione (Kneer and Zenk 1992). The activity of this enzyme is regulated by the heavy metals post-translation activation (Cobbett 2000). Heavy metals induce plant stress with the accumulation of free radicals and damage to the cell membrane. The most common radicals are represented by reactive oxygen species (ROS), reactive nitrogen species (RNS), or reactive sulfur species (RSS). The non-specific response can be represented by the increase of the detoxification enzymes such as those belonging to the ascorbate–glutathione cycle (Figure 2. S1).

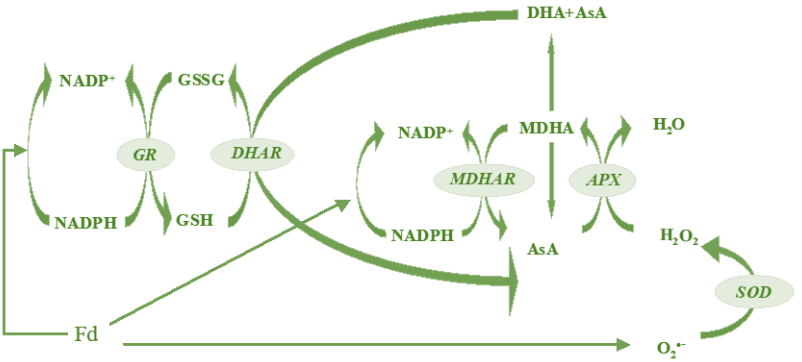


Figure 2. S1. The detoxification enzymes belonging to ascorbate-glutathione cycle. The superoxide radicals ($O_{2.}^{-}$) are removed by superoxide dismutase (SOD) which leads to hydrogen peroxidase (H_2O_2) formation. The ascorbate (AsA) is regenerated by oxidized forms by a cycle catalyzed by ascorbate peroxidase (APX) enzyme. The AsA is covered in monodehy-droascorbate (MDHA) by APX, then the monodehydroascorbate reductase (MDHAR) oxides the MDHA and reduces the $NADP^+$ that is regenerated by Ferredoxin (Fd). The MDHA can be converted to ASA and dehydroascorbate (DHA) by non-enzymatic reaction. The DHA is converted by dehydroascorbate reductase enzyme (DHAR) in ASA, contemporary the reduced glutathione (GSH) is transformed in oxidized glutathione (GSSG) by NADPH.

Free radicals are highly reactive and can damage the cell membrane and the phospholipid double layers. The damage of ROS on the cell membrane is specifically due to loss of compartment integrity and enzymes coming into contact with substrates generating products that can be responsible for several physiological disorders, compromising the visual appearance (Nazir et al. 2020). Membrane integrity and low lipid peroxidation are also good markers for the estimation of ornamental plant tolerance to heavy metal concentrations. At the nursery level, the selection of plants tolerant to heavy metals is carried

out by exposing the plants to increasing doses and monitoring the lipid peroxidation, phytochelatin accumulation, and enzymatic response. The distribution of plants in the planning area must be done considering the concentration and distribution of pollution in the soil. The shadows of buildings or tall plants in green areas can have negative effects on other plants. Therefore, the combination of different plant species such as herbaceous plants, shrubs, and woody ornamentals must be carefully considered. The visual appearance and aesthetical quality of the area depend on the health status of plants and their correct distribution. It is important to identify the correct exposure to ensure adequate light intensity. Plant distribution and combination must be carried out considering their shading tolerance. Buildings and trees can be responsible for shading and light limitations. Many ornamental plants can have a plasticity degree that allows the adaptation of plants to lower light intensities. At the nursery level, the ornamental plants can be prepared for low-light environments by progressive light reduction using black nets with a shading percentage from 50 to 90%. The intensity of shading depends on the shading in the urban area. The shade adaptation must be achieved by slow light intensity reduction (Middleton 2001). Plants under shade contribute to the ornamental value through the increase of chlorophyll concentration. At the physiological level, leaves under progressive shading intensity reduce the light compensation point (Figure 2.1). This means that a lower amount of light is required to compensate the respiration process (Fonteno and McWilliams 1978). Ornamental plants that have good light plasticity can be used for green planning in the shaded areas inside urban and peri-urban environments. If plants are not tolerant to shade, under shading conditions, the respiration can be higher than photosynthesis with a negative sugar accumulation balance in a 24 h period. This negative balance, if prolonged, can lead to plant death. Shade plants can survive at low light availability since they have low light compensation points.

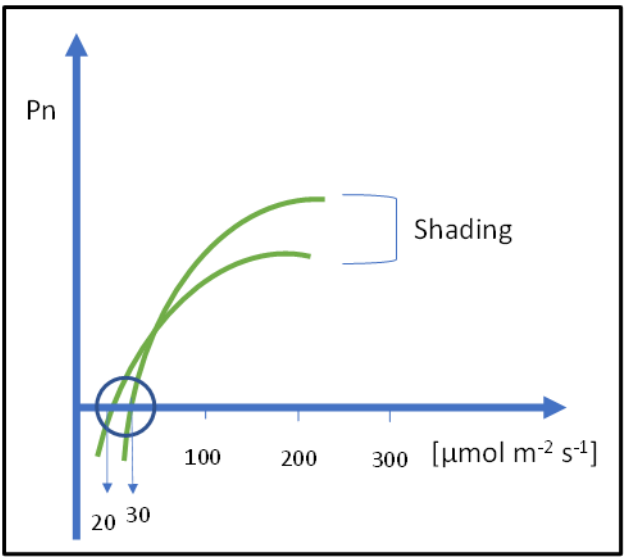


Figure 2.1. Schematic of light saturation curves and light compensation points lowered by shading treatments. Shading lowers the light compensation point from 30 to 20 mol m^{-2} s^{-1}. The lowering of light compensation point can require several days or weeks.

Plants tolerant to shade are typical of underbrush conditions (Table 2.4) the light compensation point from 30 to 20 µmol m^{-2} s^{-1}. The lowering of light compensation point can require several days or weeks.

Table 2.4. Effect of shadow on ornamental plant quality and traits associated to tolerance.

Target organs	Stress effects	Tolerance or adaptation response	References
Roots	Decrease of roots biomass	-	Zhao et al. 2012
Stem	Increase of stem elongation	Increase the firmness of cell walls	Zhao et al. 2012
Leaves	Reduction of thickness and leaf number	Increase the chlorophyll concentration and lowering the light compensation point	Lugassi-Ben-Hamo et al. 2010; Zhao et al. 2012;
Flowers	Reduction of flower number	Increase the flower longevity and turnover	Lugassi-Ben-Hamo et al. 2010

High and low temperatures can induce damages that compromise the ornamental value of plants. Temperature is an important environmental parameter that can induce speciation and affect plant distribution in diverse geographical areas. Plant growth and development are tightly correlated with temperature, and many species are synchronised with the environment for foliation and flowering. Each species has an optimal range of development; the minimum and maximum temperatures must be considered in the selection of plant species to use in certain regions or geographical areas. The temperature has a direct impact on primary and secondary metabolism. In an urban context, the reduction of growth is not a problem if there is any change of visual or external quality. In fact, slow growth can reduce the cost of management due to pruning. Unfortunately, the wrong ornamental plant selection exposed to low temperature can suffer cold stress or chilling injury during winter. On the contrary, plant species sensitive to high temperature, as well as for

low temperature, can also show some physiological disorders such as leaf abscission or senescence.

Cold stress can be dramatically deleterious depending on the phenological stage of plants. Deciduous ornamental plants are strongly tolerant to low temperatures during winter when they are in the dormant stage. In spring, if new vegetation appears early, eventual low temperatures can induce chilling injury. Based on temperature data recorded in recent years, it is possible to distribute plants in areas considering their sensitivity to low temperatures. The combination of ornamental species from woody trees, shrubs, and herbaceous plants can protect each other.

High temperatures during summer can negatively affect ornamental plant performance (Table 2.5).

Table 2.5. Effect of high or low temperature on ornamental plant quality and traits associated to tolerance.

Target organs	**Stress effects**	**Tolerance or adaptation response**	**References**
Roots	Decrease of roots functionality	Increase the roots uptake of ions	Franco et al. 2006; Francini et al. 2021
Stem	Increase or decrease of stem growth	Increase the firmness of cell walls	Vaid and Runkle 2013
Leaves	Increase of thickness, leaf necrosis	Reduction the chlorophyll concentration	Vaid and Runkle 2013
Flowers	Reduction of flower numbers	-	Khodorova and Boitel-Conti 2013

The negative effect of heat stress depends on the solar radiation intensity and duration. In summer, the temperature can rapidly

increase during the day, and the highest values can be observed from 12:00 to 2:00 pm. It can happen that ornamental plants can be exposed to high temperatures for 4–6 h per day during the hottest summer months.

The localisation and light exposure of plants in the urban environment can mitigate or increase high temperature stress. At physiological and biochemical levels, the tolerance of plants to high temperatures depends on the transpiration rate and the thermoregulation efficiency of plants (Wahid et al. 2007). At the molecular level, the tolerance of plants to heat stress is associated to the accumulation of heat shock proteins (HSPs) and genes encoded for the detoxification of ROS (Toscano et al. 2023).

2.5. Strategies to improve the shrub tolerance to abiotic stress

The improvement of shrub tolerance can involve the identification of new ornamental species among wild or spontaneous plants in specific geographical areas. These works involve genotype characterisation or agronomic strategies such as the use of biostimulants or mulching.

2.5.1. Plant species and/or cultivar choice

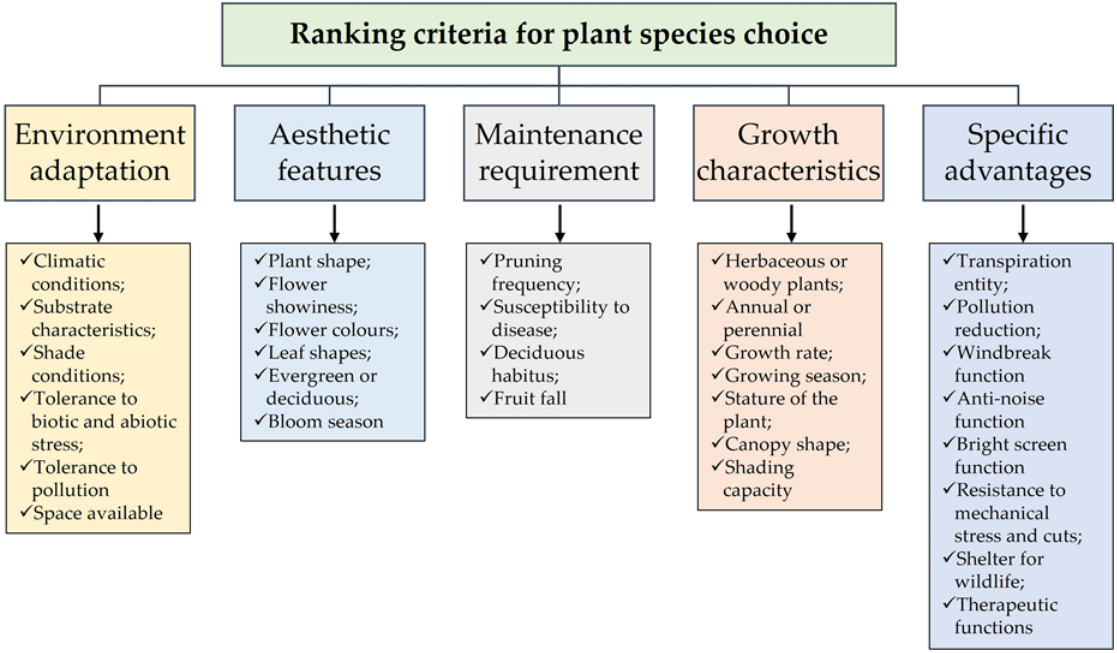
Due to their characteristics, woody plants, trees, and shrubs can ensure numerous ecosystem services. Plants in cities provide valuable ecosystem services, which can improve quality of life, but they also face numerous stresses, such as heat, salt, drought, extreme winds, and pests, which can reduce or cancel these benefits (Allen et al. 2010). In order to ensure ecosystem services, a key factor is plant selection to obtain more stable and resilient green infrastructures. The possibility of fully utilising the benefits of green infrastructures and their ecosystem services is reduced by the lack of technical criteria for the selection of plant species in urban areas (Vogt et al. 2015; Núñez-Florez et al. 2019; Farrell et al. 2022). As recorded by Capotorti et al.

(2020), it is essential to put 'the right plant in the right place'. There is not much research on the requirements and characteristics of ornamental plants in urban environments (McPherson et al. 2018). The lack of a clear reference framework (Ferrante et al. 2021; Toscano et al. 2022) leads to unsuitable choices and often an increase in the maintenance costs of green areas. The selection of plant species is, in fact, influenced by subjective elements, such as the availability of plants in nurseries or personal preferences, which determines the planting of species unsuitable for the environment and which, therefore, do not ensure the desired ecosystem services.

The choice of a single species can be done for urban parks on the basis of their adaptability to environmental conditions, their aesthetic and ecological values, their reduced maintenance requirements, and the advantages they can bring (Figure 2.2). The selection of appropriate species of ornamental plants becomes the key factor for the creation of a sustainable green space, i.e., a space that adapts well to the environmental conditions of the place (Asgarzadeh et al. 2014; Ghafari et al. 2020). In this context, it is essential to identify a diversified mix of woody species well-adapted to the conditions determined by climate change. The adaptation of the plant depends, in addition to the characteristics of the genotype, on the environmental conditions and the amount of care it will receive (Van der Veken et al. 2008); it should also be remembered that rates of climate change can be more rapid and extreme in cities than in rural areas.

Miller (1997) proposed a species selection scheme that included site (i.e., environmental and cultural constraints), social factors (i.e., aesthetics, functions, and disruptions), and economic factors (i.e., planting and maintenance costs).

Figure 2.2. Ranking criteria for plant species choice for sustainable green areas.



Roloff et al. (2009) focused on drought tolerance and cold hardiness as critical for the future survival of trees in a changing climate, based on the climatic conditions of the species' places of origin. This reasoning, although correct, must, however, take into account, in addition to the characteristics of the environments of origin and therefore the needs of the different species, the physiological plasticity of a plant, i.e., the range of habitats to which a species can adapt. It should also be taken into account that one stress can accentuate the severity of another (Toscano et al. 2021); higher temperatures, for example, increase the evapotranspiration demand and therefore drought stress, predisposing the plant to attacks by parasites (Dale and Frank 2014). In addition, salinity from recycled irrigation water or coastal flooding can adversely affect soil health and shrub growth (Farieri et al. 2016). Species with narrow tolerance ranges may be most adversely affected. Some shrubs, thanks to their plasticity, often appear more suitable than trees, which have a poor genetic ability to adapt due to their long life.

The complexity of choosing the correct plant species determines a reduction in the number of species used, considered more 'reliable'. However, the biodiversity of urban green spaces is important as it reduces the risks deriving from pests and diseases and from climate change and therefore improves the resilience of ecosystem services ensured by green infrastructures. To manage and enhance biodiversity, Santamour's proposed 10/20/30 'rule of thumb' (Santamour 1990) has been widely accepted, which states that urban forests should comprise no more than 10% of any particular plant species, 20% of any genus, or 30% of any single botanical family (Kendal et al. 2014).

Urban environments are capable of sustaining plant species biodiversity due to environmental and land cover heterogeneity, socioeconomic factors, and possible species introductions. Urban green spaces include many different types of habitats, from intact patches of native vegetation to highly constructed habitats such as

green roofs (Aronson et al. 2017). Improving our understanding of the contribution of urban biodiversity could enable green infrastructure to deliver the ecosystem services needed to sustain an urbanising global population.
A very important contribution to the biodiversity of the urban forest can be ensured by shrubs, due to their small size, which allows for the presence of numerous individuals within the green area, and their resilience to numerous biotic and abiotic stresses.

2.5.2. Agronomic tools and management plans

The tolerance of ornamental plants can be induced or activated by different applications of microbic and non-microbic biostimulants (Toscano et al. 2018b) or other strategies that can directly or indirectly reduce abiotic stress intensities.

2.5.2.1. Biostimulants and arbuscular mycorrhizas

Landscape plants are produced in nurseries in a relatively controlled environment. Upon transplanting to the landscape, they are subjected to high levels of post-transplant stress, caused by such factors as root loss, water stress, insects and disease, and soil changes (Koller 1977). Therefore, in the first period of establishment, it is essential to minimise stress for plants with the best growing conditions possible. In recent decades, globally, high temperatures, drought, salinity, and heavy metals are considered main stresses that have negative effects on plants. In order to overcome these stress conditions, plants implement various mechanisms (morphological, physiological, biochemical); to relieve these stressful conditions, biostimulants have been widely used in recent years. The use of biostimulants in agriculture has been emphasised, which are products that contain active ingredients or organic agents free of pesticides, capable of acting, directly or indirectly, on all or part of the cultivated plants, increasing their productivity. According to EU Regulation 2019/1009

(2019), biostimulants means a product stimulating plant nutrition processes, independently of the product's nutrient content, with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: (a) nutrient use efficiency; (b) tolerance to abiotic stress; (c) quality traits; (d) availability of confined nutrients in soil or rhizosphere. Biostimulants are products based on natural raw materials, such as hydrolysed proteins and amino acids from animal and plant by-products, microalgae and seaweed extracts, humic substances, plant extracts, and microorganisms (La Torre et al. 2016). Biostimulants are applied as a foliar spray and enhance plant growth; freezing, drought, and salt tolerance; photosynthetic activity; and resistance to fungi, bacteria, and virus, improving the yield and productivity of many crops. Unfortunately, new landscape plantings are often installed in settings with poor soil (e.g., heavy clay, low organic matter, and poor nutrition) and receive slight or no supplemental irrigation, which may decrease their chance of survival. Plants treated with commercial biostimulants including mycorrhizae, hydrogel, and/or biostimulants may be more resistant to such stressful conditions, necessitate less additional nutrients and irrigation, and have improved disease resistance (Newman and Davies 1987). Biostimulants reduce the need for fertilizers for the plants and increase their productivity and resistance to water stress since they act as a hormonal and nutritional increment (de Vasconcelos and Chaves 2020).

In shrubby plants, production can be improved by biostimulant application. Hibiscus (*Hibiscus* spp.) treated with commercial biostimulants showed an increase in gas exchange with higher photosynthetic activities (Massa et al. 2018). Hibiscus plants treated with hydrolysed substances obtained from green compost and a fraction of urban solid wastes (i.e., FORSU) showed an enhanced photosynthetic rate that turned into a higher relative growth rate and

biomass accumulation under optimal growing conditions (Massa et al. 2016).

Actiwave, a biostimulant based on carbon nitrogen, was utilised in the nursery for improving the rooting of *Camellia japonica* L. cuttings because the rooting stage in this species is long and requires more than three months if no rooting promoting treatments are applied (Ferrante et al. 2013). Camellia cuttings treated with Actiwave®, Valagro, Atessa (Chieti), Italy as a spray, showed a speeded uprooting and growth.

In a study on woody ornamental plants using *Lantana camara* L. treated with humic substances, the genetic analyses (MADS-box AGAMOUS-like) highlighted the relationship between the above substances and the activation of genes involved in plant flower and fruit development (Costa et al. 2008).

Arbuscular mycorrhizal (AMF) and humic substances are two of the seven main categories of biostimulants, which have been used separately in previous studies to improve plant nutrient uptake, growth, and development, as well as to enhance tolerance and resistance to abiotic stress and to promote soil structural stability (du Jardin 2015; Aalipour et al. 2020).

On gardenia (*Gardenia jasminoides* J. Ellis), a calcifuge woody plant, the effects on the growth of two strains of *Rhizophagus irregularis* (AMF) were analysed to evaluate the possibility to reduce phosphate fertilization. Under reduced phosphate fertilization, inoculation with arbuscular mycorrhizal fungi favoured the growth of gardenia plants, especially in the high-peat substrate (Papafotiou et al. 2021).

Many studies indicate that the symbiotic relationship between plants and the arbuscular mycorrhizal fungi (AMF) is a key factor in helping plants tolerate and/or resist abiotic stress. Yang et al. (2015), in a study conducted on *Robinia pseudoacacia* L. under lead stress, showed that AMF symbiosis had enhanced the physiological and biochemical properties of this woody plant via increased ROS scavenging capacity.

Earlier rooting of photinia cuttings was observed in plants treated with the rhizobacteria *Azospirillum brasilense* (Larraburu et al. 2007). In a study conducted by Loconsole et al. (2023) on *Lantana camara* L. and *Abelia grandiflora* (Rovelli ex André) Rehder, the treatments with Goteo®, (Goteo—Goactiv, UPL, Cesena, Italy) a commercial seaweed-based biostimulant, stimulated adventitious rooting and provided better rooting quality and shoot development of stem cuttings. Similarly, Rathore et al. (2009) showed that the plants of *Glycine max* (L.) Merr. treated with seaweed extract (prepared from *Kappaphycus alvarezii*) showed beneficial effects with improved vegetative growth.

The application of biostimulators commonly results in an increased concentration of photosynthetic pigments that are strictly connected with the plant's photosynthetic activity and carbohydrate levels (Abd-Elkader et al. 2022).

In this regard, Augé (2001) stated, also, that the positive effect of AMF relies mainly on the uptake and transport of water and on an improved uptake of nutrients, especially of available soil P and other immobile mineral nutrients, resulting in the hydration of plant tissues, sustainable physiology, and a clear promotion of growth. AMF have also been shown to regulate several plant growth-controlling processes both under normal and stressful conditions (Yand et al. 2015; Abd-Elkader et al. 2022). Arbuscular mycorrhizal (AM) symbiosis can also increase host resistance to drought stress, although the effect is not always predictable.

The positive effect of plant growth-promoting bacteria (PGPB) occurs through the activation of the 1-aminocyclopropane-1-carboxylate deaminase enzyme that reduces ethylene production and increases auxin concentration in roots (Glick 2014). Plant growth regulators (PGRs) act at very low concentrations to stimulate, inhibit, or otherwise modify plant growth. PGRs are commonly used for root induction and development in cuttings propagated in ornamental shrub

nurseries. Auxins are particularly capable of stimulating simultaneous and steady root formation.

2.5.2.2. Mulching

Mulching in urban areas has multiple functions. The mulch can be used for weed control, avoiding competition among ornamental plants and spontaneous species. The mulch can also be useful for reducing water loss from the soil and improving the water use efficiency of ornamental plants used in the green area. This barrier effect in reducing water evaporation has been studied in zinnia plants mulched with pine needles, volcanic stone (scoria), a plastic polyethylene layer, and wood chips (Kazemi and Safari 2018). Results showed that the water use efficiency of plants increased in plants mulched with plastic polyethylene and wood chips. Weed development was absent in plastic and wood chip mulching. The flowering periods were longer with a lower number of flowers in plants mulched with plastic polyethylene. In woody ornamental plants, such as *Tilia europaea* L. and *Aesculus* × *carnea* Zeyh., trees mulched with two organic mulch coarse composts derived from green material left after sifting (coarse compost) and pine bark were analysed in terms of growth or eco-physiological response (Ferrini et al. 2008). The two organic mulches increased the height increment and the transpiration of plants compared to the control treated with herbicide.

In a study performed using cobblestone, water permeable brick, pine bark, green waste compost, and living (turf grass) mulches on the growth of *Sophora japonica* L. in urban trees, results indicated that organic mulching improved soil fertility and physical properties, but no differences were found in the growth (Qu et al. 2019). Similar results were observed in tea olive [*Osmanthus fragrans* (Thunb.) Siebold]. Mulching used was inorganic, such as round gravel, and organic, such as wood chips, and manila turf grass demonstrated that organic mulches improved soil organic matter (Ni et al. 2016).

2.5.2.3. Association among different species

The combination of plants is an important parameter that must be carefully considered in urban areas to reduce management costs and avoid agronomic problems. The combination must be planned to have at least no negative interference among the plants at the roots and aerial parts. As for herbaceous plants, the combination of plants should be planned avoiding competition for nutrients or water at the root level. Plant density and species distribution should be done by placing plants with superficial and deep roots closer to exploit nutrients at different depths. The herbaceous plants and trees should be combined favouring the use of plants belonging to *Fabaceae* family that can provide nitrogen by symbiosis with *Rhizobium* that are able to fix the atmospheric nitrogen. This is important for avoiding the supply of nitrogen fertilizers and lowering management costs. Positive interaction can be also represented by the improvement of soil structure. Trees can improve water infiltration in soil with their roots, avoiding flooding, with benefits for species suffering in heavy and compact soils. In polluted soils, species that have high heavy metal uptake can reduce the concentration for sensitive species, avoiding the appearance of phytotoxicity symptoms.

Interactions among plants belonging to different species (interspecific) and among those belonging to the same species (intraspecific) shall be investigated in ornamental plants for combination in urban environments. Antagonistic species that produce allelopathic molecules should be planted at a minimum distance to avoid negative effects on the closer plants. All antagonism situations must be avoided because they can induce ornamental value losses such as leaf yellowing and stunted growth. In particular, it has been reported that *Lantana camara* L. showed allelopathic effects on several species (Kato-Noguchi and Kurniadie 2021), especially those that belong to *Liliaceae* family (Hussain et al. 2011). The allelopathic substances can

cause the death of trees or herbaceous species around the producing plants. Black walnut produces natural products that can induce the death of white pine (*Pinus strobus* L.) and red pine (*P. resinosa* Aiton), or black alder (*Alnus rugosa* Spreng.) and whips of white birch (*Betula* L. spp.) (Chick and Kielbaso 1998). However, further research studies are required for planning a better combination of ornamental plants and avoiding those that have antagonist effects.

At the areal part, the canopy of plants should be independent without branch crossing that can limit air circulation with an increase of disease incidence or insect infestations. The intersection of the branches can be avoided by increasing the planting distance.

High density induces higher maintenance costs, especially those related to pruning. The combination of plants with canopies at different heights can provide several positive benefits. In shade plants, the shadow of higher plants can show dark green leaves. The canopy of higher plants can protect the shorter ones.

2.5.2.4. Transplanting modalities

The transplanting modalities are crucial to assure the full establishment of plants. Information about this issue is wider for re-establishment of woody species in degraded environments than in urban green areas. The season of transplanting is crucial: in the Mediterranean climate, fall transplant before the rain season helps the establishment of the plants. The inability of container-grown seedlings to develop deep and well-structured root systems rapidly after planting out (Vallejo et al. 2000) hampers the plant's survival. Different tools to increase the root system's ability to capture and transport water efficiently are adopted, like arbuscular mycorrhizal (AM) symbioses (Caravaca et al. 2003; Green et al. 2005).

Drought stress imposed in the nursery phase can allow the seedlings to develop root biomass and branching (Green et al. 2005). Gilman et al. (2009) analysed the effects of different volumes and frequencies of

irrigation applied to the root ball, in view to understand their influence on canopy growth, plant health, survival, and attractive features, and found that irrigation frequency affected only one species (*Viburnum odorotissimum* Ker Gawl) among the shrub species analysed (*Ilex cornuta* Lindl. & Paxt. 'Burfordii Nana', *Pittosporum tobira* Thunb. 'Variegata'). Irrigation every four days with three litres was able to establish shrubs in north Florida, where rainfall occurs after planting. The survival or growth was not increased if applying more water volume or irrigating more frequently. Only a slight improvement of the aesthetical value of shrubs was obtained in the first year after planting with more intense irrigation.

To promote the establishment of some native Mediterranean shrubs (*Medicago arborea* L., *Quercus coccifera* L., and *Pistacia lentiscus* L.), alpha grass (*Stipa tenacissima* L.) was adopted. The grass significantly reduced photosynthetically active radiation and soil temperature, improving shrub survival near *S. tenacissima* than in the open areas. Predawn water potentials measured before and after the summer were significantly higher in the area with the alpha grass (Maestre et al. 2001).

Micro-catchment water harvesting (Reddy 2016) can also represent a solution in very dry climates to capture rainwater and improve soil moisture and vegetation establishment because it allows deep root development and reduces the mortality rate of shrubs (Ali and Yazar 2007). The slope length useful to capture water can be inserted by the plant composition of the green infrastructure.

In order to understand the influence of irrigation and organic mulching on the survival of shrubs after transplanting, Montague et al. (2007) analysed the gas exchange and growth of some shrubs [*Lagerstroemia indica* L. 'Victor', *Forsythia* × *intermedia* Zabel 'Lynwood', *Spiraea vanhouttei* (Briot) Zabel, and *Photinia* × *fraseri* Dress] placed in landscaped beds. After transplanting, the plants were irrigated twice a week, with a return of 100%, 75%, and 50% of the reference

evapotranspiration (ETO). Although the plants that had used mulch and greater quantities of water had a greater stomatal conductance, all the plants — the trial took place in Texas in a place with high temperatures (>32 °C) and low rainfall (26.3 cm) — survived, and they appeared healthy throughout the growing season, fully responding to their ornamental function.

2.6. Conclusions

Abiotic stress mitigation can be achieved by a multi-action approach, such as agronomic management and suitable ornamental genotypes. In a short period, the use of biostimulants, mulching, or appropriate species combination could provide positive effects on abiotic stress mitigation, especially in urban and peri-urban areas. Beside the agronomic strategies, the selection of tolerant species against the different abiotic stresses could provide positive effect on urban environments and human welfare. Wild or underutilised species can be opportunely selected for improving or preserving the ornamental quality.

Further studies must be carried out for understanding the ornamental plant interactions and responses under abiotic stresses in urban environments.

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3. Which plant species for green roofs in the Mediterranean environment?

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Which plant species for green roofs in the Mediterranean environment?

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Abstract
In recent years, owing to intense urbanization and global change with the consequent extreme climate effects, interest in green roofs, even extensive ones, in the Mediterranean environment has increased. To this end, the choice of plant species is crucial because, owing to the identification of the most suitable plants, it will be possible to expand this type of green infrastructure and increase its ecosystem services in the urban environment. In this context, the objective of the review, through a critical analysis of some of the references on the topic, is to identify suitable criteria for plant species selection that are simple to apply and able to respond to the need to have plants capable of surviving, ensuring a suitable aesthetic effect, and providing essential ecosystem services. We also investigated whether, and to what extent, associations of different species can better adapt to the difficult environmental conditions of Mediterranean green roofs. Two possible strategies to identify the plant idiotype were analyzed: the analysis of plants present in habitat analogues or the identification of morpho-functional characters capable of discriminating the response to abiotic stress, and in particular to drought stress. The use of plant

communities, rather than a single species, seems capable of improving aesthetic effects, plant survival, and ecosystem services.

Keywords: plant species selection; ecosystem services; plant species survival; plant community; aesthetic effects; extensive green roofs

3.1. Introduction

The growth of the global population (in 2022, 8 billion people on Earth were exceeded) and the rate of urbanization (the UN Urbanization Statistics in 2018 (Urbanization Statistica UN 2018) estimate that over 60% of the world population lived in urban areas and that by 2030, the number of megacities with more than 10 million inhabitants will be equal to 43) indicate that the problems linked to cities have become central to the quality of human life. In fact, overpopulation determines that cities have become heterotrophic organisms with their own metabolism (Odum 1983), which bases their growth and expansion on the indiscriminate use of resources (energy and raw materials, often non-renewable), favored by the proliferation of means of transport and supported by industrial development and today's technologies. Urban agglomerations return heat and pollution to the environment, alter bio-geo-chemical cycles, and cause irreversible loss and fragmentation of natural habitats (Fischer and Lindenmayer 2007). Furthermore, cities not only consume the resources immediately available within their physical boundaries, but also have a pervasive effect on large areas, linked to the production of commercial goods and services necessary for their sustenance and development. To mitigate the failures of intense urbanization, a fundamental role for the environmental quality of the city is assured by green infrastructures and ecosystem services (Francini et al. 2022). Green roofs play an important role among the green infrastructures. Since building roof surfaces cover 20–25% of urban areas (Abass et al. 2020), green roofs can effectively contribute to increasing ecosystem services (reduction in air temperature,

interception of rainfall, reduction in pollution, etc.). This contribution varies among the different types of green roofs, which, based on the height of the substrate and the level of maintenance requirements, can be divided into extensive (height of the substrate often less than 10 cm, cheaper, weight approximately 60–150 kg m^{-2}), semi-intensive (15–30 cm, expensive, weight 25% above or below 150 kg m^{-2}), and intensive (>30 cm, very expensive, weight 200–500 kg m^{-2}) (Abass et al. 2020).

Vegetation can ensure the long-term effect of a green roof, also through its evolution, as a result of interactions with the environment and between the different species used. Therefore, the identification of the most suitable plant species is one of the most important aspects of green roofs, which involves green roof designers, who must evaluate numerous parameters and individuate the advantages and disadvantages of possible plant species choices. In recent years, ecological aspects have become more prevalent than aesthetic aspects in the selection of plant species. The positive environmental effects of these types of green infrastructure in urban areas have been highlighted above all (Snodgrass and Snodgrass 2006), which strongly influence the same criteria for choosing plants. Green roofs are considered indispensable ecosystem structures for cities, and are capable of ensuring heat island mitigation, temperature balancing of buildings, better management of water runoff, and greater urban biodiversity (Arabi et al. 2015; Shafique et al. 2018). Based on the function that is considered prevalent, the selection criteria change, sometimes significantly; therefore, it is necessary to be well aware of the objectives that underlie the identification of different species (Snodgrass and Snodgrass 2006).

3.2. The environmental conditions of green roofs

It is often thought that a green roof is a simple extension or continuation of the vegetal landscape found at ground level (Burke 2003), but this is not true because the microclimatic conditions on green roofs are profoundly different; not considering this aspect can lead to high plant mortality (Butler et al. 2012). Green roofs are difficult environments for plants, which must cope with shallow substrates, poor availability of nutrients and water, high solar radiation, and high pollutant levels (Aguiar et al. 2019).

Green roofs are a useful solution for covering private (terraces, roofs, and garages of residential complexes), public buildings (offices, industries, and shopping centers), or other elements that, in an urban environment, are preferred to be hidden. In all of these cases, the area explored by the root systems of the plants is strongly limited by the narrowness of the available substrate, particularly in depth. The approach used for the choice of plant species for green roofs could be compared, given the similarity of the conditions, with many situations of urban forestry, such as the same street trees in which the root systems of the plants suffer similar limitations in their development. The main consequences determined by this critical factor are different, and among the most influential:

- limited growth of vegetation, determined by reduced substrate depth and rapid loss of humidity;
- difficulty anchoring plants with larger sizes caused by the reduced expansion of the root systems;
- inadequate drainage caused by substrate compaction.

The survival of plants on green roofs is related to different factors that modify plant physiology and growth, such as solar radiation level (Getter et al. 2009), air quality (Francini et al. 2022), and substrate characteristics (Olly et al. 2011).

While shading in most natural habitats reflects the structure of vegetation, shading in urban environments also reflects the

heterogeneity in buildings and other built structures (Cadenasso et al. 2007). Consequently, the solar radiation reaching the plants can vary both within and between green roofs and can affect the survival of the plants as well as the temperature of the substrate. The effects of building structures can be exerted on the deposition of particulate matter (Cohen et al. 2004), altering growth medium conditions and plant survivorship. Although the conditions of the green roof substrate may change over time, its composition and depth, at least in the initial phase, are designed to satisfy both the construction aspects and needs of the dominant vegetation on the green roof (Green Roofing Guideline 2018). Therefore, differences in survival among plant species can be caused by abiotic factors associated with the design of a roof (for example, composition and depth of substrate) and by those connected to green roof sites due to the heterogeneity of the urban landscape (Aloisio et al. 2017).

Although natural soils are typically replaced by engineered substrates owing to their high weight, they can provide important ecological advantages for green roof systems, thereby increasing habitat vitality. Even when they cease to be biologically active, natural soils can still benefit from green roofs by mimicking the mineral properties of their natural habitat. Problems related to the infiltration of fine particles, the increased load on roofs, and the impossibility of predicting their biological activity limit their use on green roofs (Best et al. 2015). In addition, the compost substrate, owing to organic matter, can promote plant growth and help plants overcome the harsh conditions of a green roof (Dunnet and Nagase 2010; Papafotiou et al. 2018). The positive effects of deeper substrates on plant growth on extensive green roofs have been shown in various studies (Eksi et al. 2017; Papafotiou et al. 2018) and were mainly attributed to increased water-holding capacity. The characteristics of the cultivation substrates are able to promote urban biodiversity and counteract habitat loss. Schradera and Böning (2006) analyzed 10 green roofs of different ages (“old roofs” were

built between 1990 and 1994 and "young roofs" between 1998 and 1999) in Germany, and found that the old roofs environment is more stable due to the evolution of the soil and the greater presence of collembolans. Differences in species richness were small, but species diversity appeared to be dynamic over time, resembling that of recently reclaimed areas. A specific type of urban soil, called edifisols, is formed on buildings as a result of early and relatively natural soil formation processes occurring on artificial substrates (Charzyński et al. 2015). Due to organic pollutants, plant residues, and bird droppings, a new type of humus, techno humus, is formed on these often ephemeral and young edifisols (Markiewicz et al. 2018).

3.3. Objectives of the plant species choice

The choice of plant species in relation to the type of green roof is strictly linked to the objectives you intend to achieve that vary from the simple survival of the plants to the improvement of the aesthetic effect, the reduction in costs and, above all, the achievement of particular ecosystem services.

3.3.1. Plant survival and cost reduction

Plant survival on green roofs is a key parameter in the choice of plant species and clearly influences maintenance costs. Succulent plants, often characterized by the CAM photosynthetic cycle, represent the most used group of plants on green roofs (Oberndorfer et al. 2007) and are suitable for growth and survival in xeric environments, which are typical conditions of green roofs (Beattie and Berghage 2004). For example, the survival of forbs and grasses is typically lower than that of drought-resistant succulent plants (Butler et al. 2012), and among herbaceous plants, forbs often have a lower survival rate than grasses, at least in the natural environment (Luenroth and Adler 2008). Plant species with C4 photosynthesis, which are more adapted to thermal and water stress than those with C3 photosynthesis (Sage 2004), can

ensure greater survival on roofs in hot and dry climates. Furthermore, compared with succulents, which often form a low layer of vegetation, forbs and grasses generally have a taller stature (Olly et al. 2011), although this parameter can vary considerably between different plant species. Greater plant height and coverage of herbaceous plants could influence both the interactions between plant species mixtures and the consequent positive ecosystem functions (Cook-Patton and Bauerle 2012). Positive interactions can be observed between plant species (Bertness and Callaway 1994). For example, it has been analyzed how a greater sward height can increase the survival rate of plants on green roofs, owing to the reduction in the temperature of the soil and increased biodiversity (Stewart et al. 2001). Therefore, plant survival depends on the composition of the plant species, characterized by different growth habitus (Lundholm et al. 2010); biotic and abiotic factors (e.g., light radiation, temperature, and substrate characteristics) interact to influence plant survival (Heim et al. 2014; Aloisio et al. 2017).

In the absence of irrigation, the selection and survival of plants depends mainly on the substrate depth of the green roof system (Dvorak and Volder 2010). In semi-arid regions, roofs with higher substrate depths may be required, ranging from 15 cm for succulents to over 30 cm for grasses and herbs (Williams et al. 2010). However, a compromise must be found between substrate depth and weight because building structures often cannot support excessive weight loads (Benvenuti and Bacci 2010). The inclusion of a water retention layer, in which the water available to plants is stored for a longer period, could be a valid solution (Dvorak and Volder 2010). Furthermore, placing the green roof in a more sheltered position reduces the rate of evapotranspiration and helps keep the substrate moist for a longer period (Getter et al. 2009). Solar radiation exposure influences the floral quality of green roofs in terms of vegetative

cover, plant species richness, and plant species composition (Van Mechelen et al 2015).

To identify plants capable of surviving on extensive green roofs in a Mediterranean environment, the inclusion of native plants has been proposed with positive results, even if it is not always possible to match the survival ensured by non-native species of the *Sedum* genus (Durhman et al. 2007; Latocha and Batorska 2007). The choice of native plants, however, should not be made based only on the fact that these plants are adapted to the climatic conditions of the site (Dunnet 2006) because the conditions in which they evolved, and above all the depth and characteristics of the soils, are very different from those of the substrate used in green roofs, which are generally shallow and well drained. Some strategies implemented by native plants, such as deepening the root system to cope with water shortages, are not applicable when there is just over 10 cm of growing substrate. Therefore, it is necessary to pay attention to plants originating from habitats characterized by shallow and well-drained substrates, such as rocky or ruderal habitats; in the communities that settle in these contexts, there may be plant species suitable for extensive green roofs, in particular.

The use of native plant species on green roofs, especially in the Mediterranean area, is also limited by the lack of references regarding their performance and necessary cultivation care. Furthermore, the seeds of native plant species do not simply bud on rooftops (White and Snodgrass 2003).

The identification of plants with characteristics and physiology suitable for the toleration of stress can significantly reduce irrigation needs and related maintenance costs, a particularly important aspect in a climate, such as the Mediterranean climate where water is scarce in summer (Brandão et al. 2017).

3.3.2. Aesthetic effects

To achieve interesting ornamental effects, it is best to aim for the presence of a plant community rather than a single species. *Sedum* monocultures, for example, are widely adopted in extensive greenery, and have a monotonous effect (Dunnet and Nolan 2004), which is not always pleasant. Furthermore, similar to agricultural monocultures, the presence of a single plant species increases the risk of parasitic attack. Abscission of leaves in response to drought, typical of many native Mediterranean species, can improve the response to water stress (Toscano et al. 2019); however, from an aesthetic perspective, it is an aesthetically unpleasant adaptation.

Obtaining a space of high aesthetic value is an important ecosystem benefit provided by green roofs (Fernandez-Cañero et al. 2013), although this is not always recognized. The contribution of green roofs to stress reduction in modern cities was analyzed (through the psychological and physiological parameters of heart rate variability and systolic blood pressure). Based on these results, it was observed that a space with an open and structured vegetation design that includes both grass and shrubs may have more potential for stress reduction than a monotonous vegetation model; the increase in vegetation biomass was not necessarily linked to higher psychological and physiological benefits (Lee et al. 2023).

Several studies have suggested that future research on plant breeding should better consider and quantify plant architecture and shape, flowering duration, variation in color and vegetation throughout the year, biomass production, and how irrigation and other cultivation techniques influence the vegetation and aesthetic performance of green roofs (Benvenuti and Bacci 2010; Nagase and Dunnet 2010).

3.3.3. Ecosystem services

There are numerous ecosystem services provided by green roofs. Among the main and typical ones, there is rainfall interception and

prevention of flash floods in urban areas, consequently decreasing the load on sewer systems. To this end, vegetation plays an important role because it significantly influences the moisture content of the substrate and runoff rate (Stovin et al. 2015; Abuseif 2023). The reduction in runoff occurs through various processes such as water interception, plant transpiration, root absorption, retention, and storage of water in plant tissues (Stovin et al. 2012). Plant water consumption is determined by transpiration capacity, whereas root biomass influences its ability to store water (Nagase and Dunnet 2012). Increasing the plant cover on a green roof improves rainwater retention (Berndrsson 2010); however, species richness does not significantly affect water storage unless diverse plants are characterized by higher water consumption (Monterusso et al. 2004). Beyond the choice of plant species, there is no doubt, however, that an element that influences the interception of water is the depth of the substrate: Soulis et al. (2017), using three species with different characteristics—*Sedum sediforme* (Jacq.) Pau, a succulent plant, *Origanum onites* L., a xerophyte plant, and *Festuca arundinacea* Schreb., a turfgrass—noticed how the total runoff reduction (%) for the entire study period was equal for the three species to 50.8%, 63.6%, and 54.9% with a substrate depth of 8 cm, and to 60.3%, 81.1%, and 68.8%, respectively when the substrate was 16 cm deep. However, it should be emphasized that at the same depth of substrate, xerophyte species were the most efficient in intercepting rainwater.

The amount of runoff can be reduced by increasing the number of plant species with different characteristics (Riven and Mulder 2005). The presence of taller species can lead to a higher rate of water interception and evapotranspiration, whereas the presence of shorter plants in contact with soil determines the presence of a layer of humidity beneath the canopy. The capacity to store water changes based on the characteristics of the plant (orientation and size of the leaves, roughness and hydrophobicity of the bark, rigidity of the

branches, and density of the foliage). Plant community structure can also influence water interception (Nagase and Dunnet 2012). To this end, the characteristics of the root system are important because water retention in the substrate is influenced by the root structure. Plants that form dense and fibrous roots, such as grasses (Nagase and Dunnet 2012), reduce the porosity of the substrate and the volume available to retain water (MacIvor and Lundholm 2011), thus proving to be more efficient in reducing rainwater runoff than succulent or forb species. Evapotranspiration is the other plant-related process that influences runoff production (Lundholm and Richardson 2010). Species with high evapotranspiration rates that reduce water from the growing medium create more space for the capture of water in subsequent rain events (MacIvor and Lundholm 2011).

Another ecosystem function of green roofs is the increase in biodiversity; green roofs can also be used as an urban space where plant biodiversity can be preserved (Dunnet and Kingsbury 2008). Higher plant species biodiversity can make extensive green roofs attractive to native arthropods and avian species (Wolf and Lundholm 2008). Having different plant species, especially in extensive green roofs, may allow them to adapt to variable moisture conditions and maximize the evaporative cooling benefit, thus extending the benefits of green roofs (Compton 2006).

Plants absorb a significant percentage of solar radiation through their biological functions such as photosynthesis, transpiration, respiration, and evaporation (Vijayaraghavan 2016). Similarly, it has been suggested that the presence of different plant species with lower reflectance to increase diversity would only reduce the albedo of green roof (Cook-Patton and Bauerle 2012). Therefore, the choice of plant species has an important effect on temperature (Bevilacqua et al. 2015). It has been suggested that plant characteristics, such as height, leaf area index, and plant responses to drought, greatly influence the thermal insulation capacity of green roofs (Raimondo et al. 2015).

Plants with a high canopy density and height can provide greater shading and cooling via transpiration, suggesting that plant phenology, plant water consumption, and their interactions are key points for understanding seasonal differences in the relative contribution of each plant, especially for roof thermal performance (Bevilacqua et al. 2015).

The greater thermal regulation capacity of vegetated roofs compared with non-vegetated roofs has been largely attributed to the shading effect caused by plants or greater evapotranspiration (Blanusa et al. 2013). Since these two factors are not always directly related, the selection of plant species and adoption of models to facilitate this selection are complex. In this regard, Azeñas et al. (2018) highlighted how *Sedum sediforme* (Jacq.) Pau, a species that consumes little water and forms a dense, multi-layered foliage of fleshy leaves, has a higher thermal regulation capacity than *Brachypodium phoenicoides* (L.) Roem. & Schult., a herbaceous species with higher transpiration rates than *Sedum* sp. This suggests that canopy architecture, leaf anatomy, and spatial arrangement of leaves would have a greater effect on thermal regulation than the transpiration capacity of plants. Although both species show similar surface coverage, *B. phoenicoides* has erect leaves that would allow for the passage of a greater quantity of incident radiation compared to *S. sediforme*, a species that presents creeping behavior, with maximum development and flowering during the warm season. If shading has positive effects on summer thermal regulation, it has negative effects in winter because it reduces the temperature. Nevertheless, the performance of the plants must always be considered based on the climatic conditions of the site. In fact, some models have indicated that taller plants have a greater insulating effect in Mediterranean European cities due to the height of the foliage and higher LAI values (Ascione et al. 2013). However, these models usually do not consider the differences in leaf anatomy and canopy architecture, which affect the thermal regulation capacity of plants.

Blanusa et al. (2013) analyzed different types of plants (a mixture of *Sedum*, *Stachys byzantina* K.Koch, *Bergenia cordifolia* Sternb., and *Hedera hibernica* Carrière) to evaluate whether and to what extent the plants differ in their "cooling potential". They wanted to understand whether leaf morphology influenced leaf temperature, and how the substrate water content altered this response. The relationship between the temperatures of the leaf surface and those of the air immediately above the canopy and the influence of the type of plant on the temperature of the substrate below the canopy (i.e., potential to provide aerial cooling) were also studied. It was found that *S. byzantina* offered the best results in terms of cooling the leaf surface (even in the drying substrate, e.g., 5 °C cooler than *Sedum*), substrate cooling under the canopy (up to 12 °C), and air above the canopy (up to 1 °C, when soil moisture was not limited). Based on these results and the significance of the reduction in temperature, the authors suggested that the choice of plant species on extensive green roofs should not be anchored only to the survival of the plants, but should also consider the contribution in terms of ecosystem services. Plant selection should therefore be based not only on "what survives", but also on "what provides the best ecosystem service" (Blanusa et al. 2013).

The effectiveness of the temperature reduction is also a function of the height of the plants, and the highest temperature reductions were obtained with plants 35 cm tall, followed by those 15 cm tall, and then by those 10 cm tall. Plants with green leaves are more efficient at reducing temperatures than those with purple/red leaves (Liu et al. 2012).

Another ecosystem service is the ability of the green roof to control and reduce sound pressure (Azkorra et al. 2015); the reduction in the noise level can reach 8–10 dB (Abass et al. 2020) in relation to the vegetation layer characteristics.

Finally, we cannot forget that green roofs can be used as supplements for other sources of food production in urban areas. Urban agriculture carried out on roofs can improve numerous ecosystem services, increase biodiversity, and reduce food insecurity (Chowdhury et al. 2020).

3.4. Criteria for plant species selection

The selection criteria, or rather the idiotype of plant species or plant community, change for extensive or intensive green roof; for the first, plant species that tolerate water deficit in the substrate are recommended, with a low and prostrate habit (to obtain good coverage), evergreen foliage, and a long flowering season. However, in intensive green roofs, it is possible to use a wide range of plants, including shrubs and trees, thus increasing the ability of plant cover to reduce pollution and improve air quality (Rowe 2011). It must be taken into account that fertilization, carried out in an intensive green roof to promote plant growth, makes plants more vulnerable to drought (Rowe et al. 2006). The use of numerous plant species to improve biodiversity favors vegetation establishment (Lundholm and Richardson 2010).

The selection of plants must consider various aspects (Table 3.1), such as weight tolerated by the structure, plant cover, level of maintenance, and tolerance to the difficult environmental conditions that occur in green roofs, such as high exposure to the sun, shallow growing substrates, limited water availability, increased wind speed, and prolonged drought periods (Chow and Bakar 2018).

Table 3.1. Key traits of plant species suitable for green roofs in Mediterranean environment.

Ecological	Ecosystem Service	Morpho-Biometric	Aesthetic	Physiological	Cultivation
- plant diversity and symbiosis; - native origin; - competitive or ruderal Grime's strategies.	- good rainfall interception; - good dust capturing ability; - resistant to pollution and capable of absorbing or retaining pollutants; - reduction in noise level; - thermal regulation; - increase biodiversity.	- low plant height with cushion forming habit; - shallow and spreading roots; - root system adapts to limited substrate depth; - leaf shape and orientation; - high LAI value; - structure wind tolerant; - low shrubs, forbs, grass, groundcover plants, geophytes, and climbing plants.	- plant shape and aesthetic appearance; - relatively stable growth rate and high ornamental value; - high ground cover; - establish quickly; - long flowering season; - variation in color and vegetation throughout the year.	- succulent leaves with ability to store water; - certain degree of succulence of roots and stems; - flooding tolerance; - tolerance to high/load temperature; - drought tolerance and ability to utilize excess water; - tolerate extreme environmental conditions; - CAM or C4 photosynthesis; - ability to adapt the transpiration rate to environmental conditions; - plasticity in the use of water; - light-loving plants.	- easy to transplant; - short period of establishment and fast reproduction; - have been used locally and commonly; - have been successfully introduced; - simple maintenance and with a slow growth rate, and low nutrient requirements.

Species of the *Sedum* genus, which are succulent groundcovers, are widely used in green roofs because they can survive in difficult environments (Villarreal and Bengtsson 2005); however, some researchers suggest favoring native plant species due to their high adaptability to local climatic conditions (Chow et al. 2018). To expand the list of plants suitable for green roofs, it is possible to include species that live in habitats similar to those found on roofs, such as coastal dunes and rocky outcrops exposed to high temperatures and shallow soils (Lundholm 2006). According to Balachowski and Volaire (2018), it is good to consider both the climate of origin and the characteristics of the plant when choosing the species, as the former indicates the environment in which a plant is adapted, but the latter allows for predicting the probable ecological strategies that the plant will use in response to resource limitation (Chu and Farrell 2022).

There are different approaches to selecting plants suitable for green roof environments: they can be based on the characteristics of the plants, through a morpho-physiological and ecological approach (Nagase and Dunnet 2012; Caneva et al. 2015; Lundholm 2015), or on the identification of natural habitats that express conditions similar to those of green roofs (Lundholm 2006; Lundholm and Walker 2018). Some physiological approaches have tested the ability of plants to tolerate drought and utilize excess water when present, as these strategies are functional for selecting plant species for green roofs where stormwater management is relevant (Farrell et al. 2013). Another strategy is based on climatic characteristics of the area of origin. Climate is the key determinant of large-scale ecosystem composition and structure, and temperature and precipitation significantly influence plant species distribution (Birks and Birks 2014). Aridity indices, such as the heat moisture index (HMI) (Du et al. 2019), can be useful because they represent the aridity of the environment and consider the interaction between temperature and

precipitation (Dobbs et al. 2014). Plants from climates with a higher HMI tolerate drier and hotter conditions, which may indicate greater tolerance to water deficit conditions (Aspimwall et al. 2013). Shrubs can also be very drought tolerant, for example, those from dry rocky habitats have been found to survive water-deprived conditions when placed in a green roof with a shallow substrate (Farrell et al. 2013). Although several studies have analyzed the drought tolerance of shrubs in arid and semi-arid habitats (Papafotiou et al. 2013; Raimondo et al. 2015; Savi et al. 2016), no studies have evaluated shrubs based on physiological approaches and climate of origin. Regarding stormwater retention, ideal shrub species for green roofs should have high evapotranspiration under well-irrigated conditions, but tolerate water deficit conditions, as attested by a more negative midday water potential (Farrell et al. 2013). This strategy has been demonstrated for monocotyledonous and herbaceous species from rocky outcrops, which showed high water use under well-irrigated conditions and drought tolerance by reducing water potential under water-deficit conditions. However, it is unclear whether this combination of high water use and high drought tolerance also exists in shrubs because drought tolerance is often negatively correlated with transpiration rate (Tardieu 2005).

3.4.1. The idiotype of plant species

Several methods have been proposed to select plant species on a green roof, such as the "habitat template" approach proposed by Lundholm and Walker (2018). This approach is based on the concept that artificial ecosystems can be modified to present conditions similar to those of a natural habitat so that plants living in that environment are able to adapt better to green roofs (Lundholm and Richardson 2010). However, another approach comprises selecting a certain characteristic of the plant that appears strategic (Van Mechelen et al. 2014; Caneva et al. 2015; Ksiazek-Mikenas and Köhler 2018), such as

the size of the plant, aesthetic value (Benvenuti and Bacci 2010), or resistance to abiotic stress (Toscano et al. 2020). The key assumption of the latter approach is that abiotic stresses are critical factors that limit plant growth and survival, particularly in green roofs.

Another factor that influences the plant selection process is canopy structure. Plants with a mostly horizontal leaf distribution and/or extensive foliage development should be selected to reduce the transmission of solar radiation. Other important factors include growth rate, nutrient requirements, and sensitivity to pollution. In addition to drought, plants on green roofs must tolerate intense radiation and high temperatures in both air and substrate. In particular, high thermal levels in the root zone can have detrimental effects on plant physiology, growth, and biomass (Liu and Huang 2005). Savi et al. (2016) reported a significant positive correlation between the root vulnerability to heat stress and plant mortality. Ideal plants should be drought, heat and wind tolerant, pest resistant, light loving, have low height, good coverage, and shallow root system (Xiao et al. 2014).

It is advisable to favor plants that express the following characteristics: ease of transplant, simple maintenance, slow growth rate, resistance to pollution, and capability to absorb or retain pollutants. Plants must be able to survive even in the presence of large quantities of water, because there is no good drainage system on some roofs (Xiao et al. 2014). Being taller, trees are more exposed to the action of wind than other types of plants; the size of the canopy and leaves influences their susceptibility to wind; therefore, it is often necessary to provide anchoring (Getter and Rowe 2006).

Ideal plants for extensive green roofs must be established quickly, provide high ground cover, tolerate extreme environmental conditions, and adapt to limited substrate depths (Durhman et al. 2007). Therefore, it is not surprising that succulents are among the most intensively studied taxa, as they have shallow root systems, high water use efficiency, and are able to tolerate the extreme conditions

found on extensive green roofs in Northern regions with growth substrates of 7–10 cm, but also 5 cm. The *Sedum* genus has proven to be very reliable, as it has high drought tolerance. Many species tolerate up to one month without precipitation and some *Sedum* species have survived for up to four months without water in greenhouse experiments in the Great Lakes region of the United States (Durhman et al. 2006). The genus *Delosperma* also appeared to be suitable and *D. cooperi* (Hook.f.) L.Bolus and *D. nubigenum* (Schltr.) L.Bolus proved to be suitable in many regions (Butler and Orians 2009). Some perennial grasses and herbaceous flowers also have effective adaptive measures to cope with drought. The answer depends on climatic conditions and substrate depth; with substrates of 15 cm or more, many native herbaceous plants survive in northern climates without irrigation (Lundholm 2009).

Geophytes can survive periods of drought thanks to the accumulation of water in underground organs, such as bulbs and rhizomes (Nagase and Dunnet 2013). Especially in a Mediterranean climate, therophytes appear promising because their short life cycle allows them to spend the summer months dry as seeds and to germinate again after autumn rain (Van Mechelen et al. 2014).

Green roofs are often installed to reduce urban stormwater runoff; to achieve this, plants must use water when it is available, but reduce transpiration when water itself is in limited supply. Succulent species often fail to achieve these goals. To this end, Farrel et al. (2013) observed the behavior of species found in granite outcrop environments to evaluate the water use strategies they implemented under contrasting conditions of water availability. The investigated species exhibited good plasticity with respect to water use. The authors developed a conceptual model using physiological traits to select species suitable for green roofs. Ideal plant species are those that use high quantities of water and can tolerate drought simultaneously. In particular, the parameters analyzed were (i) water use strategy under

well-watered conditions (WW), (ii) status rate of water use under water-deficit (WD), and (iii) maintenance of water under WD conditions. Suitable plant species are those with high use of water under conditions of good water availability, which are therefore able to reduce the runoff of rainwater and maintain a good water status under conditions of water deficit. Species with slow rates of water use under water deficit conditions are more tolerant to drought.

In the study, carried out in Australia, in contrast to the succulent (*Sedum pachyphyllum* Rose), used as a control, all the species of the granite outcrop showed plasticity in the use of water; however, the most drought-resistant species proved to be the four monocotyledons [*Arthropodium milleflorum* (Redouté) J.F.Macbr., a geophyte, *Stypandra glauca* R.Br., *Dianella admixta* Gand., *Lomandra longifolia* Labill., hemicryptophyte grasses] and the flowering herbaceous plant, also hemicryptophyte, *Isotoma axillaris* Lindl., present in the surface depressions on the granite outcrops. This was achieved through reduced transpiration and/or a high root mass fraction (RMF), proving that high water users can also be drought resistant, making them the most suitable plant species for green roofs. All species analyzed had a certain degree of root, stem, or leaf succulence. Therefore, plant selection for green roofs should prioritize these traits in non-succulent species. Shrubs, such as *Grevillea alpina* Lindl. or *Correa reflexa* Vent., present in these habitats, are generally found in deeper depressions in the soil or in cracks (Biedinger et al. 2000) and can avoid water stress by accessing water stored deeper in the underlying rocky substrate (Schwinning 2010). This strategy, which is useful for granite outcrops, is not effective for green roofs with shallow substrates; therefore, the high water potential and anisohydric response of shrubs to water deficit is a risky strategy (Farrell et al. 2013). Other selection criteria, such as plant shape and aesthetic value, can be considered, although an approach based on the physiological response to water availability also has great potential to

improve the ecosystem services provided by plants (Farrell et al. 2013).

Lundholm et al. (2015) used four characteristics (height, individual leaf area, specific leaf area (SLA), and leaf dry matter content) to evaluate whether these characteristics could predict adaptability to green roof conditions and ecosystem services. Six indicators were considered for the latter: thermal, hydrological, water quality, and carbon sequestration functions. Species average height and SLA were the most useful traits for predicting several services via their effects on canopy density or growth rate. The characteristics of plants, which are easily measurable, can therefore be used to select species suitable for optimizing the performance of green roofs, as well as from an ecosystem service perspective (Lundholm et al. 2015).

3.4.2. Choose the plant species or the plant community?

Although it might be tempting to use only the optimal plant species for green roofs, it is better to use plants of different biological forms and, therefore, different drought tolerance strategies to ensure both better performance and resilience to stress (MacIvor et al. 2011).

The performance of green roofs, in fact, could be improved by expanding the range of plant species currently used (Lundholm et al. 2010; Nagase and Dunnet 2010; Butler and Orians 2011). Plant diversity, in terms of both life form and drought tolerance, can improve ecosystem functioning, due to the complementarity of performance (Lundholm et al. 2010; Van Mechelen et al. 2015). Numerous studies underline the importance of biological interactions between different plant species that influence the survival and growth of plants; therefore, it is preferable, when choosing the plant species, to refer to the entire plant community rather than to a single plant species (McIntire and Fajardo 2014). Species composition of plant communities is determined by their interactions with biotic and abiotic factors. Historically, abiotic factors (soil moisture, temperature,

nutrients, and pH) were thought to have a more significant influence on the establishment and survival of plant species, and the role of biotic interactions, whether negative (competition) or positive (facilitation), was underestimated in plant development and in the coexistence and structure of plant communities (Grime 1973; Callaway 1995).

Sedum species can facilitate the survival of non-succulent plants by reducing high soil temperatures during drought periods (Butler and Orians 2011). Their particular photosynthetic pathway (CAM) and succulent leaves allow them to survive prolonged periods of drought, making them the main choice for extensive green roofs (Durhman and Rowe 2006). Even bryophytes, which can spontaneously colonize substrates and survive extreme droughts, can mitigate the thermal conditions of extensive green roofs, thereby facilitating the survival of other vascular plants (Butler and Orians 2011).

The presence or absence of these interactions influences relationships within and between plant communities (Brooker and Callaghan 1998). In particular, in the context of green roofs, great interest has been aroused by the so-called "nurse plants" which facilitate the growth of other plants that live within their canopy, modifying the microclimate (López et al. 2007), providing shade in conditions of high temperatures (Butler and Orians 2011), increasing soil nutrients with an increase in decomposing organic matter (Anthelme and Dangles 2012), reducing the negative action of the wind (Le Roux and McGeoch 2010) or increasing the soil moisture content (Callaway 2007). Furthermore, the presence of nurse plants increases the biodiversity of species, abundance of plant formation, and survival of other plants (Aguiar et al. 2019). Butler and Orias (2011) analyzed the use of *Sedum album* L. as a nurse plant to facilitate the growth of *Agastache rupestris* (Greene) Standl. and *Asclepias verticillata* L. and noted how *S. album* acted as a competitor during periods of abundant water, reducing the biomass of the other two species, while improving

their performance during the summer water deficit, and reducing their mortality.

When plants with different habitus are adopted (succulents, forbs, and grasses), better management of rainwater and greater cooling are achieved compared to monocultural systems (Dvorak and Volder 2010). One of the most important interaction effects between plants is connected to the shading exerted by taller individuals on shorter ones. In the absence of this protective action, high solar radiation can reduce photosynthetic activity due to the photoinhibition of PSII (Murata et al. 2007), with consequent delay in plant growth (Demmig-Adams and Adams 1992), whereas the high leaf temperatures that are reached lead to the closure of the stomata and inactivation of the enzymes, with a consequent reduction in growth (Zandalinas et al. 2018). In particular, in hot climates, shade is observed to increase the growth of many plant species, owing to the involvement of microclimatic conditions (Aguiar et al. 2019). The level of shade tolerable by plants is affected by their light compensation point (LCP), and the LCP of different species can be adopted for the selection of ornamental plants that can be used in urban and peri-urban areas (Francini et al. 2023) and green roofs. Furthermore, in green roofs, along with the shading caused by vegetation, the shading cones of the neighboring structures are of significant importance and can significantly modify the microclimatic conditions.

Linking plant water use to leaf traits and Grime's CSR strategies (Grime 1973) could facilitate the selection of green roof plant. To this end, Lönnqvist et al. (2023) determined the growth (shoot biomass, relative growth rate, and leaf area), leaf characteristics (leaf dry matter content, specific leaf area, and succulence), and CSR strategies of 10 common species on European green roofs [of which three succulents: *Phedimus spurium* (M.Bieb.) 't Hart, *Hylotelephium telephium* (L.) H.Ohba, *Sedum acre* L.; one grass: *Poa alpina* L., and six forbs: *Campanula rotundifolia* L., *Galium verum* L., *Hypericum perforatum*

L., *Lotus corniculatus* L., *Tanacetum vulgare* L., and *Viola tricolor* L.], and correlated them with their use of water under conditions of full availability (WW) or water deficit (WD). All three succulent species included in the experiment mostly showed stress tolerance traits, and their water loss was lower than that of the unvegetated substrate, probably due to substrate cover. Plants with higher water use under WW had ruderal and competitive strategies, and greater leaf area and shoot biomass than those with lower water use under WW. However, the four species with the highest water use under WWconditions (*T. vulgare*, *V. tricolor*, *P. alpina*, and *L. corniculatus*) were able to reduce their water use under WD, indicating that they could both retain more precipitation than surviving periods of water limitation. This study indicates that for optimal rainwater retention, the selection of green roof plants in the regions, at least in the northern European latitudes, should focus on the selection of nonsucculent plants with predominantly competitive or ruderal strategies to maximize the long day length during the short growing season.

The diversity of plant species and functional traits have been shown to enhance green roof ecosystem services. Differences between plant species that contribute to ecosystem services are products of evolutionary change and phylogenetic diversity (PD). MacIvor et al. (MacIvor et al. 2018) analyzed six combinations of communities with different levels of PD, using 28 plant species from 12 different botanical families. They found that the minimum and average roof temperatures in the plant community decreased with an increase in PD. The increase in PD also led to an increase in the volume of rainwater captured, although it was not proportional to the amount of water lost due to evapotranspiration 48 h following the rainfall event (MacIvor et al. 2018).

Nagase and Dunnet (2010) investigated the influence of biological diversity on the survival of plants subjected to water stress. Twelve plant species were selected from the three main functional groups

commonly used for extensive green roofs (*Sedum*, forbs, and grasses). For each group, four species were selected and planted in combination with increasing diversity and complexity: monocultures, four-species mixtures, and twelve species mixtures. Three irrigation regimes were imposed: watering every one, two, or three weeks. The results demonstrated that a more diversified mixture was more advantageous than a monoculture in terms of survival and presence of vegetation. Combinations of functionally diverse species can achieve this goal more effectively than plants of the same taxonomic group, which, compete for the same resources when grown together (Nagase and Dunnet 2010).

If a single function improves in a more plant species-rich community than in a monoculture, this is called a "mixture advantage" which is based on two factors: the first is connected to the benefits associated with the cultivation of different species; the contribution of a plant species to a certain function improves when the species itself is cultivated with another species (Tilman et al. 2001); the second is niche complementarity (Tilman et al. 2001) and occurs when two or more plant species, due to differences in resource use, can better exploit available resources. Therefore, it is important for plant species to exhibit functional differences. In constructed ecosystems, designers are unlikely to rely on chances to improve ecosystem function; therefore, it is important to identify the most important functional traits that determine ecosystem functioning and to determine whether species combinations can reliably improve ecosystem function. If the plant species in a mixture are relatively similar, for example, all succulent plants, functional diversity will be low despite the high taxonomic variability.

3.5. Plant species for green roofs in the Mediterranean area

In many regions with hot and dry climates, including the Mediterranean, green roof technology is not widespread (Williams et

al. 2010), mainly due to the difficult climate (summer drought and high temperatures) and the limited availability of water. These characteristics impose severe restrictions on the growth and survival of green roofs (Benvenuti and Bacci 2010; Butler and Orians 2011). Plants are assumed to not survive in semi-arid climates on unirrigated green roofs with substrate depths of less than 5 cm, especially during the summer drought or establishment phases (Thuring et al. 2010; Williams et al. 2010). Furthermore, summer water scarcity is a recurring problem in the Mediterranean and climate change will lead to even more severe water scarcity because summer precipitation is expected to decrease by 5% per decade (Vanuytrecht et al. 2014). This can lead to irrigation becoming an unsustainable, regulated, and limited option. Therefore, it is necessary to select plant species that are capable of adapting to the absence of irrigation (Van Mechelen et al. 2014). Mediterranean areas contain habitats rich in native plant species that have the potential to be used on extensive green roofs (Van Mechelen et al. 2014) because they are believed to be better adapted to local climatic conditions and require little maintenance (Dvorak and Volder 2010). From a biodiversity perspective, one could also assume that green roofs constitute a new habitat for some Mediterranean plants whose natural environments are in danger. The choice can rely on the fact that many native plant species of the Mediterranean (in particular, the xerophytes) express morpho-functional and physiological adaptations that make them particularly suitable for green roofs; in fact, they present changes in the structures of the leaves (imbricated or often linear, with thick and waxy cuticle, sunken stomata, pubescent surface) and roots (deep roots, large root hair, rapid development in young plants), reduction in photosynthesis, and leaf drop phenomena under conditions of drought, high solar radiation, and high temperatures in summer (Brandão et al. 2017).

Although research in the Mediterranean environment is less extensive than that carried out in a continental climate, numerous native species

have been considered. Azeñas et al. (2018), for example, analyzed the response of five Mediterranean species—*Brachypodium phoenicoides* (L.) Roem. & Schult., *Crithmum maritimum* L., *Limonium virgatum* (Willd.) Fourr., *Sedum sediforme* (Jacq.) Pau, and *Sporobolus pungens* Kunth—grown in a non-limiting water regime or restoring 50% of evapotranspiration. The plant species were selected because they grow in natural habitats characterized by shallow soils with low organic substance content, high solar radiation, and extreme temperatures, namely, under conditions similar to those found on a green roof. Furthermore, some of these (*S. sediforme*) were CAM or CAM-facultative succulent species. The behavior of these plant species was monitored for two years. All plant species survived and exhibited a suitable aesthetic performance and vegetation coverage. *S. sediforme* recorded the least changes in appearance, the highest biomass production, and the lowest water consumption. However, *B. phoenicoides* appears to be an interesting alternative due to its valuable aesthetic characteristics and water consumption during the rainy season, suggesting a potential role of this species in the regulation of rainwater related to runoff reduction. *S. pungens* performed well in summer but presented poor aesthetic value during winter. *L. virgatum*, a plant that grows on rocky coasts, has shown good aesthetic value, both for its flowering and compact shape, and a high carbon sequestration capacity. In contrast, the use of C4 species, such as *S. pungens*, in urban green roofs in the Mediterranean climate is limited by the difficulty of this species to survive in winter and regrow in early spring (Azeñas et al. 2018).

To broaden the diffusion of green roofs in the Mediterranean environment, the contribution of four native plant species in Portugal—*Antirrhinum linkianum* Boiss. & Reut., *Asphodelus fistulosus* L., *Centranthus ruber* (L.) DC. and *Sedum sediforme* (Jacq.) Pau—resilient and drought tolerant, was analyzed. Growth, and aesthetic value were evaluated under two irrigation regimes (return of

100 and 60% evapotranspiration). *A. linkianum* had the highest number of flowers, longest seed production duration, and the highest area coverage, demonstrating its suitability for use. The level of irrigation did not significantly affect flowering and green coverage for any of the plant species and irrigation costs could be reduced by adopting deficit irrigation (Esfahani et al. 2022).

Attention has also been paid to therophyte species; annual plants contribute significantly to the vegetation of the Mediterranean basin, but their presence on green roofs has been limited to date (Van Mechelen et al. 2014), which is due to the brevity of their cycle, the difficulty of regeneration, and the lack of competitiveness compared to perennial plants. The absence of these plants during the summer months results in a modest cooling effect during the hot season. Van Mechelen et al. (2014) analyzed the plants present in natural habitats in southern France, that presented characteristics similar to those of green roofs, and identified 372 potentially usable species on the basis of some functional parameters; of these 35% are therophytes, which indicates that many annual species can be taken into consideration. Therophytes have interesting properties such as a short flowering period and the production of many seeds. Their conservation value may also be important, as many annual plants are threatened in Mediterranean areas (Lavergne et al. 2006). Other traits such as CAM metabolism, stress tolerance, and succulence have been shown to be important for successful green roofs (Oberndorfer et al. 2007).

The screening tool provides a potential list; however, definitive proof is obtained through experimental tests. Despite these limitations, the plant characteristics approach offers interesting possibilities for Mediterranean regions and can also help adapt green roof designs to future climate change (Van Mechelen et al. 2014).

Sage species native to Greece—i.e., *Salvia fruticosa* Mill., *S. officinalis* L., *S. pomifera* ssp. *pomifera*, *S. ringens* Sm., *S. tomentosa* Mill., and interspecific hybrids—were evaluated for their inclusion in

an extensive green roof in a Mediterranean climate in the summer period with regular or reduced irrigation (every 2–3 days with substrate humidity of 16–22% v/v and 4–5 days with substrate humidity of 7–11% v/v). Regardless of the irrigation frequency, *S. pomifera* ssp. *pomifera* x *S. ringens* and *S. officinalis* x *S. pomifera* ssp. *pomifera* showed the highest survival rate among all hybrids and species, as well as satisfactory growth, while *S. fruticosa* recorded the lowest survival, demonstrating that numerous *Salvia* species can be used in extensive green roofing in arid regions (Papafotiou et al. 2022). The possible use of two Mediterranean shrubs, *Arbutus unedo* L. and *Salvia officinalis* L., on green roofs was analyzed. The first species presented a substantial isohydric response (owing to the reduction in the stomatal opening at the first signs of stress, it was able to contain an excessive lowering of the water potential) and the second anisohydric (the plant appeared capable of withstanding strong variations in water potential while only partially limiting stomatal closure). Both species can be used on the Mediterranean green roof, even if the anisohydric species appear to be more sensitive to the characteristics of the substrate (Raimondo et al. 2015).

Reducing soil temperature while maintaining a relatively high air temperature has been shown to improve the growth and functional status of both roots and shoots and enhance plant survival (Huang et al. 2012). This contrasts with the need to reduce the depth of the substrate to limit weight and installation costs (Cao et al. 2014). However, the depth of the substrate is not always a limiting factor in the adoption of shrubs. Savi et al. (2015) investigated the behavior of two drought-adapted shrubs of two-years-old (*Cotinus coggygria* Scop. and *Prunus mahaleb* L.) grown in experimental modules with a 10 or 13 cm deep substrate. The results highlighted how the reduced depth of the substrate translated into less severe water stress than hypothesized and that the shallower substrate indirectly stimulates lower water consumption as a consequence of the reduced plant

biomass; therefore, it is possible to hypothesize a green roof with the use of stress-resistant shrubs in sub-Mediterranean areas, even in the presence of a substrate only 10 cm deep.

The performance of native Mediterranean plants on green roofs could be improved by adopting a plant community instead of a monoculture. Varela-Stasinopoulou et al. (2023) analyzed the growth, flowering, and self-reproduction rate of three plant communities, artificially created and made up of native Mediterranean plants, placed in substrates of different depths (8 and 15 cm) and with two irrigation regimes (high, 20% ETo and low, 10% ETo). The plant communities simulated those on the islands of Crete and Greece. Each of the three artificial plant communities comprised nine species and subspecies. Deeper substrates significantly improve the growth, flowering, and survival of most taxa. The irrigation regime was not significant for any species except for one, indicating that minimal amounts of water may be sufficient for irrigation. Four species failed to flower, whereas 15 species managed to self-reproduce.

Information on the plant species proposed for extensive green roofs in the Mediterranean region is presented in Table 3.2. The selected papers were experimental trials conducted in the Mediterranean environment. No information has been reported on plant species performance because the operative and stressful conditions are quite different. The list is full of over 180 species and/or cultivars belonging to over 40 families, most of them of Mediterranean origin, attesting to a large number of plant species that can be counted even with substrate depths of less than 20 cm. Among biological forms, chamaephytes (~40% of the total) and hemicryptophytes (~30%) stand out.

Table 3.2. Plant species studied for extensive green roofs[1] in the Mediterranean region.

Plant Species[2]	Botanical Family[3]	Plant Life Form[4]	Chorotypes[5]	Substrate Depth (cm)	References
Achillea millefolium L.	*Asteraceae*	H	Eurosib.	4, 7, 10, 19	Eksi and Rowe 2019; Chu et al. 2022
Aeonium arboretum Webb & Berthel.	*Crassulaceae*	NP	Macarones.	6	Gurrea-Ysasi et al. 2022
Allium carinatum L.	*Amaryllidaceae*	G	Stenomedit.	20	Benvenuti 2014
Allium roseum L.	*Amaryllidaceae*	G	Stenomedit.	10	Vannucchi et al. 2022
Allium sphaerocephalon L.	*Amaryllidaceae*	G	Paleotemp.	5, 10	Van Mechelen et al. 2015
Alyssum alyssoides L.	*Brassicaceae*	T	Eurimedit.	5, 10	Van Mechelen et al. 2015; Vannucchi et al. 2022
Alyssum saxatile L.	*Brassicaceae*	C	Medit.	14	Provenzano et al. 2010
Anemone hortensis L.	*Ranunculaceae*	G	N-Eurimedit.	20	Benvenuti 2014
Anthemis arvensis L.	*Asteraceae*	T	Stenomedit.	20	Benvenuti 2014
Anthemis maritima L.	*Asteraceae*	H	W-Medit.	15, 20	Benvenuti and Bacci 2010
Anthyllis vulneraria L.	*Fabaceae*	H	Eurimedit.	10	Vannucchi et al. 2022
Antirrhinum majus L.	*Plantaginaceae*	C	W-Medit.	20	Benvenuti 2014
Antirrhinum linkianum Boiss. & Reut	*Plantaginaceae*	C	Endem. Portugal	11	Eshahani et al. 2022
Aptenia cordifolia (L.f.) Schwantes	*Aizoaceae*	C	Africa	5, 6	Schweitzer and Erell 2014; Gurrea- Di Miceli et al. 2022; Ysasi et al. 2022
Arbutus unedo L.	*Ericaceae*	P	Stenomedit.	18	Raimondo et al. 2015
Armeria maritima (Mill.) Willd.	*Plumbaginaceae*	H	Subcosmopol.	4, 7, 10, 11 ± 1	Vestrella et al. 2015; Eksi and Rowe 2019

Armeria maritima (Miller) Willdenow subsp. *maritima*	*Plumbaginaceae*	H	Subcosmopol.	19	Chu et al. 2022
Armeria maritima 'Rosea'	*Plumbaginaceae*	H	Subcosmopol.	14	Provenzano et al. 2010
Armeria pungens Hoffmanns. & Link	*Plumbaginaceae*	C	W-Europ.	15, 20	Benvenuti and Bacci 2010
Artemisia absinthium L.	*Asteraceae*	C	E-Medit.	7.5, 10, 15	Papafotiou et al. 2013; Papafotiou et al. 2018
Arthrocnemum macrostachyum (Moric.) K.Koch	*Amaranthaceae*	C	Medit.	10	Paraskevopoulou et al. 2015; Paraskevopoulou et al. 2023
Asphodelus fistulosus L.	*Asphodelaceae*	H	Subtrop.	11	Eshahani et al. 2022
Atriplex halimus L.	*Amaranthaceae*	P	Stenomedit.	7.5, 10, 15	Papafotiou et al. 2018; Tassoula et al. 2021
Atriplex portulacoides L.	*Amaranthaceae*	C	Circumbor.	10	Paraskevopoulou et al. 2021
Ballota acetabulosa Benth.	*Lamiaceae*	C	W-Asia	8	Kokkinou et al. 2016
Blackstonia perfoliata (L.) Huds.	*Gentianaceae*	T	Eurimedit.	10	Vannucchi et al. 2022
Brachypodium phoenicoides (L.) Roem. & Schult.	*Poaceae*	H	W.Stenomedit.	15	Dunnet and Nolan 2004; Azeñas et al. 2018a; Azeñas et al. 2018b; Azeñas et al. 2019
Brachyscome multifida DC.	*Asteraceae*	H	Australia	19	Chu et al. 2022
Calamintha nepeta Savi	*Lamiaceae*	C	Medit.-Mont.	15, 20	Benvenuti and Bacci 2010; Benvenuti 2014
Calendula arvensis L.	*Asteraceae*	H	Eurimedit.	10	Vannucchi et al. 2022

Carpobrotus edulis (L.) N.E.Br.	*Aizoaceae*	C	S-Africa	5, 6, 9, 12, 15	Palermo et al. 2019; Di Miceli et al. 2022
Carpobrotus rossii (Haw.) Schwantes	*Aizoaceae*	C	S-Africa	10	Razzaghmanesh et al. 2014
Carthamus carduncellus L.	*Asteraceae*	H	NW-Medit.	5, 10	Van Mechelen et al. 2015
Centaurea cyanus L.	*Asteraceae*	T	Stenomedit.	20	Benvenuti 2014
Centranthus macrosiphon Boiss.	*Caprifoliaceae*	H	W.Stenomedit.	10	Vannucchi et al. 2022
Centranthus ruber (L.) DC.	*Caprifoliaceae*	C	Stenomedit.	11, 11 ± 1, 15, 20	Benvenuti and Bacci 2010; Vestrella et al. 2015; Eshahani et al. 2022
Cerastium tomentosum L.	*Caryophyllaceae*	C	Endem. Italy	6, 9, 12, 14, 15	Provenzano et al. 2010; Palermo et al. 2019
Chrysanthemum myconis L.	*Asteraceae*	T	Stenomedit.	20	Benvenuti 2014
Chrysocephalum apiculatum (Labill.) Steetz	*Asteraceae*	H	Endem. Italy	19	Chu et al. 2022
Cistus salviifolius L.	*Cistaceae*	NP	Stenomedit.	10, 13	Savi et al. 2016
Clinopodium acinos Kuntze	*Lamiaceae*	T	Eurimedit.	5, 10	Van Mechelen et al. 2015
Colchicum autumnale L.	*Colchicaceae*	G	C-Europ.	20	Benvenuti 2014
Consolida regalis Gray	*Ranunculaceae*	T	Eurimedit.	20	Benvenuti 2014
Convolvulus cneorum L.	*Convolvulaceae*	C	N-Medit.	7.5, 10, 15	Tassoula et al. 2015; Papafotiou et al. 2018; Tassoula et al. 2021
Convolvulus sabatius Viv.	*Convolvulaceae*	G	W.Stenomedit.	19	Chu et al. 2022

Cotinus coggygria Scop.	*Anacardiaceae*	NP	Medit.-Turan.	10, 13	Savi et al. 2015; Savi et al. 2016
Crithmum maritimum L.	*Apiaceae*	C	Eurimedit.	7.5, 15, 20	Benvenuti and Bacci 2010; Nektarios et al. 2016; Martini et al. 2017; Azeñas et al. 2018b; Azeñas et al. 2019
Crocus vernus (L.) Hill	*Iridaceae*	G	Eurimedit.	20	Benvenuti 2014
Delosperma cooperi (Hook.f.) L.Bolus	*Aizoaceae*	C	S-Africa	14	Provenzano et al. 2010
Delosperma N.E.Br. 'Kelaidis'	*Aizoaceae*	C	S-Africa	14	Provenzano et al. 2010
Delosperma N.E.Br. sp.	*Aizoaceae*	C	S-Africa	14	Provenzano et al. 2010
Dianella caerulea Sims 'Breeze'	*Asphodelaceae*	H	Australia	10	Razzaghmanesh et al. 2014
Dianthus carthusianorum L.	*Caryophyllaceae*	H	C-Europ.	15, 20	Benvenuti and Bacci 2010; Benvenuti 2014
Dianthus deltoides L.	*Caryophyllaceae*	H	Euroasiat.	10	Vannucchi et al. 2022
Dianthus deltoides L. 'Leuchtfunk'	*Caryophyllaceae*	H	Euroasiat.	14	Provenzano et al. 2010
Dianthus fruticosus subsp. *fruticosus*	*Caryophyllaceae*	H	Endem. Greece	7.5, 15	Nektarios et al. 2011
Dianthus gratianopolitanus Vill.	*Caryophyllaceae*	H	C-Europ.	6, 9, 12, 15	Palermo et al. 2019
Dianthus superbus L.	*Caryophyllaceae*	H	Euroasiat.	5, 10	Van Mechelen et al. 2015
Dorycnium hirsutum (L.) Ser.	*Fabaceae*	C	Eurimedit.	14	Provenzano et al. 2010
Drosanthemum floribundum (Haw.) Schwantes	*Aizoaceae*	C	S-Africa	5, 6, 8, 10, 11 ± 1, 12	Vestrella et al. 2015; Di Miceli et al. 2022; Marouli et al. 2022

Dymondia margaretae Compton	*Asteraceae*	H	S-Africa	11 ± 1	Vestrella et al. 2015; Vestrella et al. 2018
Echium plantagineum L.	*Boraginaceae*	H	Eurimedit.	8	Latini et al. 2022
Echium vulgare L.	*Boraginaceae*	H	Europ.	8	Latini et al. 2022
Emerus major Mill.	*Fabaceae*	NP	C-Europ.	10, 13	Savi et al. 2016
Erodium cicutarium (L.) L'Hér.	*Geraniaceae*	T	Subcosmop.	10	Vannucchi et al. 2022
Erophila verna (L.) DC.	*Brassicaceae*	T	Circumbor.	5, 10	Van Mechelen et al. 2015
Euphorbia characias L.	*Euphorbiaceae*	NP	Stenomedit.	15, 20	Benvenuti and Bacci 2010
Euphorbia cyparissias L.	*Euphorbiaceae*	H	C-Europ.	5, 10	Van Mechelen et al. 2015
Euphorbia pithyusa L.	*Euphorbiaceae*	C	W-Medit.	15, 20	Benvenuti and Bacci 2010
Festuca arundinacea Schreb.	*Poaceae*	H	Paleotemp.	8, 16	Soulis et al. 2017
Frankenia laevis L.	*Frankeniaceae*	C	Stenomedit.	11 ± 1	Vestrella et al. 2015; Vestrella et al. 2018
Geranium molle L.	*Geraniaceae*	H	Eurasiat.	10	Vannucchi et al. 2022
Glaucium flavum Crantz	*Papaveraceae*	H	Eurimedit.	15, 20	Benvenuti and Bacci 2010
Halimione portulacoides (L.) Aellen	*Amaranthaceae*	C	Circumbor.	5, 10	Schweitzer and Erell 2014; Paraskevopoulou et al. 2015
Hardenbergia violacea (Schneev.) Stearn	*Fabaceae*	P	Australia	19	Chu et al. 2022
Helianthemum Gray 'Fire Dragon'	*Cistaceae*	C	-	14	Provenzano et al. 2010
Helianthemum nummularium Mill.	*Cistaceae*	C	Europ.-Caucas.	5, 10	Van Mechelen et al. 2015

Helichrysum italicum (Roth) G.Don	*Asteraceae*	C	S-Europ.	7.5, 10, 15, 20	Benvenuti and Bacci 2010; Papafotiou et al. 2013; Monteiro et al 2017; Papafotiou et al. 2018
Helichrysum italicum subsp. *microphyllum* (Willd.) Nyman	*Asteraceae*	C	Eurimedit.	15, 20	Benvenuti and Bacci 2010
Helichrysum orientale (L.) Gaertn.	*Asteraceae*	C	Endem. Medit.	7.5, 8, 10, 15	Papafotiou et al. 2013; Kokkinou et al. 2016; Papafotiou et al. 2018
Helichrysum stoechas (L.) Moench	*Asteraceae*	C	Stenomedit.	11 ± 1, 15, 20	Benvenuti and Bacci 2010; Vestrella et al. 2015
Hemerocallis L. 'Stella de Oro'	*Asphodelaceae*	G	-	14	Provenzano et al. 2010
Heuchera L. 'Electra'	*Saxifragaceae*	H	-	10	Monteiro et al. 2017b
Heuchera L. 'Obsidian'	*Saxifragaceae*	H	-	10	Monteiro et al. 2017b
Hypericum calycinum L.	*Hypericaceae*	C	Medit.-Mont.	14	Provenzano et al. 2010
Hypochaeris radicata L.	*Asteraceae*	H	Europ.-Caucas.	10	Vannucchi et al. 2022
Hyssopus officinalis L.	*Lamiaceae*	C	Orof. Eurasiat.	19	Chu et al. 2022
Hyssopus officinalis subsp. *aristatus* (Godr.) Nyman	*Lamiaceae*	C	Medit.	14	Provenzano et al. 2010
*Indigofera australis*Willd.	*Fabaceae*	P	Australia	19	Chu et al. 2022
Iris chamaeiris Bertol.	*Iridaceae*	G	NWStenomedit.	20	Benvenuti 2014
Iris lutescens Lam.	*Iridaceae*	G	NWStenomedit.	5, 10, 11 ± 1	Van Mechelen et al. 2015; Vestrella et al. 2015; Vestrella et al. 2018
Jacobaea maritima (L.) Pelser & Meijden	*Asteraceae*	C	Stenomedit.	19	Chu et al. 2022

Lagurus ovatus L.	*Poaceae*	T	Eurimedit.	5, 10	Van Mechelen et al. 2015; Ondoño et al. 2016
Lampranthus spectabilis (Haw.) N.E.Br.	*Aizoaceae*	C	S-Africa	6, 8, 10, 12	Marouli et al. 2022
Lavandula angustifolia Mill.	*Lamiaceae*	NP	Stenomedit.	6, 8, 10, 12	Marouli et al. 2022
Lavandula dentata L.	*Lamiaceae*	NP	Paleosubtrop.	15	Monteiro et al. 2017b
Lavandula stoechas L.	*Lamiaceae*	NP	Stenomedit.	15, 20	Benvenuti and Bacci 2010
Lavandula stoechas subsp. *luisieri* (Rozeira) Rozeira	*Lamiaceae*	NP	Endem. Spain	15	Brandão et al. 2017
Leontodon tuberosus L.	*Asteraceae*	H	Stenomedit.	15, 20	Benvenuti and Bacci 2010; Benvenuti 2014
Ligustrum vulgare L.	*Oleaceae*	NP	Eurasiat.	10 or 13	Savi et al. 2016
Limonium virgatum (Willd.) Fourr.	*Plumbaginaceae*	C	Eurimedit.	11 ± 1, 15	Vestrella et al. 2015; Azeñas et al. 2018b; Azeñas et al. 2019
Linum bienne Mill.	*Linaceae*	H	Eurimedit.	5, 10	Van Mechelen et al. 2015
Lobularia maritima (L.) Desv.	*Fabaceae*	C	Stenomedit.	5, 10	Van Mechelen et al. 2015; Vannucchi et al. 2022
Lomandra longifolia Labill. 'Tanika'	*Asparagaceae*	H	Australia	10	Razzaghmanesh et al. 2014
Lomelosia cretica (L.) Greuter & Burdet	*Caprifoliaceae*	C	Stenomedit.	7.5, 10, 15	Papafotiou et al. 2018; Tassoula et al. 2021
Lotus creticus L.	*Fabaceae*	C	Stenomedit.	10, 11 ± 1	Vestrella et al. 2015; Ondoño et al. 2016
Medicago arborea L.	*Fabaceae*	P	NE-Medit.	6, 8, 10, 12	Marouli et al. 2022
Melissa officinalis L.	*Lamiaceae*	H	Eurimedit.	8	Kokkinou et al. 2016

Muscari comosum (L.) Mill.	*Asparagaceae*	G	Eurimedit.	10	Vannucchi et al. 2022
Myoporum parvifolium R.Br.	*Scrophulariaceae*	C	Australia	10	Razzaghmanesh et al. 2014
Narcissus tazetta L.	*Amaryllidaceae*	G	Stenomedit.	20	Benvenuti 2014
Nepeta cataria L.	*Lamiaceae*	H	E-Medit.	19	Chu et al. 2022
Nigella damascena L.	*Ranunculaceae*	T	Eurimedit.	20	Benvenuti 2014
Olearia axillaris (DC.) Benth.	*Asteraceae*	T	Australia	19	Chu et al. 2022
Origanum dictamnus L.	*Lamiaceae*	H	Endem. Crete	7.5, 10, 15	Papafotiou et al. 2018; Tassoula et al. 2021
Origanum majorana L.	*Lamiaceae*	H	Saharo-Sind.	7.5, 10 or 15	Papafotiou et al. 2018
Origanum onites L.	*Lamiaceae*	C	E-Medit.	8 or 16	Soulis et al. 2017
Origanum vulgare L.	*Lamiaceae*	H	Eurasiat.	19	Chu et al. 2022
Ornithogalum umbellatum L.	*Liliaceae*	G	Eurimedit.	10, 20	Benvenuti 2014; Vannucchi et al. 2022
Otanthus maritimus (L.) Hoffmanns. & Link	*Asteraceae*	C	Medit.-Atlant.	10, 20	Benvenuti and Bacci 2010
Paliurus spina-christi Mill.	*Rhamnaceae*	P	SE-Europ.	10, 13	Savi et al. 2016
Pallenis maritima (L.) Greuter	*Asteraceae*	H	W-Medit.	7.5, 10, 11 ± 1, 15	Vestrella et al. 2015; Ondoño et al. 2016b; Papafotiou et al. 2017; Papafotiou et al. 2018; Azeñas et al. 2019
Pennisetum clandestinum Hochst. ex Chiov.	*Poaceae*	H	E-Africa	5	Schweitzer and Erell 2014

Petrorhagia prolifera (L.) P.W.Ball & Heywood	*Caryophyllaceae*	T	Eurimedit.	5 and 10	Van Mechelen et al. 2015
Petrorhagia saxifraga Link	*Caryophyllaceae*	H	Eurimedit.	10	Vannucchi et al. 2022
Phillyrea angustifolia L.	*Oleaceae*	P	Stenomedit.	10, 13	Savi et al. 2016
Phlox douglasii Hook. 'McDaniels Cushion'	*Polemoniaceae*	H	N-America	14	Provenzano et al. 2010
Pistacia lentiscus L.	*Anacardiaceae*	P	S-Medit.	10, 13	Savi et al. 2016
Plantago afra L.	*Plantaginaceae*	T	Stenomedit.	5, 10	Van Mechelen et al. 2015
Prunus mahaleb L.	*Rosaceae*	P	S-Europ.	10, 13	Savi et al. 2015; Savi et al. 2016
Pyrus pyraster (L.) Burgsd.	*Rosaceae*	P	Eurasiat.	10, 13	Savi et al. 2016
Rosmarinus officinalis L.	*Lamiaceae*	NP	Stenomedit.	8, 15, 19	Kokkinou et al. 2016; Brandão et al. 2017; Chu et al. 2022
Salvia fruticosa Mill.	*Lamiaceae*	C	E-Medit.	8, 10	Kokkinou et al. 2016; Martini et al. 2022; Papafotiou et al. 2022
Salvia L. hybr	*Lamiaceae*	C	-	10	Papafotiou et al. 2022; Martini et al. 2022
Salvia officinalis L.	*Lamiaceae*	C	Stenomedit.	10, 13, 18	Raimondo et al. 2015; Savi et al. 2016; Martini et al. 2022; Papafotiou et al. 2022
Salvia officinalis L. 'Berggarten'	*Lamiaceae*	C	Stenomedit.	10	Monteiro et al. 2017b
Salvia pomifera subsp. *pomifera*	*Lamiaceae*	C	Endem. Crete	10	Papafotiou et al. 2022
Salvia ringens Sm.	*Lamiaceae*	C	Endem. Greece	10	Martini et al. 2022; Papafotiou et al. 2022

Salvia tomentosa Mill.	*Lamiaceae*	C	NE-Medit.	10	Papafotiou et al. 2022
Santolina chamaecyparissus L.	*Asteraceae*	NP	Medit.	7.5, 10, 15	Papafotiou et al. 2018
Santolina rosmarinifolia L.	*Asteraceae*	NP	Medit.	11 ± 1	Vestrella et al. 2015
Saponaria ocymoides L.	*Caryophyllaceae*	H	Orof. S-Europ.	14	Provenzano et al. 2010
Satureja illyrica Host	*Lamiaceae*	C	S-Europ.	14	Provenzano et al. 2010
Satureja montana L.	*Lamiaceae*	C	W-Medit.	15, 20	Benvenuti and Bacci 2010; Monteiro et al. 2017a
Scabiosa columbaria L.	*Dipsacaceae*	H	Eurasiat.	15, 20	Benvenuti and Bacci 2010; Benvenuti 2014
Scilla autumnalis L.	*Asparagaceae*	G	Eurimedit.	20	Benvenuti 2014
Scrophularia canina L.	*Scrophulariaceae*	H	Eurimedit.	15, 20	Benvenuti and Bacci 2010
Scrophularia peregrina L.	*Scrophulariaceae*	T	Stenomedit.	10	Vannucchi et al. 2022
Sedum acre L.	*Crassulaceae*	C	Europ.	4, 5, 6, 7, 10, 12	Van Mechelen et al. 2015; Eksi and Rowe 2019; Nektarios et al. 2021; Vannucchi et al. 2022
Sedum album L.	*Crassulaceae*	C	Eurimedit.	4, 5, 6, 7, 10, 12, 14	Provenzano et al. 2010; Van Mechelen et al. 2015; Eksi and Rowe 2019; Pérez et al. 2020; Nektarios et al. 2021; Di Miceli et al. 2022; Vannucchi et al. 2022
Sedum floriferum Praeger	*Crassulaceae*	C	Siberia	14	Provenzano et al. 2010
Sedum hispanicum L.	*Crassulaceae*	C	SE-Europ.	5, 14	Provenzano et al. 2010; Di Miceli et al. 2022
Sedum L. spp.	*Crassulaceae*	C	-	6, 10	Monteiro et al. 2017b; Gurrea-Ysasi et al. 2022

Sedum ochroleucum Chaix	*Crassulaceae*	C	Medit.-Mont.	5	Di Miceli et al. 2022
Sedum reflexum L.	*Crassulaceae*	C	C-Europ.	6, 12, 14	Provenzano et al. 2010; Nektarios et al. 2021
Sedum rupestre L.	*Crassulaceae*	C	C-Europ.	15, 20	Benvenuti and Bacci 2010
Sedum sediforme (Jacq.) Pau	*Crassulaceae*	C	Stenomedit.	5, 6, 7.5, 8, 10, 11, 12, 13, 15, 16, 19	Nektarios et al. 2015; Soulis et al. 2017; Azeñas et al. 2018a; Azeñas et al. 2018b; Azeñas et al. 2019; Schindler et al. 2019; Pérez et al. 2020; Esfahani et al. 2022; Di Miceli et al. 2022; Marouli et al. 2022
Sedum sexangulare L.	*Crassulaceae*	C	C-Europ.	6, 10, 12, 14	Provenzano et al. 2010; Pérez et al. 2020; Nektarios et al. 2021
Sedum spurium M.Bieb.	*Crassulaceae*	C	Europ.-Caucas.	14	Provenzano et al. 2010
Sedum spurium M.Bieb. cf. [6] 'Coccineum'	*Crassulaceae*	C	Europ.-Caucas.	10	Pérez et al. 2020
Sedum spurium M.Bieb. cf. [6] 'Summer Glory'	*Crassulaceae*	C	Europ.-Caucas.	10	Pérez et al. 2020
Sempervivum L. 'Reinhard'	*Crassulaceae*	C	-	10	Monteiro et al. 2017b
Sesuvium verrucosum Raf.	*Aizoaceae*	C	America	5	Schweitzer and Erell 2014
Sideritis athoa Papan. & Kokkini	*Lamiaceae*	C	Macarones.	7.5, 10, 15	Papafotiou et al. 2018; Tassoula et al. 2021
Sideritis hyssopifolia L.	*Lamiaceae*	C	NW-Medit.	5, 10	Van Mechelen et al. 2015
Silene conica L.	*Caryophyllaceae*	T	Paleotemp.	5, 10	Van Mechelen et al. 2015
Silene gallica L.	*Caryophyllaceae*	T	Eurimedit.	10	Vannucchi et al. 2022

Silene vulgaris (Moench) Garcke	*Caryophyllaceae*	H	Paleotemp.	5, 10	Ondoño et al. 2016
Spartium junceum L.	*Fabaceae*	P	Eurimedit.	10, 13	Savi et al. 2016
Sporobolus pungens Kunth	*Poaceae*	G	Subtrop.	15	Azeñas et al. 2018b; Azeñas et al. 2019
Stachys byzantina K.Koch	*Lamiaceae*	H	E-Asia	10, 14	Provenzano et al. 2010; Monteiro et al. 2017b
Sternbergia lutea (L.) Ker Gawl. ex Spreng.	*Amaryllidaceae*	G	Medit.-Mont.	20	Benvenuti 2014
Teucrium chamaedrys L.	*Lamiaceae*	C	Eurimedit.	14	Provenzano et al. 2010
Teucrium fruticans L.	*Lamiaceae*	NP	Stenomedit.	19	Chu et al. 2022
Thymus caespititius Brot.	*Lamiaceae*	C	Iberian Peninsula	15	Monteiro et al 2017
*Thymus marschallianus*Willd.	*Lamiaceae*	C	Eurasiat.	14	Provenzano et al. 2010
Thymus pseudolanuginosus Ronniger	*Lamiaceae*	C	S-Europ.	15	Monteiro et al 2017
Thymus serpyllum L.	*Lamiaceae*	H	Eurasiat.	11 ± 1	Vestrella et al. 2015
Trifolium arvense L.	*Fabaceae*	T	Paleotemp.	10	Vannucchi et al. 2022
Trifolium campestre Schreb.	*Fabaceae*	T	Paleotemp.	10	Vannucchi et al. 2022
Tuberaria guttata (L.) Fourr.	*Cistaceae*	T	Eurimedit.	20	Benvenuti 2014
Verbascum blattaria L.	*Scrophulariaceae*	H	Cosmopol.	10	Vannucchi et al. 2022
Verbascum thapsus L.	*Scrophulariaceae*	H	Europ.-Caucas.	15, 20	Benvenuti and Bacci 2010
Veronica prostrata L.	*Plantaginaceae*	H	Eurasiat.	14	Provenzano et al. 2010

Zoysia matrella (L.) Merr.	*Poaceae*	H	E-Asia	7.5, 15	Ntoulas et al. 2013

[1] We considered that substrate layer thickness of extensive green roof was from 5 to 20 cm (Pérez et al. 2020);
[2] the names of the species have been corrected based on the indications of "World flora online" (https://www.worldfloraonline.org/); we preferred to keep the names indicated by the authors of the papers, except when multiple synonyms of the same species were used; in this case, we have only reported the correct botanical name;
[3] according to "World flora online" (https://www.worldfloraonline.org/);
[4] according to The Life Forms of Plants of Raunkiaer (1934); H = Hemicryptophyte, NP = Nanophanerophyte, G = Geophyte, T = Therophyte, C = Chamaephyte, P = Phanerophyte;
[5] references are mainly related to Pignatti (1982);
[6] "cf." means that, according to the authors, the cultivar cannot be affirmed with certainty but the phenotype is compatible.

3.6. Conclusions

Green roofs are increasingly being considered for their contribution to ecosystem services, which are essential for the quality of life of urban areas. Many of its functions are connected to the vegetation that is installed, making the choice of species one of the most strategic aspects of the design. Even in the Mediterranean environment, sustainable solutions are possible for the creation of a green roof, which must be based on a reduction in the height of the substrate to allow it to be installed in a greater number of structures, and a more rational use of water resources. For the identification of species, the analysis of both plant species is present in habitat analogs and some morpho-functional characteristics can be considered a useful strategy. However, only experimental trials in representative contexts can provide conclusive information to understand whether these choices are functional. Another strategy is to increase biodiversity by replacing a single species with a plant community to make the best use of the positive relationships that can be established between different plant species. However, even in this case, only specific experimental trials would be able to provide a framework of knowledge indispensable for spreading the presence of green roofs in the Mediterranean environment.

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4. The use of Mediterranean native shrubs for improving the sustainability of urban environments

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The use of Mediterranean native shrubs for improving the sustainability of urban environments

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Abstract
The Mediterranean Basin is home to one of the highest levels of biodiversity on Earth, with approximately 25,000 plant species. The typical vegetation in this region is predominantly consists of shrubs. Due to their anatomical, morphological, physiological, and biochemical characteristics, these plants can tolerate abiotic stress, particularly drought. Consequently, incorporating them could enhance the sustainability of urban green spaces. However, species native to the Mediterranean Basin are not yet widely represented in green spaces, despite their attractive ornamental traits. To quantify the

contribution of Mediterranean native shrubs, a survey was conducted on Italian flora. Using a selection grid, species of potential interest were identified; these species belong to the Mediterranean chorotype and exhibit appealing ornamental characteristics. The overall attributes of these plant species support their ornamental use, owing to their form and the features of their leaves, flowers, or fruits. The investigation identified 369 species that are not currently available in commercial nurseries. The most represented families included *Rosaceae*, *Asteraceae*, *Fabaceae*, *Lamiaceae*, and *Cistaceae*. Although the flowering period of each species is relatively short, the blooms of different species are distributed throughout the year. Therefore, by simultaneously utilizing various species, it is possible to ensure a continuous ornamental display.

4.1. Introduction

The Mediterranean Basin is one of the richest regions in the world in terms of plant diversity, boasting approximately 25,000 plant species (Myers et al. 2000). Due to their high biological diversity, Mediterranean ecosystems have been recognized as biodiversity hotspots and prime targets for conservation efforts (Myers et al. 2000; Tavşanoğlu and Pausas 2018). However, the climate makes the Mediterranean Basin one of the most vulnerable areas to both abiotic and biotic stress. Drought, combined with extreme temperatures, constitutes abiotic stress that negatively affects the survival, growth, development, and productivity of Mediterranean woody species (Ogaya and Peñuelas 2003). These environmental constraints are expected to intensify in the near future due to global climate change (Pinheiro et al. 2014).

Mediterranean plants can tolerate stressful abiotic conditions and, therefore, are somewhat resilient to the warming and increased drought associated with climate change (Toscano et al. 2019). However, only species with significant phenotypic plasticity—

particularly those capable of rapid evolutionary changes in both their functional traits and plasticity—will persist under rapidly changing environmental conditions induced by global change (Matesanz and Valladares 2014). These characteristics are especially common in shrubs, which are characterized by having "more active meristems, and more potential points for stem regeneration" (Givnish 1984). These traits enable shrubs to tolerate abiotic stress better than trees. Shrubs are often associated with disturbed and stressful environments; for these reasons, they are more suitable for green infrastructures in Mediterranean environments, especially where maintenance is limited. The high biodiversity and aesthetic value of these plants suggest that their use is the key component of sustainable landscape (Romano and Scariot 2021).

Among Mediterranean ecosystems, shrublands represent a characteristic vegetation type that is widespread across various habitats. Due to several factors—including physiological, morphological, reproductive, phenological, and regenerative traits, as well as inter- and intraspecific interactions—each shrub species constitutes a significant component of the plant community. Shrubs play a vital ecological role (Pasalodos-Tato et al. 2015) in multiple ways, such as soil protection (Bochet et al. 2006; Francini et al. 2021), enhancing biodiversity (Mangas et al. 2007), nutrient production, carbon storage (Chapin 1983), and ecosystem restoration (Rey et al. 2009).

The spread of shrubs in the Mediterranean region is attributed to their adaptability to harsh climatic conditions, characterized by hot summers and low rainfall, resulting in a long, dry season (Paz et al. 2016). The use of native Mediterranean shrub species offers a potential solution to drought (Munné-Bosch and Peñuelas 2004), salt stress (Cassaniti et al. 2013), and heat stress. These native shrubs can withstand severe drought, a critical factor influencing plant survival and species distribution (Filella et al. 1998). Due to their unique

morphophysiological traits, woody plants are well-suited for ornamental purposes. It is no coincidence that shrubs are commonly found in degraded environments where abiotic stresses are frequent. From an ornamental perspective, shrubs exhibit various features—such as a high number of twigs influencing their growth patterns; pulvinate (cushion-like) shapes that reduce transpiration and enhance the aesthetic appeal of green infrastructures; distinctive leaf characteristics; varied flowering periods; diverse flower colors—that contribute interest and variety to the landscape (Leotta et al. 2023a).

Despite widespread appreciation, the vast plant diversity of the Mediterranean region largely consists of neglected and underutilized species (Padulosi et al. 2013). Only a small fraction of these resources is currently employed in the agricultural and ornamental horticulture sectors due to poor documentation of their potential and the lack of coordinated efforts (Rivera et al. 2006; Scariot et al. 2006). Globally, the ornamental horticulture industry seeks new crops—often of exotic origin—that exhibit appealing ornamental traits, adaptability to diverse conditions, and low maintenance costs (Fascetti et al. 2014; Maloupa et al. 2008; Junqueira and Peetz 2017). Unfortunately, native plants receive insufficient attention, partly because of limited information regarding their performance and propagation.

Studies on the ornamental value of new crops generally focus on the plants' habit and characteristics, such as morphological and phenological traits, development and growth, ecological characteristics including tolerance to various factors, aesthetic features like flower and leaf attributes, and cultivation methods encompassing agricultural practices such as ease of propagation and adaptability to different cultivation conditions (Krigas et al. 2021). For plants intended for landscaping and gardening, resistance to biotic and abiotic stresses is of particular importance (Krigas et al. 2021).

Another factor supporting the use of native plants in green spaces is the potential to aid populations of declining native species, thereby

helping to prevent their extinction. Gardens, which are common in both urban and rural environments, are increasingly recognized for their role in conserving biodiversity (Gratzfeld 2017). Recently, the concept of conservation gardening has emerged as a novel approach to biodiversity restoration. This approach focuses on cultivating declining native species in private gardens, integrating commercial horticulture with conservation efforts, and promoting public engagement (Segar et al. 2022; Staude 2022; Munschek et al. 2023). Awareness of the importance of biodiversity and the benefits of incorporating local flora into urban landscape design is growing (Lovell and Johnston 2009; Ahern 2013). By doing so, we can safeguard pollinators that have co-evolved with many genera or species of native plants (Hall et al. 2017; Senapathi et al. 2017).

An increasingly informed public is demanding the inclusion of native plants in green spaces, driven by the awareness that this can reduce dependence on resources—primarily water—in urban and suburban environments. Although native flora encompasses a variety of species with diverse water requirements, some species, particularly shrubs, demonstrate greater water-use efficiency (Chaves et al. 2003; Evans et al. 2013; Martinson 2020).

Recently, the checklist of vascular flora native to Italy has been updated (Bartolucci et al. 2018). The checklist now includes 8,241 species and subspecies, belonging to 1,111 genera and 153 families (Bartolucci et al. 2024). According to Bartolucci et al. (2024), the taxa currently confirmed to occur in Italy number 7,591, as the remaining taxa have not been recently verified, are doubtful, or are affected by errors or incomplete information. In any case, the Italian flora is particularly rich in plant species, which can serve as a valuable resource for revitalizing the biological diversity of ornamental nurseries and for individuating new species better suited for urban greenery.

In this context, the objective of this study was to review the shrub species present in the Italian flora and examine some of their morphological and functional characteristics to conduct a preliminary assessment of their adaptability to various landscape use modalities.

4.2. Materials and methods

4.2.1. Literature sources

The survey began with a review of relevant literature sources (Pignatti 1982; Raimondo et al. 2010; Conti et al. 2005; Bartolucci et al. 2018, 2024), which facilitated the identification of the initial group of species. All data collection and literature searches were performed within the period November 2024 until February 2025. All identified plants belonged to the category commonly referred to as bushes. These plants correspond to chamaephytes, caespitose phanerophytes, and nanophanerophytes, according to Raunkiaer (1934). Although some individual accessions could be classified under other biological forms (e.g., rhizomatous geophytes, caespitose hemicryptophytes, hemicryptophytes with rosettes, scapose hemicryptophytes), all listed taxa fall within these biological categories. The taxa considered were limited to species spread in Italy and classified as indigenous, naturalized archaeophytes, or cryptogenic. From the preliminary list, certain accessions were selected for further study based on specific morpho-biological characteristics, flowering season, and distribution within the national territory. For each accession, synonym names were verified to eliminate duplicates using Plants of the World Online (https://powo.science.kew.org/). However, a double taxonomic check (accepted name versus heterotypic synonym) has not been performed. Families were listed according to Plants of the World Online. Information on origin and flowering period was referenced from Pignatti (1982) and Acta Plantarum (https://www.actaplantarum.org/). Based on literature data (Pignatti 1982; Pignatti et al. 2017–2019; Acta Plantarum), plant height was categorized into classes (< 20 cm; 20–60

cm; 60–100 cm; 100–140 cm; > 140 cm), along with flower color and the organ of greatest showiness (flowers, fruits, or leaves). For the latter information, we relied on iconography available online from reliable websites (Acta Plantarum, iNaturalist).

4.2.2. Morphological traits

Considering morphological traits (height, prominence of flowers, fruits, and leaves) and functional characteristics (origin environments, flowering season), the potential use of these species for ornamental purposes in green areas—such as green roofs, naturalistic gardens, pot cultivation, slopes, xeriscaping, wildflowers, —was proposed. The list of selected plants was then compared with a previous list of species found in twenty-seven nurseries nationwide that marketed ornamental plants used for the arrangement of green spaces (Romano 2021), in order to identify which of these accessions were available on the market.

4.3. Results

The review identified 1,137 taxa belonging to 271 genera and 74 different botanical families (Table 4.1). Based on specific morphobiological characteristics, flowering season, and distribution within the national territory, a selection process identified 544 taxa belonging to 199 genera and 68 botanical families (Table 4.2). Among these, 159 taxa were subspecies, accounting for 29.2% of the total (Table 4.1S). The most represented botanical families are *Rosaceae* (44 genera and 56 species), *Asteraceae* (16 genera and 55 species), *Fabaceae* (17 genera and 50 species), *Lamiaceae* (16 genera and 45 species), *Cistaceae* (3 genera and 38 species), and *Caryophyllaceae* (12 genera and 30 species).

To highlight the extensive biodiversity observed, it should be noted that 36 botanical families (52.9% of the total) were represented by a single genus, and 23 botanical families (33.8%) were represented by a

single species (Table 4.1). The highest number of genera was found in the *Rosaceae* family, followed by *Asteraceae*, *Fabaceae*, and *Lamiaceae*.

Table 4.1. Number of taxa and genera for botanical families of Italian flora of potential ornamental value.

Botanical family	N. genera	N. taxa	Botanical family	N. genera	N. taxa
Amaranthaceae	12	21	*Hypericaceae*	1	5
Anacardiaceae	3	6	*Lamiaceae*	21	89
Apiaceae	3	4	*Lauraceae*	1	1
Apocynaceae	5	9	*Linaceae*	1	5
Aquifoliaceae	1	1	*Linnaeaceae*	1	1
Araliaceae	1	1	*Loranthaceae*	1	1
Arecaceae	1	1	*Malvaceae*	2	9
Aristolochiaceae	1	1	*Myrtaceae*	2	2
Asparagaceae	2	7	*Oleaceae*	5	6
Asteraceae	22	93	*Orobanchaceae*	1	2
Berberidaceae	1	2	*Phyllanthaceae*	1	1
Betulaceae	5	10	*Plantaginaceae*	8	32
Blechnaceae	1	1	*Plumbaginaceae*	4	68
Boraginaceae	4	8	*Polygalaceae*	1	1
Brassicaceae	17	54	*Polygonaceae*	1	4
Buxaceae	1	2	*Ranunculaceae*	1	5
Campanulaceae	3	4	*Rhamnaceae*	5	14
Cannabaceae	2	4	*Rosaceae*	18	149
Capparaceae	1	2	*Rubiaceae*	4	6
Caprifoliaceae	3	14	*Rubiaceae*	2	11
Caryophyllaceae	19	81	*Rutaceae*	4	8
Celastraceae	1	3	*Salicaceae*	1	39
Cistaceae	4	44	*Santalaceae*	1	1
Convolvulaceae	2	3	*Sapindaceae*	1	3
Coriariaceae	1	1	*Saxifragaceae*	1	1
Cornaceae	1	4	*Scrophulariaceae*	1	1
Crassulaceae	5	37	*Selaginellaceae*	1	1

Cupressaceae	1	5	*Smilacaceae*	1	1
Elaeagnaceae	1	1	*Solanaceae*	2	4
Ephedraceae	1	3	*Staphyleaceae*	1	1
Ericaceae	11	28	*Styracaceae*	1	1
Euphorbiaceae	2	27	*Tamaricaceae*	3	7
Fabaceae	22	133	*Thymelaeaceae*	2	16
Fagaceae	1	3	*Ulmaceae*	2	3
Frankeniaceae	1	2	*Valerianaceae*	1	3
Grossulariaceae	1	8	*Viburnaceae*	2	5
Hydrangeaceae	1	1	*Vitaceae*	1	1
			TOTAL	**271**	**1137**

Table 4.2. Number of taxa and genera for botanical families of selected plants, according to the number of taxa.

Botanical family	**N. genera**	**N. taxa**	**Botanical family**	**N. genera**	**N. taxa**
Rosaceae	44	56	*Convolvulaceae*	2	3
Asteraceae	16	55	*Ephedraceae*	1	3
Fabaceae	17	50	*Grossulariaceae*	1	3
Lamiaceae	16	45	*Malvaceae*	1	3
Cistaceae	3	38	*Orobanchaceae*	1	3
Caryophyllaceae	12	30	*Ranunculaceae*	1	3
Brassicaceae	9	29	*Solanaceae*	3	3
Euphorbiaceae	2	19	*Cornaceae*	1	2
Plumbaginaceae	4	15	*Frankeniaceae*	1	2
Crassulaceae	3	14	*Rutaceae*	1	2
Amaranthaceae	8	11	*Viburnaceae*	2	2
Caprifoliaceae	4	9	*Amaranthaceae*	1	1
Ericaceae	3	9	*Apocynaceae*	1	1
Amaranthaceae	2	7	*Aquifoliaceae*	1	1
Asparagaceae	2	7	*Araliaceae*	1	1
Plantaginaceae	4	7	*Arecaceae*	1	1
Rhamnaceae	3	7	*Aristolochiaceae*	1	1
Salicaceae	1	7	*Asphodelaceae*	1	1

Boraginaceae	2	6	*Berberidaceae*	1	1
Rubiaceae	5	6	*Blechnaceae*	1	1
Tamaricaceae	2	6	*Celastraceae*	1	1
Thymelaeaceae	2	6	*Lauraceae*	1	1
Anacardiaceae	3	5	*Loranthaceae*	1	1
Apocynaceae	4	5	*Myrtaceae*	1	1
Campanulaceae	3	5	*Phyllanthaceae*	1	1
Cupressaceae	1	5	*Polygonaceae*	1	1
Hypericaceae	1	5	*Santalaceae*	1	1
Oleaceae	4	5	*Sapindaceae*	1	1
Apiaceae	3	4	*Saxifragaceae*	1	1
Betulaceae	4	4	*Scrophulariaceae*	1	1
Capparaceae	1	4	*Selaginellaceae*	1	1
Fagaceae	1	4	*Smilacaceae*	1	1
Ulmaceae	2	4	*Vitaceae*	1	1
Cannabaceae	2	3	*Zygophyllaceae*	1	1
Convolvulaceae	2	3	**TOTAL**	**199**	**544**

Most of the species were from the Mediterranean area, based on the criteria adopted for inclusion in the list (Figure 4.1). Endemic or subendemic species account for 29% of the total. Steno-Mediterranean species are also well represented, comprising 21% of the total.

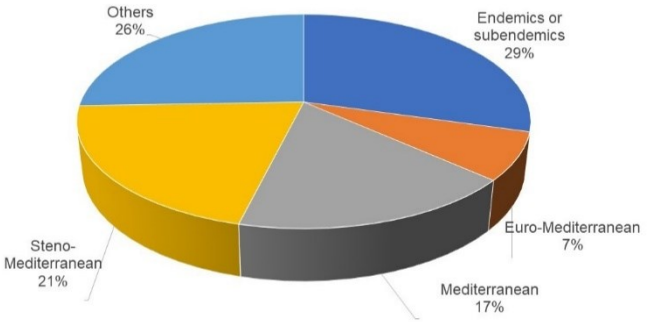

Figure 4.1. Distribution of shrub in the Mediterranean area, in wild, urban, peri-urban areas.

The most common biological form was chamaephytes, which accounted for 58% of the total, followed by cespitose phanerophytes (22%), and nanophanerophytes (20%) (Figure 4.2).

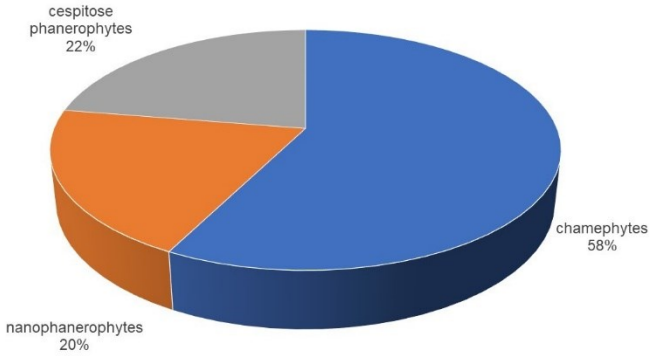

Figure 4.2. Distribution of shrub for biological forms.

Over 50% of the plants were under 60 cm in height, while 30% exceeded 100 cm (Figure 4.3).

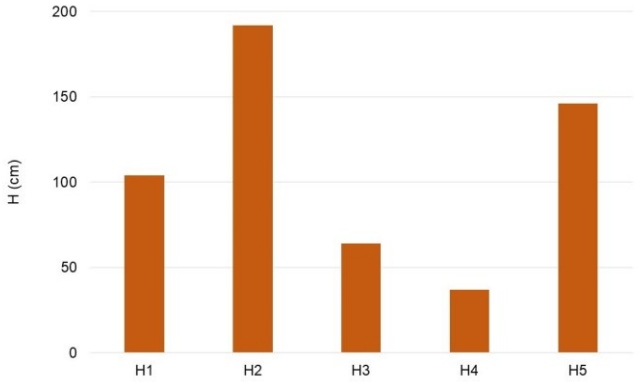

Figure 4.3. Distribution of shrub for height: H1= < 20 cm; H2= 20-60 cm; H3= 60-100 cm; H4= 100-140 cm; H5= > 140 cm.

The flowering period of different species lasted an average of 3.7 months. In terms of distribution across the months, the highest concentration of flowering occurred between April and June, accounting for over 50% of the blooms. The lowest concentrations were observed in November and December, at 1.9% and 1.2%, respectively (Figure 4.4).

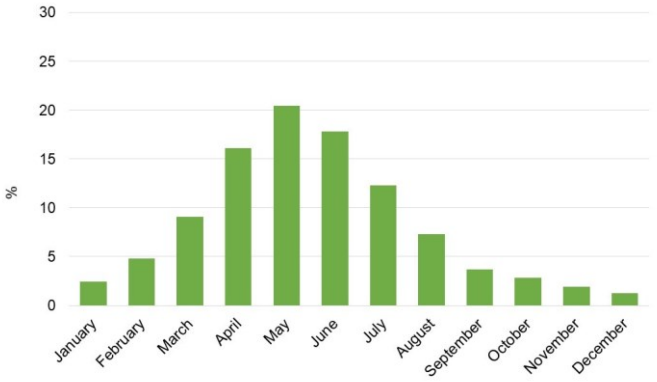

Figure 4.4. Distribution of flowering in the different months of the year.

The conspicuousness supporting the ornamental characteristics was attributed to the flowers in 86.4% of cases, to the fruit in 20.6%, and to the foliage in 23.7% (Table 1S). In fact, some species were valued for multiple ornamental traits.

The color of the flowers, the most conspicuous organ, was white in 134 species (24.6% of the total), yellow in 196 species (30.0%), pink-red in 103 species (18.9%), and violet-lilac in 46 species (8.5%) (Table 1S).

Based on their morphological and phenological traits, the species can be primarily used in naturalistic gardens (39%), xeriscaping (28%),

slope stabilization (14%), and green roofs (11%). Their use for pot cultivation and wildflowers is marginal (Figure 4.5). Each species supports multiple applications, averaging 2.1 uses per species, highlighting their potential value in green spaces.

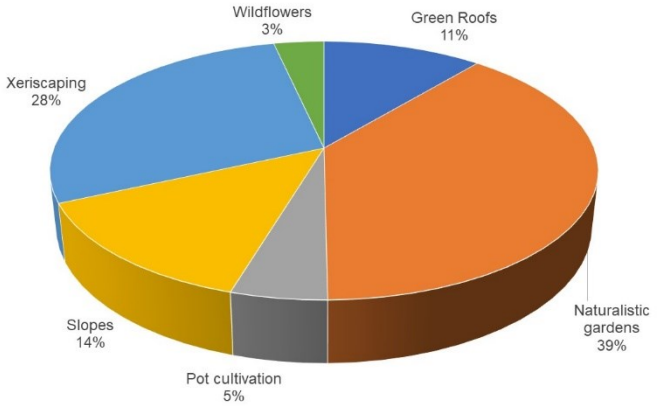

Figure 4.5. Distribution of shrub for the possible uses in green areas.

Despite the small size of the flowers, many species are appreciated for their abundant blooms and, occasionally, for the distinctive characteristics of their fruits and foliage, highlighting the aesthetic appeal of many recorded taxa (Figure 4.6).

Many of the ornamental species surveyed are not listed in the catalogs of major Italian commercial nurseries. A total of 369 plant species, representing 67.8% of the recorded accessions, were absent from these commercial nursery catalogs. However, it cannot be ruled out that some of these unlisted species are cultivated on a marginal scale.

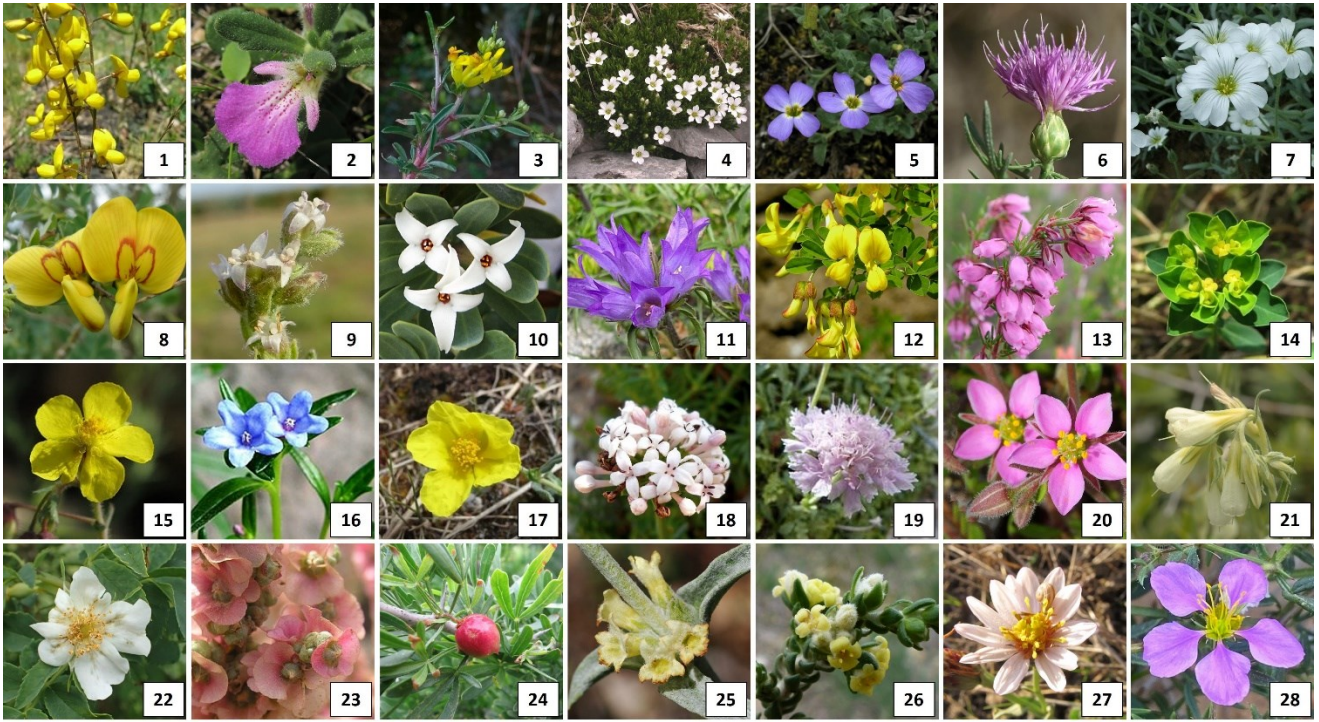

Figure 4.6. Top left to right: 1) *Adenocarpus complicatus* subsp. *bivonae* (C.Presl) Peruzzi; 2) *Ajuga iva* subsp. *iva*; 3) *Anthyllis hermanniae* L.; 4) *Arenaria grandiflora* subsp. *grandiflora*; 5) *Aubrieta columnae* Guss.; 6)

Centaurea aeolica Guss. Ex Lojac.; 7) *Cerastium tomentosum* L.; 8) *Colutea arborescens* L.; 9) *Cressa cretica* L.; 10) *Daphne oleoides* Schreb.; 11) *Edraianthus graminifolius* A.DC.; 12*) Emerus major* Mill.; 13) *Erica cinerea* L.; 14) *Euphorbia gasparrinii* Boiss.; 15) *Fumana laevipes* Spach; 16) *Glandora rosmarinifolia* (Ten.) D.C.Thomas; 17) *Helianthemum croceum* (Desf.) Pers.; 18) *Hexaphylla rupestris* (Tineo) P.Caputo & Del Guacchio; 19) *Lomelosia crenata* (Cirillo) Greuter & Burdet; 20) *Minuartia geniculata* Thell.; 21) *Onosma echioides* (L.) L.; 22) *Rosa caesia* Sm.; 23) *Salsola vermiculata* L.; 24) *Searsia pentaphylla* (Jacq.) F.A.Barkley ex Moffett, 25) *Sideritis italica* (Miller) Greuter & Burdet; 26) *Thymelaea hirsuta* Endl.; 27) *Tripolium sorrentinoi* (Tod.) Raimondo & Greuter; 28) *Zygophyllum creticum* (L.) Christenh. & Byng.

4.4. Discussion

The survey highlighted the floristic diversity of the Mediterranean region, which is influenced by various factors such as topographical, biogeographical, ecological, and climatic heterogeneity, as well as human interference that has significantly impacted the vegetation. The current levels of endemism—many of the taxa identified in the review are endemic—and biodiversity are the result of these interactions (Rundel and Cowling 2013; FAO 2018). Italy's geographical position as a peninsula with numerous islands is a key factor explaining its biodiversity (Conti et al. 2005; Tavşanoğlu and Pausas 2018).

Urban populations rely on various ecosystem services provided by natural areas. However, the expansion of urban environments and the consequent destruction of natural habitats have increased the reliance on ornamental green spaces to deliver ecosystem services for the benefit of city residents (Francini et al. 2022). Among these services, biodiversity conservation plays a crucial role. Introducing native plants can contribute significantly to conservation efforts, especially when these plants are rare or threatened. Many species identified in our study possess these characteristics, which enhances the value of their use. Incorporating native plants into urban landscapes not only boosts biodiversity but also creates environments capable of providing essential ecosystem goods and services within cities.

Public awareness of the importance of biodiversity and the benefits derived from incorporating regional flora into urban landscape design is now well established (Lovell and Johnston 2009; Ahern 2013; Phondani et al. 2016).

The demand for native plants in landscaping and gardening, driven by ecological concerns, is increasing. Therefore, research is necessary to identify suitable native plants and to evaluate whether their use can enhance biodiversity resilience and improve the capacity of native plant landscapes to provide ecosystem services for a growing urban

population (Martinson 2020). The most common benefits of green spaces include the reduction of pollution (particulate matter, heavy metals, ozone), mitigation of high temperatures, reduction of surface runoff by preventing erosion, slope stabilization, carbon dioxide sequestration, and oxygen emission (Francini et al. 2021, 2022; Leotta et al. 2023a).

Green infrastructure (GI) plays a crucial role in biodiversity conservation. Conservation gardening has recently been promoted as a strategy to mitigate the decline of native plant species (Affolter 1997; Gratzfeld 2017; Ismail et al. 2021; Mounce et al. 2017). This concept has emerged as a participatory approach to restoring biodiversity by cultivating declining native species in private gardens, integrating commercial horticulture with conservation efforts, and encouraging public engagement (Munschek et al. 2023; Segar et al. 2022). Investigating the role of gardens as dispersal corridors for native and endangered plant species could provide valuable insights for collectively addressing the biodiversity crisis (Staude 2022).

The species identified in our review could therefore contribute to enhancing the native biodiversity of cities; however, the feasibility of their actual inclusion still needs to be evaluated.

The long-term sustainability of landscapes utilizing native plant resources requires management to consider not only ornamental purposes but also ecological and environmental values (e.g., soil, water, biodiversity) and socio-economic factors (Muhtaman et al. 2000; Burlando et al. 2025).

The biodiversity of urban green spaces is crucial because it mitigates the risks posed by pests, diseases, and climate change, thereby enhancing the resilience of the ecosystem services provided by green infrastructure (Leotta et al. 2023a).

Depending on their hardiness, shrubs play a fundamental ecological role in green areas (Pasalodos-Tato et al. 2015). In natural environments, the shrub stage represents a secondary succession that

can stimulate, facilitate, or positively interact with community dynamics. In fact, shrub species, which act as nurse plants, can promote the germination and growth of woody forest species beneath their canopy by improving the water status of seedlings through reducing radiation, lowering soil temperature, conserving moisture, and protecting them from herbivore damage. These effects are also influenced by the shrubs' functional traits and morphological characteristics (Bruno et al. 2003; Castro et al. 2004; Manaut et al. 2011). Shrubs are pioneer, branched plant species that are highly adaptable and strongly interactive with other species (Götmark et al. 2016).

In Mediterranean ecosystems, shrubs are a characteristic type of vegetation, widespread across various habitats. Due to multiple factors—including physiological, morphological, reproductive, phenological, and regenerative traits, as well as inter- and intraspecific interactions—each shrub species constitutes an important component of the plant community and fulfills a specific ecological role (Lombardo et al. 2000). The most critical morphological traits for abiotic stress tolerance are leaf morphology and functionality, particularly the ability to reduce gas exchange and enhance metabolite accumulation (Leotta et al. 2023a; Toscano et al. 2020).

The high biodiversity potential offered by the identified plant species—over 540 taxa belonging to 68 botanical families—can ensure the goal of enhancing the sustainability of green spaces. Native shrubs, due to their hardiness, are well-suited for new urban green design systems that reduce maintenance costs, partly through more naturalistic planting (Nam and Dempsey 2019). This approach enables an increase in biodiversity even within urban environments (Capotorti et al. 2020; Bonthoux et al. 2019; Pomatto et al. 2023).

Biological form helps us understand the characteristics of plants and their ability to adapt to marginal conditions. In our review, the most common type was chamaephytes. The specific morphological traits of

chamaephytes can vary greatly depending on the species and environmental factors. Chamaephyte plants are characterized by low growth close to the ground, which allows them to survive in harsh conditions such as cold climates, where the warmth of the soil aids their survival, or drought conditions, due to reduced transpiration resulting from the plant's cushion-like shape. Many chamaephytes possess adaptations that enable them to endure these challenging environments. Additionally, survival in harsh conditions is facilitated by a reduction in leaf size, which minimizes water loss.

The list is well represented by caespitose phanerophytes, which are plants characterized by the formation of dense clumps or groups of stems arising from a common base. Nanophanerophytes, typically low-growing woody plants that usually reach only a few meters in height, are also included in the list compiled by our study.

The potential for domesticating endemic plants for ornamental purposes is underscored by the inadequate ex situ conservation of local endemic species in botanical gardens and seed banks (Krigas et al. 2021). Another challenge is the lack of studies on assessment systems for the rapid identification of suitable landscapes and garden plants. Key characteristics to consider include plant habit and height, vegetative and flowering periods, flower traits, modes of reproduction, tolerance to biotic and abiotic stresses, and associated survival rates (Liu et al. 2016). Utilizing these plants in landscapes and gardens can also contribute to the conservation of endemic species at risk of extinction.

An interesting aspect that emerged from this study was the variability in the heights attained. In many cases, the plants did not exceed 20 cm in height; therefore, the species identified can be used as ground cover. Ground cover plants serve as components or connecting elements in other green infrastructure interventions (Woods Ballard et al. 2015), such as rain gardens, green roofs, or rainwater harvesting tanks (Nur Hannah Ismail et al. 2023).

Their small size—over 54% of the species surveyed do not exceed 60 cm—makes them particularly suitable for new types of greenery, such as extensive green roofs (Leotta et al. 2023b; Savi et al. 2016). Their limited height is linked to their biological form: in 58% of cases, they are chamaephytes. The height of the plants surveyed, often less than one meter, allows for a wide variety of uses and plays an important ecological role in plant communities. The cushion shape, which minimizes the exposed surface area of the plant relative to its volume, contributes to their tolerance of abiotic stresses, as observed in the Mediterranean region.

Based on the information available for each of the accessions surveyed, we can only hypothesize about their possible uses, which appear to be quite diverse. The different species had an average of 2.1 uses. However, only experimental trials conducted in representative contexts will be able to accurately assess the actual uses of the accessions surveyed.

Aesthetic aspects play a crucial role in urban landscape design. The beauty of plants is primarily conveyed through their visual appearance, which is inherently linked to color. Ornamental plants contribute vibrant color to urban green spaces (Whang 2021). Additionally, the landscape created by ornamental plants evolves over time, meaning that the color of plants across different seasons has a significant chromatic impact. The color of plants, particularly their flowers, is therefore a key element in crafting an appealing urban landscape (Wang 2021). The survey revealed that plants typically flower for several months; notably, it is possible to have varying blooms—and thus different colors—throughout the year. Many of the species identified were also valued for their combined aesthetic qualities related to flowers, foliage, and fruit.

Today, ornamental plants are valued not only for their aesthetic appeal but also for their ability to enhance the environment and improve quality of life (Savé 2009). A large number of Mediterranean origin

species, particularly shrubs, are well-suited for sustainable landscaping due to their capacity to thrive in harsh conditions and tolerate drought stress. Unlike agriculture, the success of an ornamental landscape is not measured by yield but by its ability to satisfy user expectations while requiring minimal maintenance. In degraded environments, plant survival may be the primary objective of cultivation (Toscano et al. 2019). Many of these plants, owing to their origin, resilience in marginal settings, distinctive morphobiometric traits, and attractive ornamental qualities, appear well equipped to meet the current demands of urban landscaping.

4.5. Conclusions

The Mediterranean region is a rich source of biodiversity for the ornamental sector, hosting a wide variety of plant species suitable for urban and peri-urban landscapes. Although morphological and physiological traits vary among botanical families, they remain of significant interest. The ornamental value is derived from leaves, flowers, and fruits, each contributing to aesthetic appeal at different times or seasons. Consequently, combining various ornamental species can extend the visual appeal of an area throughout the year. In this study, the most important genera and species were identified for use in successive experimental tests aimed at selecting native plants capable of adapting to urban environments. This approach aims to enhance the sustainability of urban green spaces while revitalizing the biological diversity of ornamental floriculture.

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Supplementary Material

Supplementary Table 1. Characteristics of Mediterranean shrub species selected for the potential landscape use.

Species	Family	Flowering phenology[1]	Biological Form[2]	Origin	Height class (cm)	Showy organ[3]	Flower color	Nursery presence	Proposed uses [4]
Acer monspessulanum L.	Sapindaceae	IV	Pcaesp/Pscap	Euri-Medit.	>140	Fr	yellowish	yes	Sl
Achillea maritima subsp. *maritima*	Asteraceae	V-VIII	Chsuffr	Medit.-Atl.(Euri-)	20-60	Le	yellow	no	Gr, Xe, Wf
Achyranthes aspera var. *sicula* L.	Amaranthaceae	II-V	Chsuffr	SW-Medit.-Mont.	60-100	Fl	red-purple	no	Xe
Adenocarpus complicatus (L.) Gay	Fabaceae	V-VI	NP/Chsuffr	Endem. Ital.	20-60	Fl	yellow-orange	yes	Gr, Xe
Adenocarpus complicatus subsp. *bivonae* (C.Presl) Peruzzi	Fabaceae	IV-VI	NP	Endem. Ital.	20-60	Fl	yellow-orange	yes	Gr, Xe
Adenocarpus complicatus subsp. *brutius* (Brullo, De Marco & Siracusa) Peruzzi & Bernardo	Fabaceae	V-VIII	Chsuffr/NP	Endem. Ital.	20-60	Fl	yellow-orange	yes	Gr, Xe
Adenocarpus complicatus subsp. *commutatus* (Guss.) Cout.	Fabaceae	IV-VII	NP	Endem. Ital.	20-60	Fl	yellow-orange	yes	Gr, Xe
Adenocarpus complicatus subsp. *samniticus* (Brullo, De Marco & Siracusa) Peruzzi	Fabaceae	IV-VI	NP	Endem. Ital.	20-60	Fl	yellow-orange	yes	Gr, Xe
Adenocarpus complicatus subsp. *tenoreanus* (Brullo, Gangale & Uzunov) Peruzzi & Bernardo	Fabaceae	V-VIII	NP	Endem. Ital.	20-60	Fl	yellow-orange	yes	Gr, Xe
Aeonium arboreum Webb & Berthel.	Crassulaceae	XII-III	NP	Macarones.	60-100	Fl, Le	yellow	yes	Gr, Po, Xe
Aethionema saxatile (L.) W.T.Aiton	Brassicaceae	III-VI	Chsuffr	Medit.-Mont.	<20	Fl	rose	no	Gr
Ajuga iva (L.) Schreb.	Lamiaceae	II-VI	Chsuffr	Steno-Medit.	<20	Fl	rose-yellow	no	Gr, Xe
Ajuga iva subsp. *iva*	Lamiaceae	III-X	Chsuffr	Steno-Medit.	<20	Fl	rose-yellow	no	Gr, Xe
Ajuga iva subsp. *pseudoiva* (DC.) Briq.	Lamiaceae	IV-VI	Chsuffr	Steno-Medit.	<20	Fl	rose-yellow	no	Gr, Xe
Alnus glutinosa (L.) Gaertn.	Betulaceae	III-IV	Pscap/Pcaeps	Eurosiber.	>140	Le	yellowish	yes	Sl
Aloe vera (L.) Burm. f.	Asphodelaceae	IV-VI	NP	Paleotrop.	100-140	Fl, Le	yellow	yes	Gr, Po, Xe
Amelanchier ovalis (Willd.) Borkh.	Rosaceae	IV-V	Pcaesp	Medit.	>140	Fl, Fr	white	yes	Sl
Anagyris foetida L.	Fabaceae	II-IV	Pcaesp	S-Medit.	100-140	Fl	yellow	no	Sl
Andrachne telephioides L.	Phyllanthaceae	II-VII	Chsuffr/NP	Euri-Medit.-Merid.	20-60	Fr	greenish	no	Gr, Xe
Anthemis aetnensis Schouw	Asteraceae	V-VIII	Chsuffr	Endem. Sic.	<20	Fl	white	no	Ng
Anthemis cotula L.	Asteraceae	V-IX	Chsuffr	Eurimedit.	20-60	Fl	white	no	Gr, Wf
Anthemis cupaniana Tod. ex Nyman	Asteraceae	IV-VI	Chsuffr	Endem. Sic.	20-60	Fl	white	no	Ng
Anthemis ismelia Lojac.	Asteraceae	IV-VI	Chsuffr	Endem. Sic.	20-60	Fl	white	no	Ng
Anthyllis barba-jovis L.	Fabaceae	III-V	Pcaesp	Steno-Medit.-Occid.	>140	Fl	whitish	yes	Sl, Xe
Anthyllis hermanniae L.	Fabaceae	IV-VII	Chfrut	Steno-Medit.	20-60	Fl	yellow	yes	Gr, Xe

Anthyllis hermanniae subsp. *brutia* Brullo & Giusso	Fabaceae	V-VII	Chfrut	Endem. Ital.	20-60	Fl	yellow	yes	Gr, Xe
Anthyllis hermanniae subsp. *corsica* Brullo & Giusso	Fabaceae	V-VII	Chfrut	Endem. Ital.	20-60	Fl	yellow	yes	Gr, Xe
Anthyllis hermanniae subsp. *ichnusae* Brullo & Giusso	Fabaceae	V-VII	Chfrut	Endem. Sar(-Cor)	20-60	Fl	yellow	yes	Gr, Xe
Anthyllis hermanniae subsp. *japygica* Brullo & Giusso	Fabaceae	V-VII	Chfrut	Endem. Ital.	20-60	Fl	yellow	yes	Gr, Xe
Anthyllis hermanniae subsp. *sicula* Brullo & Giusso	Fabaceae	V-VII	Chfrut	Endem. Sic.	20-60	Fl	yellow	yes	Ng
Antirrhinum majus L.	Plantaginaceae	I-XII	Chfrut	W-Medit.	60-100	Fl	purple	yes	Wf
Antirrhinum siculum Mill.	Plantaginaceae	I-XII	Chfrut	Endem. Ital.	20-60	Fl	white-yellowish	no	Ng, Wf
Antirrhinum tortuosum Bosc ex Lam.	Plantaginaceae	II-VI	Chfrut	W-Stenomedit.	60-100	Fl	purple	no	Wf
Apteranthes europaea subsp. *europaea*	Apocynaceae	IV-VIII	Chsucc	SW-Medit.	<20	Fl	dark red	no	Gr, Po
Arbutus unedo L.	Ericaceae	VIII-XI	Pcaesp/Pscap	Steno-Medit.	20-60	Fl, Fr, Le	white	yes	Sl, Xe
Arenaria grandiflora subsp. *grandiflora*	Caryophyllaceae	V-VII	Chsuffr	W-Medit.-Mont.	<20	Fl	white	no	Gr, Po
Aria umbellata (Desf.) Sennikov & Kurtto	Rosaceae	IV-VI	Pcaesp/Pscap	Pontica	>140	Fl, Fr	white	no	Sl
Aristolochia sempervirens L.	Aristolochiaceae	VI-VII	Plian	Steno-Medit.-Merid.	>140	Fl	purple and yellow	yes	Ng
Artemisia alba Turra	Asteraceae	VIII-X	Chsuffr	Steno-Medit.	20-60	Le	whitish	no	Gr, Xe
Artemisia arborescens L.	Asteraceae	V-VIII	NP/Pcaeps	S-Medit.	60-100	Le	yellowish	yes	Sl, Xe
Artemisia campestris L.	Asteraceae	VII-X	Chsuffr	Circumbor.	20-60	Le	greenish	no	Xe
Artemisia campestris subsp. *alpina* (DC.) Arcang.	Asteraceae	VIII-X	Chsuffr	Circumbor.	20-60	Le	greenish	no	Xe
Artemisia campestris subsp. *campestris*	Asteraceae	VIII-X	Chsuffr	Eurasiat.	20-60	Le	greenish	no	Xe
Artemisia campestris subsp. *glutinosa* (J.Gay ex Besser) Batt.	Asteraceae	VIII-X	Chsuffr	W-Medit.	20-60	Le	greenish	no	Xe
Artemisia campestris subsp. *variabilis* (Ten.) Greuter	Asteraceae	VIII-X	Chfrut/Chsuffr	Endem. Ital.	20-60	Le	greenish	no	Xe
Arthrocaulon macrostachyum (Moric.) Piirainen & G.Kadereit	Amaranthaceae	IV-IX	Chsucc	Medit.	60-100	Le	yellowish	no	Sl, Xe
Arthrocnemum macrostachyum (Moric.) K.Koch	Amaranthaceae	VIII-IX	Chsucc/Psucc	Medit.	60-100	Le	yellowish	no	Sl, Xe
Asparagus acutifolius L.	Asparagaceae	VIII-IX	Grhiz/NP	Steno-Medit.	100-140	Le	yellowish-green	no	Xe
Asparagus albus L.	Asparagaceae	VII-X	Chfrut/NP	Steno-Medit.-Occid.	60-100	Le	white	no	Xe
Asparagus aphyllus L.	Asparagaceae	VIII-X	Chfrut	S-Stenomedit.	60-100	Le	yellowish	no	Xe
Asparagus horridus L.	Asparagaceae	III-V	NP	S-Stenomedit.	100-140	Le	yellowish-green	no	Xe
Asparagus pastorianus Webb & Berthel.	Asparagaceae	XII-IV	Chfrut/NP	SW-Medit.	100-140	Le	white	no	Xe
Astragalus nebrodensis (Guss.) Strobl	Fabaceae	VI-VII	Chfrut/NP	Endem. Sic.	20-60	Fl, Le	yellow	no	Ng
Astragalus siculus Biv.	Fabaceae	VI-VIII	Chfrut/NP	Endem. Sic.	20-60	Fl	rose	no	Ng
Atriplex glauca L.	Amaranthaceae	IV-IX	NP	Saharo-Sind.	>140	Le	greenish	no	Sl, Xe
Atriplex halimus L.	Amaranthaceae	VII-X	Pcaeps	Steno-Medit.	>140	Le	greenish	yes	Sl, Xe
Atriplex portulacoides L.	Amaranthaceae	V-VII	Chfrut/Prept	Circumbor.	60-100	Le	reddish	no	Sl, Xe
Aubrieta columnae Guss.	Brassicaceae	IV-VI	Chsuffr	NE-Medit.	<20	Fl	violet	no	Gr, Po, Xe
Aubrieta columnae subsp. *columnae*	Brassicaceae	IV-VI	Chsuffr	Endem. Ital.	<20	Fl	violet	no	Gr, Po, Xe

Aubrieta columnae subsp. *italica* (Boiss.) Mattf.	Brassicaceae	V-VI	Chsuffr	Endem. Ital.	<20	Fl	violet	no	Gr, Po, Xe
Aubrieta columnae subsp. *sicula* (Strobl) M.Koch, D.A.German & R.Karl	Brassicaceae	V-VI	Chsuffr	Endem. Sic.	<20	Fl	rose	no	Ng
Ballota hispanica Benth.	Lamiaceae	III-VIII	Chfrut	NE-Medit.	20-60	Fl	rose	no	Ng, Sl
Berberis aetnensis C.Presl	Berberidaceae	V-VI	NP	Endem. Ital.	20-60	Fl, Fr	yellow	no	Ng
Brassica incana Ten.	Brassicaceae	II-IV	Chsuffr	Subendem.	60-100	Fl	yellow	no	Ng
Brassica insularis Moris	Brassicaceae	III-V	Chsuffr	Medit. Subendem.	60-100	Fl	white	no	Ng
Brassica macrocarpa Guss.	Brassicaceae	I-III	Chsuffr	Endem. Sic.	20-60	Fl	yellow	no	Ng
Brassica rupestris Raf. subsp. *rupestris*	Brassicaceae	III-IV	Chsuffr	Endem. Ital.	60-100	Fl	yellow	no	Ng
Brassica rupestris subsp. *hispida* Raimondo & P.Mazzola	Brassicaceae	III-IV	Chsuffr	Endem. Sic.	60-100	Fl	yellow	no	Ng
Brassica villosa subsp. *bivoniana* (Mazzola & Raimondo) Raimondo & P.Mazzola	Brassicaceae	III-IV	Chsuffr	Endem. Sic.	20-60	Fl	yellow	no	Ng
Brassica villosa subsp. *brevisiliqua* (Raimondo & Mazzola) Raimondo & Geraci	Brassicaceae	III-IV	Chsuffr	Endem. Sic.	20-60	Fl	yellow	no	Ng
Brassica villosa subsp. *drepanensis* (Caruel) Raimondo & P.Mazzola	Brassicaceae	II-V	Chsuffr	Endem. Sic.	20-60	Fl	yellow	no	Ng
Brassica villosa subsp. *tinei* (Lojac.) Raimondo & P.Mazzola	Brassicaceae	III-IV	Chsuffr	Endem. Sic.	20-60	Fl	yellow	no	Ng
Brassica villosa subsp. *villosa*	Brassicaceae	I-V	Chsuffr	Endem. Sic.	20-60	Fl	yellow	no	Ng
Brassica villosa Biv. ex Spreng.	Brassicaceae	I-V	Chsuffr	Endem. Sic.	20-60	Fl	yellow	no	Ng
Bupleurum dianthifolium Guss.	Apiaceae	V-VI	Chsuffr	Endem. Sic.	20-60	Fl	yellow	no	Ng
Bupleurum fruticosum L.	Apiaceae	VI-VII	NP	Steno-Medit.	100-140	Fl	yellow	yes	Gr, Ng, Xe
Calendula suffruticosa subsp. *fulgida* (Raf.) Guadagno	Asteraceae	XI-IV	Chsuffr	Endem. Sic.	20-60	Fl	orange	no	Ng
Calendula suffruticosa subsp. *maritima* (Guss.) Meikle	Asteraceae	I-XII	Chsuffr	Endem. Ital.	20-60	Fl	yellow	no	Ng
Calendula suffruticosa Vahl	Asteraceae	XII-IV	Chsuffr	SW-Medit.	20-60	Fl	orange	no	Gr, Wf
Calicotome infesta (C.Presl) Guss.	Fabaceae	III-V	Pcaesp	Stenomedit.	>140	Fl	yellow	no	Sl, Xe
Calicotome intermedia (Salzm. ex C.Presl) Boiss. ex Rchb.f.	Fabaceae	IV-V	Pcaesp	Steno-Medit.-Occid.	>140	Fl	yellow	no	Sl, Xe
Calicotome spinosa (L.) Link	Fabaceae	III-V	Pcaesp	Steno-Medit.	>140	Fl	yellow	yes	Sl, Xe
Calicotome villosa (Poir.) Link	Fabaceae	III-V	Pcaesp	Steno-Medit.	>140	Fl	yellow	no	Sl, Xe
Campanula marcenoi Brullo	Campanulaceae	VI-VIII	Chsuffr	Paleotemp.	<20	Fl	violet	no	Gr, Po
Camphorosma monspeliaca L.	Amaranthaceae	V-IX	Chfrut	Medit.	20-60	Le	greenish	no	Xe
Capparis orientalis Duhamel	Capparaceae	IV-IX	NP	Steno-Medit.	60-100	Fl	white	no	Gr, Xe
Capparis spinosa L.	Capparaceae	V-VI	NP	Eurasiat.	60-100	Fl	white	yes	Gr, Po, Xe
Capparis spinosa var. *canescens* Coss.	Capparaceae	IV-VI	NP	Steno-Medit.	60-100	Fl	white	yes	Gr, Xe
Capparis spinosa var. *ovata* (Desf.) Fici	Capparaceae	V-VI	NP	Eurasiat.	60-100	Fl	white	yes	Gr, Xe
Caroxylon agrigentinum (Guss.) C.Brullo, Brullo, Giusso, Guarino & Iamonico	Amaranthaceae	V-VII	NP	Endem. Sic.	20-60	Fl, Le	rose	no	Ng
Carpinus orientalis Mill.	Betulaceae	IV-V	Pcaeps/Pscap	Pontica	>140	Le	greenish	yes	Sl
Celtis tournefortii subsp. *aetnensis* (Tornab.) Raimondo & Schicchi	Cannabaceae	V	Pcaesp	Endem. Sic.	>140	Le	yellowish	no	Ng
Celtis tournefortii Lam.	Cannabaceae	IV-V	Pcaeps	NW-Medit.	>140	Le	yellowish	no	Sl

Centaurea aeolica Guss. ex Lojac.	Asteraceae	V-VII	Chsuffr	Endem. Ital.	20-60	Fl	rose	no	Ng, Wf
Centaurea erycina Raimondo & Bancheva	Asteraceae	V-VII	Chros	Endem. Sic.	100-140	Fl	rose-violet	no	Ng, Wf
Centaurea panormitana subsp. *umbrosa* (Fiori) Greuter	Asteraceae	V-VIII	Chsuffr	Endem. Sic.	60-100	Fl	violet	no	Ng, Wf
Centaurea panormitana Lojac.	Asteraceae	V-VIII	Chsuffr	Endem. Sic.	60-100	Fl	violet	no	Ng, Wf
Centaurea saccensis Raimondo, Bancheva & Ilardi	Asteraceae	IV-V	Chsuffr	Endem. Sic.	60-100	Fl	violet	no	Ng, Wf
Centaurea seguenzae (Lacaita) Domina, Greuter & Raimondo	Asteraceae	V-VIII	Chsuffr	Endem. Sic.	20-60	Fl	violet	no	Ng, Wf
Centaurea tauromenitana Guss.	Asteraceae	IV-VI	Chfrut	Endem. Sic.	60-100	Fl	yellow	no	Ng, Wf
Centaurea todaroi Lacaita	Asteraceae	V-VIII	Chsuffr	Endem. Sic.	60-100	Fl	violet	no	Ng, Wf
Cerastium tomentosum L.	Caryophyllaceae	VI-VIII	Chsuffr	Endem. Ital.	20-60	Fl	white	yes	Gr, Ng, Po
Ceratonia siliqua L.	Fabaceae	IX-XI	Pcaesp/Pscap	S-Medit.	>140	Le	yellow-reddish	yes	Ng, Sl
Chamaerops humilis L.	Arecaceae	V-VI	NP/Pscap	Steno-Medit.-Occid.	>140	Le	yellowish	yes	Ng, Po, Xe
Chiliadenus lopadusanus Brullo	Asteraceae	VI-VIII	Chfrut	Endem. Sic.	20-60	Fl	yellow	no	Ng, Xe
Cichorium spinosum L.	Asteraceae	V-VI	Chsuffr	Steno-Medit.	<20	Fl	light blue	no	Xe
Cistus clusii Dunal	Cistaceae	IV-V	NP	W-Medit.	60-100	Fl	white	yes	Ng, Xe
Cistus creticus L.	Cistaceae	V-VI	NP	W-Steno-Medit.	60-100	Fl	deep pink, light purple	yes	Ng, Xe
Cistus creticus subsp. *eriocephalus* (Viv.) Greuter & Burdet	Cistaceae	III-V	NP	W-Stenomedit.	60-100	Fl	deep pink, light purple	yes	Ng, Xe
Cistus creticus L. subsp. *corsicus* (Loisel.) Greuter & Burdet	Cistaceae	V-VI	NP	W-Steno-Medit.	60-100	Fl	deep pink, light purple	yes	Ng, Xe
Cistus creticus L. subsp. *creticus*	Cistaceae	V-VI	NP	W-Steno-Medit.	60-100	Fl	deep pink, light purple	yes	Ng, Xe
Cistus crispus L.	Cistaceae	IV-V	NP	W-Medit.	20-60	Fl	deep pink	yes	Ng, Xe
Cistus monspeliensis L.	Cistaceae	IV-V	NP	Steno-Medit.	60-100	Fl	white	yes	Ng, Xe
Cistus parviflorus Lam.	Cistaceae	IV-V	NP	E-Medit.	60-100	Fl	rose	yes	Ng, Xe
Cistus salvifolius L.	Cistaceae	IV-V	NP	Steno-Medit.	20-60	Fl	white	yes	Ng, Xe
Clematis cirrhosa L.	Ranunculaceae	X-XII	Plian	Medit.-Turan.	>140	Fl	white or red	yes	Ng
Clematis flammula L.	Ranunculaceae	V-VIII	Plian/Hscap	Euri-Medit.	>140	Fl	white	yes	Ng
Clematis vitalba L.	Ranunculaceae	V-VIII	Plian	Euri-Medit.	>140	Fl	whitish	yes	Ng
Clinopodium alpinum subsp. *meridionale* (Nyman) Govaerts	Lamiaceae	V-VII	Chsuffr	Orof. S-Europ.	20-60	Fl	violet or purple	no	Gr, Po
Colutea arborescens L.	Fabaceae	V-VII	Pcaesp	Euri-Medit.	>140	Fl	yellow	no	Sl, Xe
Convolvulus cneorum L.	Convolvulaceae	IV-V	Chsuffr	N-Medit.	20-60	Fl	white with yellow throat	yes	Gr, Po
Convolvulus lineatus L.	Convolvulaceae	III-VI	Chsuffr	Steno-Medit.	<20	Fl	pale pink	no	Gr, Ng
Cornus mas L.	Cornaceae	I-IV	Pcaesp	S-Europ.-Sudsib.	>140	Fl, Fr	yellow	yes	Ng, Sl

Cornus sanguinea L.	Cornaceae	IV-VI	Pcaesp	Eurasiat.	>140	Fl, Fr	creamy white	yes	Ng, Sl
Coronilla valentina L.	Fabaceae	I-V	NP	SW-Medit.	20-60	Fl	yellow	yes	Po, Xe
Corylus avellana L.	Betulaceae	III-IV	Pcaesp	Europ.	>140	Fl, Le	yellow	yes	Ng, Sl
Cotoneaster nebrodensis K.Koch	Rosaceae	IV-V	NP	Endem. Ital.	>140	Fr	white or pale pink	no	Ng, Sl
Crataegus germanica (L.) Kuntze	Rosaceae	IV-VI	Pcaesp	S-Europ.-Sudsib.	>140	Fl, Fr	white	no	Ng, Sl
Crataegus laciniata Ucria	Rosaceae	IV-V	Pcaesp/Pscap	S-Medit.	>140	Fl, Fr	white	no	Ng, Sl
Crataegus laevigata (Poir.) DC.	Rosaceae	III-VI	Pcaesp	C-Europ.	>140	Fl, Fr	white	yes	Ng, Sl
Crataegus monogyna Jacq.	Rosaceae	IV-V	Pcaesp/Pscap	Eurasiat.	>140	Fl, Fr	white	yes	Ng, Sl
Crataegus orientalis subsp. *orientalis*	Rosaceae	V-VI	Pcaesp	S-Stenomedit.	>140	Fl, Fr	white	no	Ng, Sl
Crataegus orientalis subsp. *presliana* K.I.Chr.	Rosaceae	III-V	Pcaesp	W-Medit.	>140	Fl, Fr	white	no	Ng, Sl
Crataegus orientalis (Mill.) M.Bieb.	Rosaceae	IV-V	Pcaesp	S-Stenomedit.	>140	Fl, Fr	white	no	Ng, Sl
Cressa cretica L.	Convolvulaceae	V-VII	Chsuffr	Cosmop.	20-60	Fl	pinkish white	no	Gr, Xe
Crithmum maritimum L.	Apiaceae	VI-VIII	Chsuffr	Euri-Medit.	20-60	Fl, Le	white greenish	yes	Gr, Xe
Crucianella maritima L.	Rubiaceae	V-VII	Chsuffr	S-Medit.	20-60	Le	yellow	no	Xe
Cymbalaria pubescens (J.Presl & C.Presl) Cufod.	Plantaginaceae	III-VI	Chrept	Endem. Sic.	<20	Fl, Le	white	no	Ng
Cynanchica gussonei (Boiss.) P.Caputo & Del Guacchio	Rubiaceae	V-VIII	Chsuffr	Endem. Ital.	<20	Fl	rose	no	Ng, Xe
Cynanchica peloritana (C.Brullo, Brullo, Giusso & Scuderi) P.Caputo & Del Guacchio	Rubiaceae	IV-VI	Chsuffr	Endem. Sic.	<20	Fl	white	no	Ng
Cynanchum acutum L.	Apocynaceae	VII-VIII	Plian	Paleosubtrop.	>140	Fl, Le	pinkish white	no	Ng
Cytisus aeolicus Guss.	Fabaceae	III-IV	Pcaesp/Pscap	Endem. Ital.	>140	Fl	yellow	no	Ng, Sl, Xe
Cytisus scoparius (L.) Link	Fabaceae	V-VI	Pcaesp	Europ.	>140	Fl	yellow	yes	Ng, Sl, Xe
Cytisus villosus Pourr.	Fabaceae	II-IV	Pcaesp	Steno-Medit.	100-140	Fl	yellow	no	Ng, Sl, Xe
Daphne gnidium L.	Thymelaeaceae	VII-IX	Pcaesp	Steno-Medit.	100-140	Fl, Fr	white	no	Ng, Sl, Xe
Daphne laureola L.	Thymelaeaceae	II-IV	Pcaesp	Steno-Medit.	100-140	Fl, Fr	yellowish-green	no	Ng, Sl, Xe
Daphne oleoides Schreb.	Thymelaeaceae	IV-VI	Chfrut/NP	Eurasiat.	20-60	Fl, Fr	white	no	Ng, Sl, Xe
Daphne sericea Vahl	Thymelaeaceae	II-III	NP	E-Medit.	100-140	Fl	rose	yes	Ng, Sl, Xe
Dianthus arrostoi C.Presl	Caryophyllaceae	IV-VI	Chsuffr	SW-Medit.	20-60	Fl	rose	no	Ng
Dianthus busambrae Soldano & F.Conti	Caryophyllaceae	IV-VII	Chsuffr	Endem. Sic.	<20	Fl	purple rose	no	Ng
Dianthus cyathophorus Moris	Caryophyllaceae	IV-VI	Chsuffr	Endem. Ital.	<20	Fl	pale pink	no	Ng
Dianthus cyathophorus subsp. *cyathophorus*	Caryophyllaceae	IV-V	Chsuffr	Endem. Sar(-Cor)	<20	Fl	pale pink	no	Ng
Dianthus cyathophorus subsp. *minae* (Mazzola, Raimondo & Ilardi) Raimondo	Caryophyllaceae	V-VI	Chsuffr	Endem. Sic.	<20	Fl	pale pink	no	Ng
Dianthus gasparrinii Guss.	Caryophyllaceae	VII-X	Chsuffr	Endem. Ital.	<20	Fl	rose	no	Ng

Dianthus graminifolius C.Presl	Caryophyllaceae	IV-VII	Chsuffr	Endem. Sic.	20-60	Fl	deep pink	no	Ng
Dianthus rupicola Biv.	Caryophyllaceae	V-IX	Chsuffr	Subendem.	20-60	Fl	deep pink	yes	Gr, Ng
Dianthus rupicola subsp. *aeolicus* (Lojac.) Brullo & Miniss.	Caryophyllaceae	V-IX	Chsuffr	Endem. Sic.	20-60	Fl	deep pink	yes	Ng
Dianthus rupicola subsp. *lopadusanus* Brullo & Miniss.	Caryophyllaceae	V-IX	Chsuffr	Endem. Sic.	20-60	Fl	deep pink	yes	Ng
Dianthus rupicola subsp. *rupicola*	Caryophyllaceae	V-X	Chsuffr	Subendem.	20-60	Fl	deep pink	yes	Gr, Ng
Dianthus siculus C.Presl	Caryophyllaceae	IV-VII	Chsuffr	Subendem.	<20	Fl	deep pink	no	Ng
Diplotaxis harra subsp. *crassifolia* (Raf.) Maire	Brassicaceae	IV-V	Chsuffr	S-Medit.	60-100	Fl	yellow	no	Ng, Wf
Edraianthus graminifolius subsp. *graminifolius*	Campanulaceae	V-VII	Chsuffr	E-Medit.	<20	Fl	violet	no	Ng, Po
Edraianthus graminifolius subsp. *siculus* (Strobl) Greuter & Burdet	Campanulaceae	IV-VII	Chsuffr	Endem. Ital.	<20	Fl	violet	no	Ng, Po
Edraianthus graminifolius A.DC.	Campanulaceae	IV-VII	Chsuffr	E-Medit.	<20	Fl	violet	no	Ng, Po
Emerus major Mill.	Fabaceae	I-XI	NP	C-Europ.	>140	Fl	yellow	no	Sl, Xe
Emerus major subsp. *emeroides* (Boiss. & Spruner) Soldano & F. Conti	Fabaceae	I-X	NP	E-Medit.	>140	Fl	yellow	no	Sl, Xe
Emerus major subsp. *major*	Fabaceae	IV-VI	NP	C-Europ.	>140	Fl	yellow	no	Sl, Xe
Eokochia saxicola (Guss.) Freitag & G.Kadereit	Amaranthaceae	VII-X	Chsuffr	Endem. Ital.	20-60	Le	greenish	no	Sl, Xe
Ephedra distachya L.	Ephedraceae	III-VI	NP	Steno-Medit.-Nordoccid.	60-100	Fr	yellowish	no	Sl, Xe
Ephedra fragilis Desf.	Ephedraceae	IV-V	NP	Steno-Medit.	60-100	Fr	yellowish	no	Sl, Xe
Ephedra major subsp. *major*	Ephedraceae	III-VI	NP	Steno-Medit.-Merid.	100-140	Fr	yellowish	no	Sl, Xe
Erica arborea L.	Ericaceae	III-V	Pcaesp/NP	Steno-Medit.	>140	Fl	white	yes	Sl, Xe
Erica cinerea L.	Ericaceae	V-VII	Chfrut/NP	Steno-Medit.	20-60	Fl	pink-purple	yes	Gr, Xe
Erica manipuliflora Salisb.	Ericaceae	VIII-IX	Chsuffr/NP	Steno-Medit.-Orient.	60-100	Fl	deep pink	no	Ng, Xe
Erica multiflora L.	Ericaceae	VIII-XI	NP/Pcaesp	Steno-Medit.	100-140	Fl	pinkish-purple	yes	Ng, Xe
Erica multiflora subsp. *hyblaea* Domina & Raimondo	Ericaceae	IX-XI	NP	Endem. Sic.	100-140	Fl	pinkish-purple	yes	Ng
Erica multiflora subsp. *multiflora*	Ericaceae	IX-XI	NP	Stenomedit.	100-140	Fl	pinkish-purple	yes	Ng, Xe
Erica sicula subsp. *sicula*	Ericaceae	IV	Chfrut	Endem. Sic.	100-140	Fl	white-pink	no	Ng
Erysimum cheiri Crantz	Brassicaceae	III-VI	Chsuffr	Euri-Medit.	20-60	Fl	yellow-orange	yes	Wf
Euonymus europaeus L.	Celastraceae	IV-VI	Pcaesp/Pscap	Eurasiat.	>140	Fr	white	yes	Ng, Sl
Euphorbia amygdaloides L.	Euphorbiaceae	II-VI	Chsuffr	Europ.-Caucas.	20-60	Fl	yellowish green	no	Ng, Xe
Euphorbia biumbellata Poir.	Euphorbiaceae	IV-V	Chsuffr	W-Stenomedit.	20-60	Fl	yellowish	no	Ng, Xe
Euphorbia bivonae Steud.	Euphorbiaceae	X-II	Chsuffr	Endem. Sic.	100-140	Fl	yellowish	no	Ng, Xe
Euphorbia ceratocarpa Ten.	Euphorbiaceae	III-VII	Chsuffr	Endem. Ital.	60-100	Fl	yellowish	no	Ng, Xe
Euphorbia characias L.	Euphorbiaceae	I-IV	NP	Steno-Medit.	100-140	Fl	yellowish	yes	Ng, Xe
Euphorbia characias subsp. *wulfenii* (Hoppe ex W.D.J.Koch) Radcl.-Sm.	Euphorbiaceae	I-IV	NP	Steno-Medit.	100-140	Fl	yellowish	yes	Ng, Xe
Euphorbia dendroides L.	Euphorbiaceae	X-IV	NP	Steno-Medit.	>140	Fl	yellowish	no	Sl, Xe

Euphorbia gasparrinii Boiss.	Euphorbiaceae	IV-VI	Chsuffr	Endem. Ital.	20-60	Fl	yellowish	no	Ng, Xe
Euphorbia meuselii Geltman	Euphorbiaceae	III-VI	Chsuffr	Endem. Ital.	20-60	Fl	greenish	no	Ng, Xe
Euphorbia myrsinites L.	Euphorbiaceae	IV-VI	Chrept	Pontica	20-60	Fl, Le	reddish	yes	Ng, Xe
Euphorbia papillaris (Boiss.) Raffaelli & Ricceri	Euphorbiaceae	X-II	NP	Endem. Sic.	20-60	Fl, Fr	yellowish	no	Ng
Euphorbia paralias L.	Euphorbiaceae	III-VIII	Chfrut	Eurimedit.	20-60	Fl	yellowish	no	Ng, Xe
Euphorbia pithyusa L.	Euphorbiaceae	IV-VIII	Chsuffr	W-Medit.	60-100	Fl	yellowish	no	Ng, Xe
Euphorbia pithyusa subsp. *cupanii* (Guss. ex Bertol.) Radcl.-Sm.	Euphorbiaceae	III-VII	Chsuffr	Endem. Ital.	60-100	Fl	yellowish	no	Ng, Xe
Euphorbia pithyusa subsp. *pithyusa*	Euphorbiaceae	IV-VII	Chsuffr	Steno-Medit.-Occid.	60-100	Fl	yellowish	no	Ng, Xe
Euphorbia rigida M.Bieb.	Euphorbiaceae	II-IV	Chsuffr	Pontica	20-60	Fl, Fr	yellow	no	Ng, Xe
Euphorbia segetalis L.	Euphorbiaceae	II-VI	Chsuffr	W-Medit.	<20	Fl	yellowish	no	Ng, Xe
Euphorbia segetalis var. *pinea* (L.) Lange	Euphorbiaceae	III-VI	Chsuffr	W-Medit.	<20	Fl	yellowish	no	Ng, Xe
Fontanesia phillyreoides Labill.	Oleaceae	III-V	Pcaesp	E-Medit.	>140	Fl	white	yes	Sl
Frankenia hirsuta L.	Frankeniaceae	IV-VI	Chsuffr	Steno-Medit.	20-60	Fl	purple	no	Gr, Xe
Frankenia laevis L.	Frankeniaceae	IV-VI	Chsuffr	Steno-Medit.	<20	Fl	light pink	yes	Gr, Xe
Fumana arabica Spach	Cistaceae	IV-V	Chsuffr	Medit.-Turan.	<20	Fl	yellow	no	Gr, Ng, Xe
Fumana juniperina (Lag. ex Dunal) Pau	Cistaceae	II-V	Chsuffr	W-Medit.	<20	Fl	yellow	no	Gr, Ng, Xe
Fumana laevipes Spach	Cistaceae	IV-V	Chsuffr	Steno-Medit.	<20	Fl	yellow	no	Gr, Ng, Xe
Fumana laevis (Cav.) Pau	Cistaceae	III-VI	Chsuffr	Steno-Medit.	<20	Fl	yellow	no	Gr, Ng, Xe
Fumana procumbens Gren. & Godr.	Cistaceae	V-VI	Chsuffr	Medit.-Turan.	<20	Fl	yellow	no	Gr, Ng, Xe
Fumana scoparia Pomel	Cistaceae	III-V	Chsuffr	Steno-Medit.	<20	Fl	yellow	no	Gr, Ng, Xe
Fumana thymifolia Spach	Cistaceae	IV-VI	Chsuffr	Steno-Medit.	<20	Fl	yellow	no	Gr, Ng, Xe
Genista aetnensis (Biv.) DC.	Fabaceae	V-VI	Pcaesp/Pscap	Endem. Ital.	>140	Fl	yellow	yes	Ng, Sl
Genista aristata C.Presl	Fabaceae	V-VI	Chsuff/NP	Endem. Sic.	20-60	Fl	yellow	no	Ng
Genista aspalathoides Lam.	Fabaceae	II-III	NP	Subendem.	20-60	Fl	yellow	no	Ng, Xe
Genista cupanii Guss.	Fabaceae	VI-VII	Chsuff/NP	Endem. Sic.	20-60	Fl	yellow	no	Ng
Genista demarcoi Brullo, Scelsi & Siracusa	Fabaceae	IV-VI	NP	Endem. Sic.	60-100	Fl	yellow	no	Ng
Genista ephedroides DC.	Fabaceae	V-VI	NP	Endem. Ital.	100-140	Fl	yellow	no	Ng, Sl, Xe
Genista gasparrinii (Guss.) C.Presl	Fabaceae	III-V	NP	Endem. Ital.	20-60	Fl	yellow	no	Ng, Xe
Genista madoniensis Raimondo	Fabaceae	III-V	NP	Endem. Sic.	>140	Fl	yellow	no	Ng
Genista monspessulana (L.) L.A.S.Johnson	Fabaceae	III-V	Pcaesp	Steno-Medit.	100-140	Fl	yellow	no	Ng, Xe
Genista tyrrhena Vals.	Fabaceae	III-V	Pcaesp	Endem. Ital.	>140	Fl	yellow	no	Ng, Sl, Xe
Glandora rosmarinifolia (Ten.) D.C.Thomas	Boraginaceae	XI-IV	Chsuffr	Subendem.	20-60	Fl	blue	yes	Ng, Po
Globularia alypum L.	Plantaginaceae	X-III	Chfrut/NP	Steno-Medit.	20-60	Fl	sky blue	no	Ng, Po, Xe
Gypsophila arrostii Guss.	Caryophyllaceae	IV-VI	Chsuffr	Endem. Ital.	<20	Fl	white	no	Ng, Xe
Halocnemum strobilaceum (Pall.) M.Bieb.	Amaranthaceae	VIII-X	Chsucc/Psucc	Medit.	60-100	Le	greenish	no	Xe
Hedera helix L.	Araliaceae	VIII-XII	Plian	Submedit.	>140	Fr, Le	greenish	yes	Ng
Helianthemum apenninum Mill.	Cistaceae	IV-VI	Chsuffr	Steno-Medit.	<20	Fl	white	no	Ng, Po

Helianthemum canum (L.) Baumg.	Cistaceae	V-VII	Chsuffr	Europ.-Caucas.	<20	Fl	yellow	no	Ng, Po
Helianthemum cinereum subsp. *rotundifolium* (Dunal) Greuter & Burdet	Cistaceae	IV-V	Chsuffr	SW-Medit.	<20	Fl	yellow	no	Ng, Po
Helianthemum cinereum Pers.	Cistaceae	IV-V	Chsuffr	SW-Medit.	<20	Fl	yellow	no	Ng, Po
Helianthemum croceum (Desf.) Pers.	Cistaceae	V-VII	Chsuffr	W-Medit.	<20	Fl	yellow	no	Ng, Po
Helianthemum lippii (L.) Dum.Cours.	Cistaceae	III-V	NP	S-Medit.	20-60	Fl	yellow	no	Ng, Po
Helianthemum nummularium subsp. *berteroanum* (Bertol.) Breistr.	Cistaceae	V-VIII	Chsuffr	Europ.-Caucas.	20-60	Fl	yellow	no	Gr, Ng, Po
Helianthemum nummularium subsp. *glabrum* (W.D.J.Koch) Wilczek	Cistaceae	V-VIII	Chsuffr	SE-Europ.	20-60	Fl	yellow	no	Gr, Ng, Po
Helianthemum nummularium subsp. *grandiflorum* (Scop.) Schinz &	Cistaceae	V-VIII	Chsuffr	Orof. S-Europ.	20-60	Fl	yellow	no	Gr, Ng, Po
Helianthemum nummularium subsp. *nummularium*	Cistaceae	V-VIII	Chsuffr	Europ.-Caucas.	20-60	Fl	yellow	no	Gr, Ng, Po
Helianthemum nummularium subsp. *obscurum* (Čelak.) Holub	Cistaceae	V-VIII	Chsuffr	Europ.	20-60	Fl	yellow	no	Gr, Ng, Po
Helianthemum nummularium subsp. *semiglabrum* (Badarò) M.Proctor	Cistaceae	V-VIII	Chsuffr	Alpico-Appenn.	20-60	Fl	yellow	no	Gr, Ng, Po
Helianthemum nummularium subsp. *tomentosum* (Scop.) Schinz & Thell.	Cistaceae	V-VIII	Chsuffr	Europ.-Caucas.	20-60	Fl	yellow	no	Gr, Ng, Po
Helianthemum nummularium Mill.	Cistaceae	IV-VIII	Chsuffr	Europ.-Caucas.	20-60	Fl	yellow	yes	Gr, Ng, Po
Helianthemum oelandicum subsp. *allionii* (Tineo) Greuter & Burdet	Cistaceae	IV-VII	Chsuffr	Endem. Ital.	<20	Fl	yellow	no	Gr, Ng, Po
Helianthemum oelandicum subsp. *alpestris* (Jacq.) Breistr.	Cistaceae	V-VIII	Chsuffr	Orof. S-Europ.	<20	Fl	yellow	no	Gr, Ng, Po
Helianthemum oelandicum subsp. *incanum* (Willk.) G. López González	Cistaceae	IV-VII	Chsuffr	Europ.-Caucas.	<20	Fl	yellow	no	Gr, Ng, Po
Helianthemum oelandicum subsp. *italicum* (L.) Font Quer & Rothm.	Cistaceae	IV-VII	Chsuffr	Orof. SW-Europ.	<20	Fl	yellow	no	Gr, Ng, Po
Helianthemum oelandicum subsp. *nebrodense* (Heldr. ex Guss.) Greuter & Burdet	Cistaceae	IV-VI	Chsuffr	Endem. Sic.	<20	Fl	yellow	no	Gr, Ng, Po
Helianthemum oelandicum subsp. *pourretii* (Timb.-Lagr.) Greuter & Burdet	Cistaceae	IV-VI	Chsuffr	Subendem.	<20	Fl	yellow	no	Gr, Ng, Po
Helianthemum oelandicum (L.) DC.	Cistaceae	V-VIII	Chsuffr	Europ.-Caucas.	<20	Fl	yellow	no	Gr, Ng, Po
Helianthemum sicanorum Brullo, Giusso & Sciandr.	Cistaceae	I-VI	Chsuffr	Endem. Sic.	20-60	Fl	yellow	no	Ng
Helichrysum errerae Tineo	Asteraceae	IV-VII	Chsuffr	Endem. Sic.	60-100	Fl	yellow	no	Ng
Helichrysum italicum subsp. *italicum*	Asteraceae	V-VI	Chsuffr	S-Europ.	20-60	Fl	yellow	yes	Ng, Xe, Wf
Helichrysum italicum subsp. *siculum* (Jord. & Fourr.) Galbany, L.Sáez & Benedí	Asteraceae	V-VI	Chsuffr	Endem. Sic.	20-60	Fl	yellow	yes	Ng, Xe, Wf
Helichrysum italicum subsp. *tyrrhenicum* (Bacch., Brullo & Giusso) Herrando, J.M.Blanco, L.Sáez & Galbany	Asteraceae	IV-IX	Chsuffr	Euri-Medit.	20-60	Fl	yellow	yes	Ng, Xe, Wf
Helichrysum italicum (Roth) G.Don	Asteraceae	V-IX	Chsuffr	S-Europ.	20-60	Fl	yellow	yes	Ng, Xe, Wf
Helichrysum litoreum Guss.	Asteraceae	IV-VII	Chsuffr	Endem. Ital.	20-60	Fl	yellow	no	Ng, Xe
Helichrysum pendulum C.Presl	Asteraceae	IV-VII	Chsuffr	Medit.	20-60	Fl, Le	yellow	no	Ng, Xe, Wf
Helichrysum stoechas subsp. *barrelieri* (Ten.) Nyman	Asteraceae	V-VII	Chsuffr	Steno-Medit.-Orient.	20-60	Fl	yellow	yes	Ng, Xe, Wf
Helichrysum stoechas subsp. *stoechas*	Asteraceae	IV-VII	Chsuffr	Endem. Sic.	20-60	Fl	yellow	yes	Ng, Xe, Wf
Helichrysum stoechas (L.) Moench	Asteraceae	IV-VII	Chsuffr	Steno-Medit.	20-60	Fl	yellow	yes	Ng, Xe, Wf
Herniaria fontanesii subsp. *empedocleana* (Lojac.) Brullo	Caryophyllaceae	III-V	Chsuffr	Endem. Sic.	<20	Le	greenish	no	Ng
Hexaphylla rupestris (Tineo) P.Caputo & Del Guacchio	Rubiaceae	III-V	Chsuffr	Endem. Ital.	<20	Fl	light pink	no	Ng, Xe

Hieracium lucidum subsp. *cophanense* (Lojac.) Greuter	Asteraceae	IV-XI	Chsuffr	Endem. Sic.	<20	Fl	yellow	no	Ng
Hippocrepis emerus (L.) Lassen	Fabaceae	I-X	NP	C-Europ.	20-60	Fl	yellow	yes	Ng, Sl, Xe
Humulus lupulus L.	Cannabaceae	IV-VIII	Plian	Circumbor.	>140	Fl, Le	greenish	no	Ng
Hypericum aegypticum L.	Hypericaceae	I-VI	Chfrut	S-Stenomedit.	20-60	Fl	yellow	no	Gr, Ng, Xe
Hypericum androsaemum L.	Hypericaceae	V-VII	NP	Subatl.	60-100	Fl	yellow	yes	Gr, Ng, Xe
Hypericum hircinum L.	Hypericaceae	V-VIII	NP	Steno-Medit.	>140	Fl	yellow	no	Ng, Sl, Xe
Hypericum hircinum subsp. *hircinum*	Hypericaceae	V-VIII	NP	Endem. Ital.	>140	Fl	yellow	no	Ng, Sl, Xe
Hypericum hircinum subsp. *majus* (Aiton) N.Robson	Hypericaceae	V-VIII	NP	Steno-Medit.	>140	Fl	yellow	no	Ng, Sl, Xe
Iberis semperflorens L.	Brassicaceae	IX-V	Chsuffr	C-Medit.	20-60	Fl	white	yes	Gr, Po, Xe
Ilex aquifolium L.	Aquifoliaceae	IV-V	Pcaesp/Pscap	Submedit.	>140	Fl, Fr, Le	white	yes	Ng, Sl
Jacobaea ambigua subsp. *ambigua*	Asteraceae	VII-XI	Chsuffr	Endem. Sic.	20-60	Fl, Le	yellow	no	Ng
Jacobaea ambigua (Biv.) Pelser & Veldkamp	Asteraceae	VI-VIII	Chsuffr	Endem. Sic.	20-60	Fl, Le	yellow	no	Ng
Jacobaea candida (C.Presl) B.Nord. & Greuter	Asteraceae	VI-IX	Chsuffr	Endem. Sic.	20-60	Fl, Le	yellow	no	Ng
Jacobaea gibbosa (Guss.) B.Nord. & Greuter	Asteraceae	V-VII	Chsuffr	Endem. Ital.	20-60	Fl, Le	yellow	no	Ng, Xe
Jacobaea lycopifolia (Poir.) Greuter & B.Nord.	Asteraceae	VII-XI	Chsuffr	Endem. Ital.	20-60	Fl	yellow	no	Ng, Xe
Jacobaea maritima subsp. *bicolor* (Willd.) B.Nord. & Greuter	Asteraceae	V-VII	Chsuffr	Endem. Ital.	20-60	Fl, Le	yellow	yes	Ng, Xe
Jacobaea maritima subsp. *maritima*	Asteraceae	V-VIII	Chsuffr	Steno-Medit.	20-60	Fl	yellow	yes	Ng, Xe
Jacobaea maritima subsp. *sicula* N.G.Passal., Peruzzi & Pellegrino	Asteraceae	V-VII	Chsuffr	W-Medit.-Mont.	20-60	Fl	yellow	yes	Ng, Xe
Jacobaea maritima (L.) Pelser & Meijden	Asteraceae	IV-VIII	Chsuffr	Medit.	20-60	Fl	yellow	yes	Ng, Xe
Juniperus communis L.	Cupressaceae	I-VII	Pcaesp/Pscap	Medit.-Mont.	>140	Fl, Le	greenish	yes	Ng, Sl, Xe
Juniperus macrocarpa Sm.	Cupressaceae	I-IV	Pcaesp	Eurimedit.	>140	Fl, Le	greenish	yes	Ng, Sl, Xe
Juniperus oxycedrus L.	Cupressaceae	I-IV	Pcaesp/Pscap	Euri-Medit.	>140	Fl, Le	greenish	yes	Ng, Sl, Xe
Juniperus phoenicea L.	Cupressaceae	III-IV	Pcaesp/Pscap	Euri-Medit.	>140	Fl, Le	greenish	yes	Ng, Sl, Xe
Juniperus turbinata Guss.	Cupressaceae	X-XI	Pcaesp	W-Medit.	>140	Fl, Le	greenish	no	Ng, Sl, Xe
Launaea fragilis (Asso) Pau	Asteraceae	III-VII	Chfrut	Saharo-Sind.	20-60	Fl	yellow	no	Ng
Laurus nobilis L.	Lauraceae	II-IV	Pcaesp/Pscap	Steno-Medit.	>140	Fr, Le	yellowish	yes	Ng, Sl, Xe
Lavandula multifida L.	Lamiaceae	II-IV	Chfrut	Steno-Medit.-Occid.	20-60	Fl	blue	no	Gr, Xe
Lavandula stoechas L.	Lamiaceae	III-V	NP/Pcaesp	Steno-Medit.	20-60	Fl	blue	yes	Gr, Po, Xe
Ligustrum vulgare L.	Oleaceae	III-V	NP/Pcaesp	Eurasiat.	>140	Fl, Le	white	yes	Ng, Sl
Limbarda crithmoides (L.) Dumort.	Asteraceae	VII-XI	Chsuffr	Medit.-Atl.(Steno-)	20-60	Fl	yellow	no	Gr, Xe
Limoniastrum monopetalum Boiss.	Plumbaginaceae	V-VIII	Chfrut/NP	SW-Medit.	100-140	Fl	violet	yes	Ng, Sl, Xe
Limonium albidum (Guss.) Pignatti	Plumbaginaceae	V-VIII	Chpulv	Endem. Sic.	<20	Fl	light pink	no	Ng
Limonium bocconei (Lojac.) Litard.	Plumbaginaceae	V-VII	Chsuffr	Endem. Sic.	20-60	Fl	violet	no	Ng
Limonium catanense (Tineo ex Lojac.) Brullo	Plumbaginaceae	V-VIII	Chsuffr	Endem. Sic.	20-60	Fl	blue	no	Ng
Limonium catanzaroi Brullo	Plumbaginaceae	V-VIII	Chsuffr	Endem. Sic.	20-60	Fl	violet	no	Ng
Limonium cosyrense Kuntze	Plumbaginaceae	V-VII	Chsuffr	Endem. Sic.	20-60	Fl	violet	no	Ng
Limonium dubium (Andrz. ex Guss.) Litard.	Plumbaginaceae	V-VIII	Chsuffr	Stenomedit.	20-60	Fl	rose	no	Ng

Limonium flagellare (Lojac.) Brullo	Plumbaginaceae	V-VII	Chsuffr	Endem. Sic.	20-60	Fl	violet	no	Ng
Limonium furnarii Brullo	Plumbaginaceae	V-VIII	Chsuffr	Endem. Sic.	20-60	Fl	violet	no	Ng
Limonium glomeratum (Tausch) Erben	Plumbaginaceae	V-VIII	Chsuffr	W-Steno-Medit.	20-60	Fl	rose	no	Ng
Limonium panormitanum (Tod.) Pignatti	Plumbaginaceae	V-VIII	Chpulv	Endem. Sic.	<20	Fl	sky blue	no	Ng
Limonium tenuiculum (Tineo ex Guss.) Pignatti	Plumbaginaceae	V-VII	Chsuffr	Endem. Sic.	20-60	Fl	sky blue	no	Ng
Limonium usticanum Giardina & Raimondo	Plumbaginaceae	V-VII	Chsuffr	Endem. Sic.	20-60	Fl	sky blue	no	Ng
Lomelosia crenata subsp. *crenata*	Caprifoliaceae	V-VIII	Chsuffr	Orof. S-Medit	<20	Fl	rose	no	Ng, Po
Lomelosia crenata subsp. *dallaportae* (Heldr. ex Boiss.) Greuter & Burdet	Caprifoliaceae	V-VIII	Chsuffr	Subendem.	<20	Fl	rose	no	Ng, Po
Lomelosia crenata (Cirillo) Greuter & Burdet	Caprifoliaceae	IV-VIII	Chsuffr	S-Medit.	<20	Fl	rose	no	Ng, Po
Lomelosia cretica (L.) Greuter & Burdet	Caprifoliaceae	III-VIII	Chfrut	Steno-Medit.	20-60	Fl	violet	no	Gr, Ng, Po
Lonicera etrusca Santi	Caprifoliaceae	V-VI	Plian/Pcaesp	Euri-Medit.	100-140	Fl	purple rose	yes	Ng, Sl
Lonicera implexa Aiton	Caprifoliaceae	V-VI	Plian/Pcaesp	Steno-Medit.	100-140	Fl	rose	no	Ng, Sl
Lonicera xylosteum L.	Caprifoliaceae	V-VII	Pcaesp	Eurasiat.	>140	Fl	white	yes	Ng, Sl
Loranthus europaeus Jacq.	Loranthaceae	IV-V	Pep	Europ.-Caucas.	60-100	Fr	white	no	Ng
Lotus creticus L.	Fabaceae	II-VI	Chsuffr	Steno-Medit.	<20	Fl	yellow	yes	Gr, Ng, Xe
Lotus cytisoides L.	Fabaceae	III-VI	Chsuffr	Steno-Medit.	<20	Fl	yellow	no	Gr, Ng, Xe
Lotus hirsutus L.	Fabaceae	V-VI	Chsuffr	Euri-Medit.	20-60	Fl	pinkish white	no	Gr, Ng, Xe
Lotus longisiliquosus R.Roem.	Fabaceae	III-VI	Chsuffr	S-Medit.	<20	Fl	yellow	no	Gr, Ng, Xe
Lycium europaeum L.	Solanaceae	III-IX	NP	Euri-Medit.	>140	Fl	light purple	no	Ng, Sl
Lycium shawii Roem. & Schult.	Solanaceae	III-IX	NP	S-Stenomedit.	>140	Fr	rose	no	Ng, Sl
Malva flava (Desf.) Alef.	Malvaceae	III-V	NP	Endem.	60-100	Fl	white	no	Ng, Xe, Wf
Malva olbia Alef.	Malvaceae	III-VI	Pcaesp	Steno-Medit.	100-140	Fl	rose	no	Ng, Xe
Malva unguiculata Alef.	Malvaceae	III-VI	Pcaesp	E-Medit.	>140	Fl	violet	no	Ng, Xe
Matthiola fruticulosa subsp. *fruticulosa*	Brassicaceae	II-VI	Chsuffr	Subendem.	20-60	Fl	violet	no	Ng, Xe, Wf
Matthiola fruticulosa subsp. *coronopifolia* (Sm.) Giardina & Raimondo	Brassicaceae	II-VI	Chsuffr	Subendem.	20-60	Fl	violet	no	Ng, Xe, Wf
Matthiola fruticulosa subsp. *valesiaca* (J.Gay ex Gaudin) P.W.Ball	Brassicaceae	IV-VIII	Chsuffr	Subendem.	20-60	Fl	violet	no	Ng, Xe, Wf
Matthiola fruticulosa (L.) Maire	Brassicaceae	II-VI	Chsuffr	Subendem.	20-60	Fl	violet	no	Ng, Xe, Wf
Matthiola incana subsp. *incana*	Brassicaceae	II-V	Chsuffr	Steno-Medit.	20-60	Fl	violet	no	Ng, Xe, Wf
Matthiola incana subsp. *pulchella* (Tineo ex Guss.) Brullo & Furnari	Brassicaceae	II-V	Chsuffr	Endem. Sic.	20-60	Fl	violet	no	Ng
Matthiola incana subsp. *rupestris* (Kuntze) Nyman	Brassicaceae	II-V	Chsuffr	Subendem.	20-60	Fl	violet	no	Ng, Xe, Wf
Matthiola incana (L.) W.T.Aiton	Brassicaceae	II-V	Chsuffr/NP	Steno-Medit.	20-60	Fl	violet	no	Ng, Xe, Wf
Mcneillia rosanoi subsp. *clandestina* (Port.) Del Guacchio & F.Conti	Caryophyllaceae	V-VII	Chsuffr	Endem. Ital.	<20	Fl	white	no	Ng, Xe
Mcneillia rosanoi subsp. *moraldoi* (F.Conti) Del Guacchio & F.Conti	Caryophyllaceae	V-VIII	Chsuffr	Endem. Ital.	<20	Fl	white	no	Ng, Xe
Mcneillia rosanoi subsp. *rosanoi*	Caryophyllaceae	VI-VIII	Chsuffr	Endem. Ital.	<20	Fl	white	no	Ng, Xe
Mcneillia rosanoi (Ten.) F.Conti & Del Guacchio	Caryophyllaceae	V-VII	Chsuffr	Anfiadriat.	<20	Fl	white	no	Ng, Xe

Medicago arborea L.	Fabaceae	IV-VI	Pcaesp	NE-Medit.	>140	Fl	yellow	yes	Ng, Sl
Medicago marina L.	Fabaceae	II-VIII	Chrept	Euri-Medit.	20-60	Fl	yellow	no	Ng, Xe
Mespilus germanica L.	Rosaceae	V-VI	Pcaesp/Pscap	Europ.	>140	Fl, Fr	white	yes	Ng, Sl
Micromeria cosentina (Ten.) N. Terracc.	Lamiaceae	VIII-XI	Chsuffr	Endem. Ital.	20-60	Fl	violet	no	Ng, Xe
Micromeria graeca subsp. *fruticulosa* (Bertol.) Guinea	Lamiaceae	IV-VI	Chsuffr	Endem.	20-60	Fl	rose	no	Ng, Xe
Micromeria graeca subsp. *garganica* (Briq.) Guinea	Lamiaceae	IV-VI	Chsuffr	Endem.	20-60	Fl	rose	no	Ng, Xe
Micromeria graeca subsp. *graeca*	Lamiaceae	IV-VI	Chsuffr	Steno-Medit.	20-60	Fl	rose	no	Ng, Xe
Micromeria graeca subsp. *longiflora* (C.Presl) Nyman	Lamiaceae	IV-VI	Chsuffr	Endem.	20-60	Fl	rose	no	Ng, Xe
Micromeria graeca subsp. *tenuifolia* (Ten.) Nyman	Lamiaceae	IV-VI	Chsuffr	Endem.	20-60	Fl	rose	no	Ng, Xe
Micromeria graeca (L.) Benth. ex Rchb.	Lamiaceae	IV-VI	Chsuffr	Steno-Medit.	20-60	Fl	rose	no	Ng, Xe
Micromeria juliana Benth.	Lamiaceae	V-VI	Chsuffr	Steno-Medit.	20-60	Fl	white	no	Ng, Xe
Micromeria microphylla Benth.	Lamiaceae	IV-V	Chsuffr	E-Medit.	<20	Fl	rose	no	Ng, Xe
Micromeria nervosa Benth.	Lamiaceae	IV-V	Chsuffr	S-Medit.	20-60	Fl	rose	no	Ng, Xe
Minuartia geniculata Thell.	Caryophyllaceae	III-VII	Chsuffr	Steno-Medit.	<20	Fl	rose	no	Ng, Xe
Minuartia recurva subsp. *condensata* (C.Presl) Greuter & Burdet	Caryophyllaceae	VII-VIII	Chsuffr	Europ.-Caucas.	<20	Fl	white	no	Ng, Xe
Minuartia recurva subsp. *recurva*	Caryophyllaceae	VII-VIII	Chsuffr	Europ.-Caucas.	<20	Fl	white	no	Ng, Xe
Minuartia recurva (All.) Schinz & Thell.	Caryophyllaceae	VI-VIII	Chsuffr	Europ.-Caucas.	<20	Fl	white	no	Ng, Xe
Myriolimon ferulaceum (L.) Lledó, Erben & M.B.Crespo	Plumbaginaceae	V-VII	Chsuffr	SW-Stenomedit.	20-60	Fl	rose	no	Ng, Xe
Myrtus communis L.	Myrtaceae	V-VII	Pcaesp	Steno-Medit.	>140	Fl, Fr, Le	white	yes	Ng, Sl, Xe
Nerium oleander L.	Apocynaceae	IV-VII	Pcaesp	S-Medit.	>140	Fl	rose	yes	Ng, Sl, Xe
Odontarrhena nebrodensis (Tineo) L.Cecchi & Selvi	Brassicaceae	IV-VII	Chsuffr	Endem. Sic.	<20	Fl	yellow	no	Ng
Odontites bocconei subsp. *angustifolia* (Lojac.) Giardina & Raimondo	Orobanchaceae	VIII-X	Chfrut	Endem. Sic.	20-60	Fl	yellow	no	Ng
Odontites bocconei subsp. *bocconei*	Orobanchaceae	VIII-X	Chfrut	Endem. Sic.	20-60	Fl	yellow	no	Ng
Odontites bocconei (Guss.) Walp.	Orobanchaceae	VIII-X	Chfrut	Endem. Sic.	20-60	Fl	yellow	no	Ng
Olea europaea L.	Oleaceae	III-VI	Pcaesp/Pscap	Steno-Medit.	>140	Fl, Fr, Le	whitish	yes	Ng, Sl, Xe
Ononis hispida Desf.	Fabaceae	V-VIII	NP	SW-Medit.	60-100	Fl	rose	no	Ng, Xe
Ononis minutissima L.	Fabaceae	III-X	Chsuffr	NW-Medit.	<20	Fl	yellow	no	Ng, Xe
Onosma echioides subsp. *angustifolia* (Lehm.) Peruzzi & N.G.Passal.	Boraginaceae	VI-VII	Chsuffr	Endem. Ital.	20-60	Fl	white	no	Ng, Xe, Wf
Onosma echioides subsp. *canescens* (C.Presl) Peruzzi & N.G.Passal.	Boraginaceae	VI-VII	Chsuffr	Endem. Ital.	20-60	Fl	white	no	Ng, Xe, Wf
Onosma echioides subsp. *dalmatica* (Scheele) Peruzzi & N.G.Passal.	Boraginaceae	VI-VII	Chsuffr	Illyrian	20-60	Fl	yellowish	no	Ng, Xe, Wf
Onosma echioides subsp. *echioides*	Boraginaceae	VI-VII	Chsuffr	Endem. Ital.	20-60	Fl	white	no	Ng, Xe, Wf
Onosma echioides (L.) L.	Boraginaceae	VI-VII	Chsuffr	Subendem.	20-60	Fl	white	no	Ng, Xe, Wf
Origanum onites L.	Lamiaceae	IV-VII	Chsuffr	E-Medit.	20-60	Fl, Le	white	no	Ng, Xe
Orthilia secunda (L.) House	Ericaceae	V-VII	Chsuffr	Circumbor.	<20	Fl	white	no	Ng
Ostrya carpinifolia Scop.	Betulaceae	III-VII	Pcaesp/Pscap	Pontica	>140	Fl, Le	yellowish	yes	Ng, Sl
Osyris alba L.	Santalaceae	II-VI	NP/Pcaesp	Euri-Medit.	100-140	Fl, Fr	yellowish	no	Ng, Sl, Xe

Paliurus spina-christi Mill.	Rhamnaceae	IV-VII	Pcaesp	SE-Europ.	>140	Fl, Fr, Le	yellow	yes	Ng, Sl, Xe
Periploca angustifolia Labill.	Apocynaceae	X-III	Pcaesp	S-Medit.	>140	Fl	yellow-red	no	Ng, Sl, Xe
Petrosedum ochroleucum subsp. *mediterraneum* (L.Gallo) Niederle	Crassulaceae	VI-VIII	Chsucc	Endem. Ital.	<20	Fl, Le	yellow	no	Ng, Po, Xe
Petrosedum ochroleucum subsp. *ochroleucum*	Crassulaceae	V-VIII	Chsucc	Medit.-Mont.	<20	Fl	yellow	no	Ng, Po, Xe
Petrosedum ochroleucum (Chaix) Niederle	Crassulaceae	V-VIII	Chsucc	Medit.-Mont.	<20	Fl	yellow	no	Ng, Po, Xe
Petrosedum sediforme subsp. *sediforme*	Crassulaceae	IV-VII	Chsucc	Steno-Medit.	<20	Fl	yellow	no	Ng, Po, Xe
Petrosedum tenuifolium (Sm.) Grulich	Crassulaceae	V-VII	Chsucc	Steno-Medit.	<20	Fl	yellow	no	Ng, Po, Xe
Phagnalon rupestre subsp. *morisianum* (Ces., Pass. & Gibelli) Arcang.	Asteraceae	II-VI	Chsuffr	Endem. Sar(-Cor)	20-60	Fl	yellow	no	Ng, Xe
Phagnalon rupestre subsp. *rupestre*	Asteraceae	II-IV	Chsuffr	SW-Medit.	20-60	Fl	yellow	no	Ng, Xe
Phagnalon rupestre (L.) DC.	Asteraceae	I-VI	Chsuffr	S-Medit.	20-60	Fl	yellow	no	Ng, Xe
Phagnalon saxatile (L.) Cass.	Asteraceae	II-VI	Chsuffr	Steno-Medit.	20-60	Fl	yellow	no	Ng, Xe
Phagnalon sordidum (L.) Rchb.	Asteraceae	V-VII	Chsuffr	W-Medit.	20-60	Fl	yellow	no	Ng, Xe
Phillyrea angustifolia L.	Oleaceae	II-V	Pcaesp	Steno-Medit.	>140	Fl, Fr, Le	white	yes	Ng, Sl, Xe
Phillyrea latifolia L.	Oleaceae	III-IV	Pcaesp/Pscap	Steno-Medit.	>140	Fl, Fr, Le	white	yes	Ng, Sl, Xe
Phlomis fruticosa L.	Lamiaceae	II-V	NP	Steno-Medit.-Sett.	60-100	Fl, Le	yellow	yes	Ng, Sl, Xe
Pimpinella tragium Vill.	Apiaceae	V-VII	Chsuffr	Medit.-Turan.	20-60	Fl	white	no	Ng, Xe
Pistacia lentiscus L.	Anacardiaceae	II-V	Pcaesp/Pscap	S-Medit.	>140	Fl, Fr, Le	red	yes	Ng, Sl, Xe
Pistacia terebinthus L.	Anacardiaceae	II-VI	Pcaesp/Pscap	Euri-Medit.	>140	Fl, Fr, Le	red	yes	Ng, Sl, Xe
Plantago albicans L.	Plantaginaceae	III-V	Chsuffr	S-Medit.	20-60	Le	white	no	Ng, Xe
Plantago subulata L.	Plantaginaceae	IV-VII	Chpulv	W-Medit.	20-60	Fl	greenish	no	Ng, Xe
Plocama calabrica (L.f.) M.Backlund & Thulin	Rubiaceae	V-IX	NP	S-Medit.	60-100	Fl	rose	no	Gr, Ng, Po
Plumbago europaea L.	Plumbaginaceae	VI-X	Chfrut/Grad	Steno-Medit.	60-100	Fl	sky blue	no	Ng, Xe
Polycarpon tetraphyllum subsp. *polycarpoides* (Biv.) Iamonico	Caryophyllaceae	III-VI	Chsuffr	SW-Medit.	<20	Le	white	no	Ng, Xe
Potentilla caulescens L.	Rosaceae	V-IX	Chsuffr	N-Medit.	<20	Fl	white	no	Gr, Ng, Xe
Potentilla caulescens subsp. *caulescens*	Rosaceae	VI-IX	Chsuffr	Endem. Ital.	<20	Fl	white	no	Gr, Ng, Xe
Potentilla caulescens subsp. *nebrodensis* (Strobl ex Zimm.) Arrigoni	Rosaceae	VI-IX	Chsuffr	Endem. Ital.	<20	Fl	white	no	Gr, Ng, Xe
Prunus cocomilia Ten.	Rosaceae	II-IV	Pcaesp/Pscap	NE-Medit.	>140	Fl, Fr, Le	white	no	Ng, Sl
Prunus mahaleb L.	Rosaceae	IV-V	Pcaesp/Pscap	Pontica	>140	Fl, Fr	white	yes	Ng, Sl
Prunus mahaleb subsp. *cupaniana* (Guss. ex Nyman) Raimondo & Spadaro	Rosaceae	III-V	Pcaesp	Endem. Sic.	>140	Fl, Fr	white	yes	Ng
Prunus mahaleb subsp. *mahaleb*	Rosaceae	IV-V	Pcaesp	S-Europ.-Pontica	>140	Fl, Fr	white	yes	Ng, Sl
Prunus persica (L.) Batsch	Rosaceae	III-V	Pcaesp	E-Asiat.	>140	Fl, Fr	rose	yes	Ng, Sl
Prunus spinosa subsp. *spinosa*	Rosaceae	I-IV	Pcaesp	Eurasiat.	>140	Fl, Fr	white	yes	Ng, Sl
Prunus webbii (Spach) Fritsch	Rosaceae	I-III	Pcaesp	E-Medit.	>140	Fl	rose	no	Ng, Sl
Pseudodictamnus mediterraneus subsp. *mediterraneus*	Lamiaceae	V-VII	Chfrut	Steno-Medit.-Orient.	20-60	Fr	greenish	no	Ng, Xe
Pseudoscabiosa limonifolia (Vahl) Devesa	Caprifoliaceae	V-VII	Chsuffr	Endem. Sic.	20-60	Fl	light lilac	no	Ng
Ptilostemon greuteri Raimondo & Domina	Asteraceae	IV-VI	Chfrut	Endem. Sic.	60-100	Fl, Le	rose	no	Ng

Pyrus spinosa Forssk.	Rosaceae	III-V	Pcaesp/Pscap	Eurasiat.	>140	Fl, Fr	white	no	Ng, Sl, Xe
Quercus coccifera L.	Fagaceae	III-V	Pcaesp/Pscap	Steno-Medit.	>140	Fl, Fr, Le	greenish	yes	Ng, Sl
Quercus ilex L.	Fagaceae	III-VI	Pcaesp/Pscap	Steno-Medit.	>140	Fl, Fr, Le	greenish	yes	Ng, Sl
Quercus petraea (Matt.) Liebl.	Fagaceae	III-VI	Pcaesp/Pscap	Europ.	>140	Fl, Fr, Le	greenish	yes	Ng, Sl
Quercus pubescens subsp. *pubescens*	Fagaceae	I-V	Pcaesp/Pscap	NW-Medit.	>140	Fl, Fr, Le	greenish	yes	Ng, Sl
Reaumuria vermiculata L.	Tamaricaceae	VI-IX	NP/Chsuffr	S-Medit.	20-60	Fl, Le	white	no	Gr, Ng, Xe
Retama raetam subsp. *gussonei* (Webb) Greuter	Fabaceae	III-IV	Pcaesp	Endem. Sic.	>140	Fl	white	no	Ng, Xe
Rhamnus alaternus subsp. *alaternus*	Rhamnaceae	I-IV	Pcaesp	Steno-Medit.	>140	Fl, Fr, Le	yellowish	yes	Ng, Sl
Rhamnus cathartica L.	Rhamnaceae	III-VI	Pcaesp/Pscap	Pontica	>140	Fr, Le	greenish	yes	Ng, Sl
Rhamnus lycioides subsp. *oleoides* (L.) Jahand. & Maire	Rhamnaceae	II-V	Pcaesp	S-Medit.	60-100	Fl, Fr, Le	yellowish	yes	Ng, Sl
Rhamnus saxatilis Jacq.	Rhamnaceae	III-V	Pcaesp	Pontica	60-100	Fr, Le	greenish	yes	Ng, Sl, Xe
Rhus coriaria L.	Anacardiaceae	IV-VIII	Pcaesp	S-Medit.	>140	Fl, Fr, Le	greenish	no	Ng, Sl
Ribes uva-crispa L.	Grossulariaceae	IV-VII	NP	Eurasiat.	>140	Fl, Fr	reddish	yes	Ng, Sl
Ribes uva-crispa subsp. *austroeuropaeum* (Bornm.) Bech.	Grossulariaceae	V-VII	NP	Eurasiat.	>140	Fl, Fr	reddish	yes	Ng, Sl
Ribes uva-crispa subsp. *uva-crispa*	Grossulariaceae	V-VII	NP	Eurasiat.	>140	Fl, Fr	reddish	yes	Ng, Sl
Ricinus communis L.	Euphorbiaceae	VI-X	Tscap/Pscap	Paleotrop.	>140	Fl, Fr, Le	yellowish	yes	Ng, Sl, Xe
Rosa agrestis Savi	Rosaceae	IV-VI	NP/Pcaesp	Euri-Medit.	100-140	Fl, Fr	white	no	Ng, Sl
Rosa andegavensis Bastard	Rosaceae	IV-VI	NP	Eurasiat.	>140	Fl, Fr	light pink	no	Ng, Sl
Rosa arvensis Huds.	Rosaceae	V-VII	NP	Steno-Medit.	100-140	Fl, Fr	white	no	Ng, Sl
Rosa balsamica Besser	Rosaceae	III-VI	NP	Paleotemp.	>140	Fl, Fr	white-pink	no	Ng, Sl
Rosa caesia Sm.	Rosaceae	IV-VI	NP	Europ.	>140	Fl, Fr	rose	no	Ng, Sl
Rosa canina L.	Rosaceae	III-VII	NP	Paleotemp.	>140	Fl, Fr	rose	yes	Ng, Sl
Rosa corymbifera Borckh.	Rosaceae	III-VI	NP	N-Europ.	>140	Fl, Fr	light pink	no	Ng, Sl
Rosa dumalis Bechst.	Rosaceae	IV-VII	NP	Europ.-Caucas.	>140	Fl, Fr	light pink	no	Ng, Sl
Rosa gallica L.	Rosaceae	IV-VI	NP	C-Europ.	60-100	Fl, Fr	pink-purple	yes	Ng, Sl
Rosa heckeliana Tratt.	Rosaceae	V-VII	NP	Orof. SE-Europ.	60-100	Fl, Fr	rose	no	Ng, Sl
Rosa micrantha Borrer	Rosaceae	IV-VI	NP	Euri-Medit.	100-140	Fl, Fr	light pink	no	Ng, Sl
Rosa montana Chaix ex Vill.	Rosaceae	V-VII	NP	Medit.-Mont.	100-140	Fl, Fr	light pink	no	Ng, Sl
Rosa pouzinii Tratt.	Rosaceae	III-VI	NP	W-Europ.	>140	Fl, Fr	light pink	no	Ng, Sl
Rosa pulverulenta M.Bieb.	Rosaceae	V-VII	NP	NE-Medit.	>140	Fl, Fr	pink	no	Ng, Sl
Rosa rubiginosa L.	Rosaceae	III-VII	NP	Eurasiat.	100-140	Fl, Fr	light pink	no	Ng, Sl
Rosa sempervirens L.	Rosaceae	IV-VI	NP	Steno-Medit.	>140	Fl, Fr	white	yes	Ng, Sl
Rosa sicula Tratt.	Rosaceae	IV-VI	NP	Medit.-Mont.	20-60	Fl, Fr	light pink	no	Ng, Sl
Rosa spinosissima L.	Rosaceae	IV-VII	NP	Eurasiat.	60-100	Fl, Fr	white	no	Ng, Sl
Rubia peregrina L.	Rubiaceae	IV-VI	Plian	Steno-Medit.	100-140	Fl, Fr	yellowish	no	Ng, Xe
Rubus acheruntinus Ten.	Rosaceae	-	NP	unknown	>140	Fl, Fr	white	no	Ng, Xe
Rubus aetneus Tornab.	Rosaceae	-	NP	Endem. Sic.	>140	Fl, Fr	white	no	Ng, Xe

Rubus caesius L.	Rosaceae	IV-VII	NP/Pcaesp	Eurasiat.	>140	Fl, Fr	white	no	Ng, Xe
Rubus canescens DC.	Rosaceae	IV-VII	NP	Euri-Medit.-Sett.	>140	Fl, Fr	white	no	Ng, Xe
Rubus cupanianus Guss.	Rosaceae	V-VII	NP	Endem. Sic.	>140	Fl, Fr	white	no	Ng, Xe
Rubus hirtus Waldst. & Kit.	Rosaceae	V-VII	NP	C-Europ.	>140	Fl, Fr	white	no	Ng, Xe
Rubus idaeus L.	Rosaceae	IV-VI	NP/Pcaesp	Circumbor.	>140	Fl, Fr	white	yes	Ng, Xe
Rubus ulmifolius Schott	Rosaceae	IV-VII	NP/Pcaesp	Euri-Medit.	>140	Fl, Fr	rose	no	Ng, Xe
Ruscus aculeatus L.	Asparagaceae	I-IV (X-XI)	Grhiz/Chfrut	Euri-Medit.	60-100	Fr, Le	greenish	yes	Ng, Xe
Ruscus hypoglossum L.	Asparagaceae	XI-IV	Grhiz/Chfrut	Euri-Medit.	20-60	Fr, Le	greenish	yes	Ng, Xe
Ruta angustifolia Pers.	Rutaceae	III-VII	Chsuffr	Steno-Medit.-Occid.	60-100	Fl, Le	yellow	no	Ng, Xe
Ruta chalepensis L.	Rutaceae	III-VII	Chsuffr	S-Medit.	60-100	Fl	yellow	no	Ng, Xe
Sabulina verna subsp. *grandiflora* (C.Presl) Dillenb. & Kadereit	Caryophyllaceae	V-VI	Chsuffr	Endem. Ital.	<20	Fl	white	no	Gr, Ng, Xe
Sabulina verna subsp. *verna*	Caryophyllaceae	VI-VIII	Chpulv/Chsuffr	Eurasiat.	<20	Fl	white	no	Gr, Ng, Xe
Sabulina verna Rchb.	Caryophyllaceae	III-VIII	Chpulv/Chsuffr	Eurasiat.	<20	Fl	white	no	Gr, Ng, Xe
Salicornia fruticosa (L.) L.	Amaranthaceae	VIII-IX	Chsucc	Euri-Medit.	60-100	Le	yellowish	yes	Ng, Xe
Salicornia perennis subsp. *alpini* (Lag.) Castrov.	Amaranthaceae	VII-VIII	Chsucc	Euri-Medit.	20-60	Le	yellowish	no	Ng, Xe
Salicornia perennis subsp. *perennis*	Amaranthaceae	VIII-XI	Chsucc	Euri-Medit.	60-100	Fl	yellowish	no	Ng, Xe
Salicornia perennis Mill.	Amaranthaceae	VIII-XI	Chsucc	Euri-Medit.	60-100	Fl	yellowish	no	Ng, Xe
Salix apennina A.K.Skvortsov	Salicaceae	III-V	NP	Subendem.	>140	Fl, Le	white	yes	Ng, Sl
Salix caprea L.	Salicaceae	II-VI	Pcaesp/Pscap	Eurasiat.	>140	Fl, Le	white	yes	Ng, Sl
Salix cinerea L.	Salicaceae	II-IV	Pcaeps	Paleotemp.	>140	Fl, Le	grey	yes	Ng, Sl
Salix fragilis L.	Salicaceae	I-IV	Pcaesp/Pscap	Eurosiber.	>140	Fl, Le	yellowish	no	Ng, Sl
Salix gussonei Brullo & Spamp.	Salicaceae	II-IV	Pcaeps	Endem. Sic.	>140	Fl, Le	whitish	no	Ng
Salix pedicellata Desf.	Salicaceae	II-IV	Pcaesp/Pscap	Steno-Medit.	>140	Fl, Le	whitish	no	Ng, Sl
Salix purpurea L.	Salicaceae	II-IV	Pcaesp/Pscap	Eurasiat.	>140	Fl, Le	red	yes	Ng, Sl
Salsola vermiculata L.	Amaranthaceae	V-VII	NP	S-Steno-Medit.	60-100	Fr	yellowish	no	Ng, Sl, Xe
Salsola verticillata Schousb.	Amaranthaceae	V-VIII	NP/Chcaesp	S-Medit.	60-100	Fr	yellowish	no	Ng, Sl, Xe
Salvia fruticosa subsp. *thomasii* (Lacaita) Brullo, Guglielmo, Pavone & Terrasi	Lamiaceae	IV-VI	Pcaesp	Endem. Ital.	20-60	Fl, Le	light lilac	yes	Gr, Ng, Xe
Salvia officinalis L.	Lamiaceae	II-V	Chsuffr	Steno-Medit.	60-100	Fl, Le	mauvish	yes	Gr, Ng, Xe
Salvia rosmarinus Schleid.	Lamiaceae	I-XII	NP/Pcaesp	Steno-Medit.	100-140	Fl, Le	lilac	yes	Gr, Ng, Xe
Sambucus nigra L.	Viburnaceae	IV-VI	Pcaesp	Europ.	>140	Fl, Fr, Le	white	yes	Ng, Sl
Sarcopoterium spinosum Spach	Rosaceae	II-V	NP	Steno-Medit.	20-60	Fl, Fr, Le	red-purple	no	Gr, Ng, Xe
Saxifraga callosa subsp. *callosa*	Saxifragaceae	IV-VI	Chpulv	Orof. SW-Europ.	20-60	Fl	white	no	Gr, Po
Scrophularia frutescens L.	Scrophulariaceae	III-VI	Chfrut	SW-Medit.	20-60	Fl	violet	no	Ng, Xe
Searsia pentaphylla (Jacq.) F.A.Barkley ex Moffett	Anacardiaceae	XII-IV	Pcaesp	SW-Medit.	>140	Fl, Le	yellowish	no	Ng, Sl, Xe
Searsia tripartita (Ucria) Moffett	Anacardiaceae	I-IV	Pcaesp	S-Medit.	>140	Fl, Le	greenish	no	Ng, Sl, Xe
Sedum acre L.	Crassulaceae	IV-VIII	Chsucc	Europ.-Caucas.	<20	Fl	yellow	yes	Gr, Xe

Sedum album L.	Crassulaceae	V-VIII	Chsucc	Euri-Medit.	<20	Fl	white	yes	Gr, Xe
Sedum album subsp. *album*	Crassulaceae	VI-VII	Chsucc	Euri-Medit.	<20	Fl	white	yes	Gr, Xe
Sedum album subsp. *micranthum* (Bast. ex DC.) Syme	Crassulaceae	VI-VII	Chsucc	Euri-Medit.	<20	Fl	white	yes	Gr, Xe
Sedum dasyphyllum L.	Crassulaceae	IV-VII	Chsucc	Euri-Medit.	<20	Fl, Le	white	yes	Gr, Xe
Sedum dasyphyllum subsp. *glanduliferum* (Guss.) Nyman	Crassulaceae	IV-VII	Chsucc	Euri-Medit.	<20	Fl, Le	white	yes	Gr, Xe
Sedum dasyphyllum var. *dasyphyllum*	Crassulaceae	IV-VII	Chsucc	Euri-Medit.	<20	Fl, Le	white	yes	Gr, Xe
Sedum gypsicola Boiss. & Reut.	Crassulaceae	IV-VI	Chsucc	W-Medit.	<20	Fl, Le	white	no	Gr, Xe
Selaginella denticulata (L.) Spring	Selaginellaceae	I-VII	Chrept	Steno-Medit.	<20	Fr	-	no	Ng
Senecio squalidus subsp. *aethnensis* (DC.) Greuter	Asteraceae	I-XII	Chsuffr	Endem. Ital.	20-60	Fl	yellow	no	Ng, Wf
Sideritis italica (Miller) Greuter & Burdet	Lamiaceae	IV-VII	Chsuffr	Endem. Ital.	20-60	Fr	yellowish	no	Ng, Xe
Silene fruticosa L.	Caryophyllaceae	III-VI	Chsuffr	NE-Medit.	20-60	Fl	rose	no	Gr, Xe
Sinapis pubescens subsp. *pubescens*	Brassicaceae	I-XII	Chsuffr	SW-Medit.	20-60	Fl	yellow	no	Ng, Wf
Smilax aspera L.	Smilacaceae	VIII-XI	NP/Grhiz	Subtrop.	>140	Fl, Fr	white-greenish	yes	Sl, Xe
Solanum dulcamara L.	Solanaceae	IV-VII	NP/Pcaesp	Eurosiber.	100-140	Fl	violet	yes	Ng, Xe
Sorbus aria (L.) Crantz	Rosaceae	IV-VI	Pcaesp/Pscap	Paleotemp.	>140	Fl, Fr	white	yes	Sl
Sorbus aucuparia L.	Rosaceae	IV-VI	Pcaesp/Pscap	Europ.	>140	Fl, Fr, Le	white	yes	Sl
Sorbus aucuparia subsp. *praemorsa* (Guss.) Nyman	Rosaceae	V-VI	Pcaesp	Endem. Ital.	>140	Fl, Fr, Le	white	yes	Sl
Sorbus aucuparia subsp. *aucuparia*	Rosaceae	V-VII	Pcaesp/Pscap	Europ.	>140	Fl, Fr, Le	white	yes	Sl
Sorbus aucuparia subsp. *glabrata* (Wimm. & Grab.) Hedl.	Rosaceae	V-VI	Pcaesp/Pscap	Europ.	>140	Fl, Fr, Le	white	yes	Sl
Sorbus graeca (Spach) Lodd. ex S.Schauer	Rosaceae	IV-VI	Pcaesp/Pscap	Pontica	>140	Fl, Fr, Le	white	no	Sl
Sorbus torminalis (L.) Crantz	Rosaceae	III-V	Pcaesp/Pscap	Paleotemp.	>140	Fl, Fr, Le	white	yes	Sl
Spartium junceum L.	Fabaceae	III-VII	Pcaesp	Euri-Medit.	>140	Fl	yellow	yes	Ng, Sl, Xe
Spergularia rubra J.Presl & C.Presl	Caryophyllaceae	II-VII	Chsuffr	Cosmop.	<20	Fl	violet	no	Gr, Xe
Stachys germanica L.	Lamiaceae	III-VI	Chfrut/NP	Steno-Medit.	20-60	Fl	sky blue	no	Ng, Xe
Suaeda pelagica Bartolo, Brullo & P.Pavone	Amaranthaceae	V-VII	NP	Endem. Sic.	20-60	Le	greenish	no	Ng, Xe
Suaeda vera Forssk. ex J.F.Gmel.	Amaranthaceae	V-VII	NP	Cosmop.	20-60	Le	yellowish	no	Ng, Xe
Suaeda vermiculata Forssk. ex J.F.Gmel.	Amaranthaceae	IV-VIII	NP	Subendem.	20-60	Le	yellowish	no	Ng, Xe
Tamarix africana Poir.	Tamaricaceae	IV-VI	Pcaesp	W-Medit.	>140	Fl	rose	yes	Ng, Sl, Xe
Tamarix canariensis Willd.	Tamaricaceae	III-IV	Pcaesp/Pscap	E-Medit.	>140	Fl	pinkish white	no	Ng, Sl, Xe
Tamarix gallica L.	Tamaricaceae	III-VI	Pcaesp/Pscap	W-Medit.	>140	Fl	rose	yes	Ng, Sl, Xe
Tamarix hampeana Boiss. & Heldr.	Tamaricaceae	III-V	Pcaesp	Steno-Medit.-Orient.	>140	Fl	rose	no	Ng, Sl, Xe
Tamarix tetragyna Ehrenb.	Tamaricaceae	III-X	Pcaesp	S-Medit.	>140	Fl	rose	no	Ng, Sl, Xe
Teucrium capitatum subsp. *capitatum*	Lamiaceae	III-VIII	Chsuffr	Steno-Medit.	20-60	Fl, Le	white	no	Gr, Ng, Xe
Teucrium chamaedrys L.	Lamiaceae	IV-XII	Chsuffr	Euri-Medit.	20-60	Fl	rose	yes	Gr, Ng, Xe
Teucrium flavum L.	Lamiaceae	IV-VII	Chfrut/NP	Steno-Medit.	20-60	Fl	white	no	Gr, Ng, Xe
Teucrium flavum subsp. *flavum*	Lamiaceae	IV-VII	Chfrut	Steno-Medit.	20-60	Fl	white	no	Gr, Ng, Xe

Teucrium flavum subsp. *glaucum* (Jord. & Fourr.) Ronniger	Lamiaceae	IV-VII	Chfrut	Steno-Medit.	20-60	Fl	white	no	Gr, Ng, Xe
Teucrium fruticans subsp. *fruticans*	Lamiaceae	III-V	NP/Pcaesp	Steno-Medit.	100-140	Fl, Le	sky blue	no	Gr, Ng, Xe
Teucrium luteum (Mill.) Degen	Lamiaceae	III-VIII	Chsuffr	Steno-Medit.	20-60	Fl	yellow	no	Gr, Ng, Xe
Teucrium montanum L.	Lamiaceae	IV-VIII	Chsuffr	Orof. S-Europ.	<20	Fl, Le	white	no	Gr, Ng, Xe
Thymbra capitata (L.) Cav.	Lamiaceae	I-XII	Chfrut	Steno-Medit.	20-60	Fl	violet	no	Gr, Ng, Xe
Thymelaea hirsuta Endl.	Thymelaeaceae	VIII-IV	NP/Chsuffr	S-Medit.	60-100	Fl, Le	yellow	no	Ng, Xe
Thymelaea tartonraira subsp. *tartonraira*	Thymelaeaceae	III-V	NP/Chsuffr	Steno-Medit.	60-100	Fl, Le	yellow	no	Ng, Xe
Thymus longicaulis C.Presl	Lamiaceae	V-VII	Chrept	Euri-medit.	<20	Fl, Le	violet	no	Gr, Ng, Xe
Thymus pulegioides L.	Lamiaceae	V-VIII	Chrept /Chsuffr	Eurasiat.	<20	Fl, Le	violet	yes	Gr, Ng, Xe
Thymus richardii subsp. *nitidus* (Guss.) Jalas	Lamiaceae	V-VII	Chrept	Endem. Sic.	<20	Fl	rose	no	Gr, Ng, Xe
Thymus spinulosus Ten.	Lamiaceae	IV-VII	Ch rept	Endem. Ital.	<20	Fl	white	no	Gr, Ng, Xe
Thymus striatus subsp. *acicularis* (Waldst. & Kit.) Ronniger	Lamiaceae	IV-VI	Chrept	SE-Europ.	<20	Fl	rose	no	Gr, Ng, Xe
Thymus striatus subsp. *striatus*	Lamiaceae	IV-VI	Chrept	SE-Europ.	<20	Fl	rose	no	Gr, Ng, Xe
Thymus striatus Vahl	Lamiaceae	IV-VII	Chrept	SE-Europ.	<20	Fl	rose	no	Gr, Ng, Xe
Trachelium caeruleum subsp. *lanceolatum* (Guss.) Arcang.	Campanulaceae	IV-VI	Chsuffr	Endem. Sic.	60-100	Fl	sky blue	no	Gr, Po, Xe
Tripolium sorrentinoi (Tod.) Raimondo & Greuter	Asteraceae	V-XI	Chsuffr	Endem. Sic.	20-60	Fl	rose	no	Ng, Xe
Ulmus minor Mill.	Ulmaceae	I-III	Pcaesp/Pscap	Europ.-Caucas.	>140	Fr	red-purple	yes	Ng, Sl
Ulmus minor subsp. *canescens* Bartolucci & Galasso	Ulmaceae	II-IV	Pcaesp/Pscap	E-Medit.	>140	Fr	red-purple	yes	Ng, Sl
Ulmus minor subsp. *minor*	Ulmaceae	II-III	Pcaesp	Europ.-Caucas.	>140	Fr	red-purple	yes	Ng, Sl
Valeriana rubra L.	Caprifoliaceae	V-VIII	Chsuffr	Steno-Medit.	20-60	Fl	rose	yes	Gr, Ng, Xe
Viburnum tinus subsp. *tinus*	Viburnaceae	XI-VI	Pcaesp	Steno-Medit.	>140	Fl, Fr, Le	white	yes	Ng, Sl
Vinca major subsp. *major*	Apocynaceae	II-V	Chrept	Euri-Medit.	20-60	Fl	violet	yes	Gr, Ng, Xe
Vinca minor L.	Apocynaceae	I-IV	Chrept	Europ.	20-60	Fl	violet	yes	Gr, Ng, Xe
Vitex agnus-castus L.	Lamiaceae	IV-VIII	Pcaesp/Pscap	Medit.-Turan.	>140	Fl	sky blue	yes	Ng, Sl
Vitis vinifera L.	Vitaceae	IV-VII	Plian	unknown	>140	Fr	greenish	yes	Ng
Withania somnifera (L.) Dunal	Solanaceae	IV-VIII	NP	Paleosubtrop.	>140	Fr	greenish	no	Ng
Woodwardia radicans (L.) Sm.	Blechnaceae	I-IX	NP/Hros	Steno-Medit.	>140	Le	-	no	Ng
Zelkova sicula Di Pasq., Garfi & Quézel	Ulmaceae	III-V	Pcaesp	Endem. Sic.	>140	Le	greenish	no	Ng
Ziziphora granatensis subsp. *alpina* (L.) Bräuchler & Gutermann	Lamiaceae	VI-IX	Chsuffr	Orof. S-Europ.	20-60	Fl	violet	no	Gr, Ng
Ziziphora granatensis subsp. *granatensis*	Lamiaceae	V-VII	Chsuffr	SW-Medit.	20-60	Fl	violet	no	Gr, Ng
Ziziphora granatensis (Boiss. & Reut.) Melnikov	Lamiaceae	V-VIII	Chsuffr	Orof. S-Europ.	20-60	Fl	violet	no	Gr, Ng
Ziziphus jujuba Mill.	Rhamnaceae	VI-VII	Pcaesp/Pscap	E-Asiat.	>140	Fr	white-greenish	yes	Ng, Sl, Xe
Ziziphus lotus subsp. *lotus*	Rhamnaceae	V-VII	Pcaesp	S-Medit.	>140	Fr	white-greenish	yes	Ng, Sl, Xe
Zygophyllum creticum (L.) Christenh. & Byng	Zygophyllaceae	IV-VI	Chsuffr	Subcosmop.	20-60	Fl	purple	no	Gr, Ng, Xe

Legend: [1] months of the year when flowering occurs; [2] Chcaesp = Caespitose Chamaephytes; Chfrut = Fruticose Chamaephytes; Chpulv = Cushion chamaephytes; Chrept = Reptant Chamaephytes; Chros = Chamaephytes with Rosette; Chsucc: Succulent Chamaephytes; Chsuffr: Suffruticose Chamaephyte; Grad: Root(-Bud) Geophytcs; Grhiz: Rhizomatous Geophytes; Hcaesp: Caespitose Hemicryptophytes; Hros: Hemicryptophytes with Rosette; Hscap: Scapose Hemicryptophytes; NP: Nano-Phanerophytes; Pcaesp: Caespitose Phanerophytes; Pep: Epiphytic Phanerophytes; Plian: Liana Phanerophytes; Prept: Reptant Phanerophytes; Pscap: Scapose Phanerophytes; Psucc: Succulent Phanerophytes; Tscap: Scapose Therophytes; [3] Fl = Flowers; Fr = Fruits; Le = Leaves; [4] Gr: Green Roofs; Ng: Naturalistic Gardens; Po: Pot Cultivation; Sl: Slopes; Xe: xeriscaping; Wf: wildflowers.

5. Morphological, physiological, and biochemical changes of herbaceous ornamental plants subjected to drought stress and recovery

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Morphological, physiological, and biochemical changes of herbaceous ornamental plants subjected to drought stress and recovery

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Abstract
Drought episodes, which are very frequent in the Mediterranean region, have negative effects on plant growth. To reduce irrigation, which is no longer sustainable today, it is necessary to identify plants capable of resisting water shortage. The aim of the experiment was to investigate the ability od three herbaceous plants, *Dimorphotheca hybrida*, *Pelargonium peltatum*, and *P. hybridum*, which are widely adopted for ornamental purposes in Mediterranean gardening, to recover from water stress, following periodic suspension and restoration of the water supply, and to study the mechanisms that

support this ability. Three theses were evaluated: control plants (100% WC) and drought-stressed plants (60% and 40%WC). After one week of submission to drought, stressed plants were re-watered to 100%WC, and then recovered for seven days. Subsequently, the plants were subjected to drought exposure for 14 days. The plants, after the second cycle of suspension of water, were kept in the same conditions of control plants for seven days. Plant growth, gas exchange, chlorophyll *a* fluorescence, Relative Water Content, chlorophyll and carotenoid content, and proline and MDA content were determined. The total dry biomass was influenced by species and drought treatment, but among the experimental factors there are not interaction. In correspondence with the most severe stress (40%WC), in the final part of the experiment, a reduction in total leaf area and leaf number was observed. The effect of treatments on leaf RWC at different times during the experiment determined values similar to those of the control plants in *P. hybridum*, and reduced during the second drought event in *P. peltatum* and *D. hybrida*. Net photosynthesis was significantly reduced in all plant species, especially during the second suspension. The Fv/Fm ratio in *P. peltatum* and *D. hybrida* showed significant differences only in the second drought stress period, and at the end of the second re-watering period, the stressed plants presented similar values to the control plants. In *P. hybridum*, stress-recovery treatments had no influence on Fv/Fm during the recovery period. For all species, proline content increased during each drought event compared with that in the control plants. Overall, malondialdehyde (MDA) tended to increase during the experimental period in all treatments. The chlorophyll content did not show significant modifications in relation to the water deficit treatments.

Keywords: climate change; chlorophyll *a* fluorescence; gas exchange; Relative Water Content; proline

Abbreviations:
A_N, net CO_2 assimilation rate; gs, stomatal conductance; E, transpiration rate; 100%, control 100 % of Water Container Capacity (WC); RWC, Relative Water Content; F0, minimum fluorescence; Fm, maximum fluorescence; Fv/Fm, maximum quantum efficiency of PSII; Pro, proline; MDA, malondialdehyde; S-R, suspension/rewatering.

Highlights:

- Identifying plants capable of resisting drought stress is important in the context of global change.
- Plant growth was affected only by species and drought treatment.
- Photosynthesis is the process most affected by drought stress.
- Proline content increased during each drought event compared to that in the control plants.
- Among the three species, *P. peltatum* and *D. hydrida* were the more tolerant.

5.1. Introduction

The constant increase in the level of greenhouse gases, particularly CO_2, has led to an increment in the Earth's temperature, with an enhance in the frequency and intensity of drought episodes (Eftekhari 2022; Rivero et al. 2022). The last few years have been characterised by increasingly hotter summers with frequent heat waves, and an increasingly harsh climate is expected in the near future, intensifying the frequency and severity of water stress (Raza et al. 2019).
The effects of climate change may be more intense in regions such as the Mediterranean, which is considered a hotspot for drought risk (Carrão et al. 2016; Aguilera et al. 2020). In the 21st century, the average increase in temperature was estimated to be 20% higher than the global average. A reduction in precipitation is expected to be

approximately 20 mm/°K (Lionello and Scarascia 2018); therefore, drought episodes are expected to be more frequent, long-lasting, and intense (Grillakis 2019).

These phenomena will especially affect coastal communities with high population density, due to the increase in urban population, considering that 4.2 billion people live in urban areas globally, which could spread 6.7 billion by 2050 (United Nations 2018). Promoting the reduction of water use in residential green areas for the irrigation of ornamental landscapes and gardens is essential to meet the growing demand for freshwater, as climate change and population growth are accentuating the reduction of resources (Warner et al. 2020).

Drought episodes are very frequent in the Mediterranean region, where the climate is characterised by higher temperatures, a high vapour pressure deficit, high levels of radiation, and low rainfall during the growing seasons. These conditions negatively influence the growth of plants under stressful conditions (Toscano et al. 2014). To avoid harmful effects and loss of the ornamental value of plants used in landscaping, irrigation is used in arid and semi-arid areas, often with drinking water. In some areas of the US, it has been estimated that 30-70% of all drinking water is used for the irrigation of urban landscapes (Kjelgren et al. 2000). The shortage of water in some regions limits the use of drinkable water for ornamental greens.

During drought stress conditions different morphological, physiological, biochemical and molecular changes were observed in plants that can damage both plant growth and productivity (Wang et al. 2001; Praveen et al. 2023). Many morphological modifications to water stress affect the above-ground part of the plants. Leaf growth is, in fact, the growth process of plants is most sensitive to water deficits (Jones et al. 1985). Unfortunately, many adaptation strategies affect the visual appearance of plants, and physiological alterations can lead to loss of the ornamental value of plants or green areas (Toscano et al. 2019). Therefore, the selection of plants for drought tolerance is

extremely important for landscape and urban green design. Selection of ornamental plants for drought tolerance involves identifying species and cultivars that exhibit adaptive mechanisms to withstand water scarcity. These mechanisms include morphological, physiological, and biochemical modifications, such as an increased root/shoot ratio, reduced growth, and changes in leaf anatomy, such as waxy and thick cuticula (Toscano et al. 2021). Interestingly, while some ornamental plants show traits indicative of drought tolerance, such as low leaf water potential and osmotic adjustment (Chapman and Augé 1994). The selection of key physiological traits can aid in the identification of drought-tolerant species, contributing to sustainable landscaping practices in the face of climate change (Toscano et al. 2019; Ferrante et al. 2021).

Among the most common physiological adaptations to water stress, the decrement of relative water content (RWC), leaf water potential, transpiration rate, stomatal conductance, and, hence, the decrease in the assimilation of CO_2, with inhibition of photosynthetic activity (Anjum et al. 2011; Vaseva et al. 2013) are mostly reported in the scientific literature. In species sensitive to drought stress, reductions in chlorophyll (Bano et al. 2021) and protein concentrations have been observed (Fazeli et al. 2007). Piveta et al (2020) in an experiment observed that water stress, in addition to inhibiting chlorophyll biosynthesis, reduces chlorophyll concentration because it increases chlorophyllase activity and the rate of its decomposition. Lower water availability induces photosynthesis reduction, and the excitation energy of light is higher than that used during the photosynthesis process. Due to drought stress, there is an overexcitation of photosynthetic pigments in the antennal pigments, which can cause the increasing of reactive oxygen species (ROS) in the chloroplasts (Kuźniak and Kopczewski 2020).

After the cessation of drought stress, the occurrence of small amounts of rainfall can have a positive effect on plant function, both at the

whole-plant level and on biochemical responses. Therefore, especially in herbaceous species, a group of plants is not always extensively investigated from this point of view, and the mechanisms adopted to tolerate water stress should be understood.

The capability of plants to adapt to prolonged periods of water shortage, at the end of which it recovers its functions, could be considered a mechanism of adaptation to the typical conditions of the Mediterranean environment (Galmés et al. 2007). Nevertheless, the response of plants certainly differs significantly according to the period of the stress, the plant species used, and the phase of development (Chaves et al. 2003).

Private gardens and public green areas are attractive because of the presence of colourful bedding plants (Toscano et al. 2021). As reported by Heidari et al. (2016), when plants have a small root system and are grown in pots, they can suffer more from the effects of water stress.

To reduce water consumption, which is no longer sustainable today, it is necessary to identify plants capable of resisting water shortage. However, the water needs and responses of ornamental plants to drought are not yet well specified, principally for herbaceous plants, which represent a large part of ornamental vegetation in urban green areas (Zollinger et al. 2006).

The current study concerns African Daisy (*Dimorphotheca hybrida* DC.) of Asteraceae family and two species of geraniums - ivy geranium (*Pelargonium peltatum* (L.) L'Hér.) and pot geraniums (*Pelargonium hybridum* (L.) L'Hér) of the Geraniaceae family. These species are widely adopted for ornamental purposes in Mediterranean gardening.

This experimental work started with the assumption that the final capacity of these species is linked to their ability to maintain high values of RWC under stress conditions and to recover rapidly after recovery periods through activation at the cellular level and the

biosynthesis of osmotic compounds. The response of different ornamental plants to drought stress have been well reported in different studies; however, the morpho-physiological responses of herbaceous ornamental species to progressive drought and recovery after irrigation are relatively limited.

The aim of this experiment was to evaluate the ability of three ornamental herbaceous species to recover from water stress, following periodic suspension and restoration of the water supply, and to study the mechanisms that support this ability. Additionally, the effects of stress memory on subsequent drought stress applications were also evaluated.

5.2. Materials and methods

The experiment was conducted during the 2023 growing season at the University of Catania, Italy. *Dimorphotheca hybrida* DC. 'Dalina® Special Sunshine Beauty' = *D. hybrida*, *Pelargonium peltatum* (L.) L'Hér. 'Moonflair® Red' = *P. peltatum*, and *Pelargonium hybridum* (L.) L'Hér. 'Galaxy™ Red' = *P. hybridum* rooted cuttings were provided by the Florensis Italia company, Lazzate (MB, Italy). Rooted cuttings of the species were transplanted into 14 Ø pots (one plant per pot) filled with a substrate based on peat and perlite (2/1, v/v). The irrigation of the plants was regular for one month at a well-watered (100 % WC) level. At the end of March, the plants were separated into three treatments: control plants (100% WC) and drought-stressed plants (60%WC and 40%WC). The plants subjected to drought stress, after one week were watered at 100% WC and left in these conditions for 7 days. After, the plants were subjected to exposure to drought for 14 days. After the second cycle of drought stress, the plants were irrigated at 100% for another 7 days (Figure 5.1).

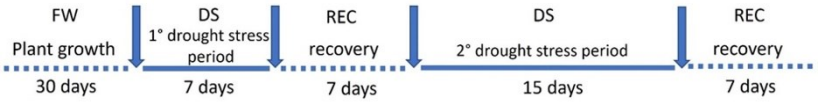

Figure 5.1. Timeline for suspension/rewatering (S-R) treatments used in the experiment. FW = full watering; DS = drought stress event; REC = recovery

Leaf materials were taken weekly for physiological and biochemical analyses. Temperature and relative humidity data were recorded using a data logger. The drought treatment was based on a gravimetric method (Toscano et al. 2018). Fresh plant material was frozen in liquid N_2 and stored at − 80°C to determined proline, chlorophyll, and malondialdehyde. At the end of the trial, roots, after being cleaned, were separated from the epigeal portion. The leaves and stems were also separated in nine plants per treatment (three per repetition). Root, leaves, and steam were dried (65 °C) and dry biomass was recorded. Total leaf area was measured with a leaf area meter (Delta-T Devices Ltd., Cambridge, UK).

The weather data was registered using a data logger CR1000 (Campbell Scientific Ltd., Loughborough, UK) during the experimental period. Mean temperatures ranged between 18 and 22 °C, and relative humidity (RH) ranged between 50 and 66% (Figure 5.2).

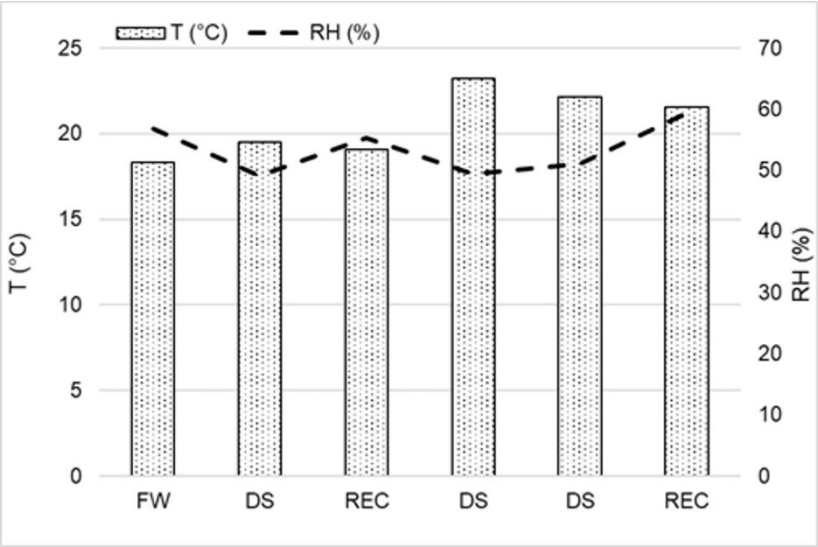

Figure 5.2. Climate trend [temperature (°C) and Relative Humidity (RH, %)] during the experimental period.

5.2.1. Physiological parameters

The leaf gas exchange parameters [net photosynthetic rate (AN), stomatal conductance (gs), and transpiration rate (E)] using a CO_2/H_2O infrared gas photosynthesis system (LCi, ADC Bioscientific Ltd., Hoddesdon, UK) was measured for every water treatment. Measurements were taken between 09:00 and 13:00.

The chlorophyll *a* fluorescence was evaluated with a modulated chlorophyll fluorimeter OS1-FL (Opti-Sciences Corporation, Tyngsboro, MA, USA). Chlorophyll *a* fluorescence was expressed as the Fv/Fm ratio, where Fm is the maximal fluorescence of the dark-adapted state, Fv is the variable fluorescence.

To determine relative water content 30 discs (10 mm in diameter) for each replicate were weighed immediately (FW), immersed in distilled water (24 h) and then turgid weight (TW) was obtained. The leaves were then dried at 75 °C for 24 h to obtain dry weight (DW). RWC

was determined using the equation: RWC%=(FW−DW/TW−DW) × 100.

5.2.2. Determination of Chl and carotenoids content

To measure the amount of chlorophyll *a*, *b*, total and carotenoids 100 mg of fresh leaf material was crushed, extracted with 5 mL of 99% methanol, and incubated in the dark (24h at 4°C). By spectrophotometer device (UV 1900i Spectrophotometer, Shimadzu, Japan) was read optical absorption in wavelengths 665.2 nm, 652.4 nm, and 470 nm. Chlorophyll content was calculated according to the equation reported by Lichtenthaler (1987).

5.2.3. Determination of proline concentration

Proline content was measured by the ninhydrin-acetic acid method according to Bates et al. (1973). The samples were quantified using a spectrophotometer at 525 nm. The toluene was used as a blank (UV 1900i spectrophotometer, Shimadzu, Japan).

5.2.4. Estimation of lipid peroxidation

Lipid peroxidation was obtained by measuring the amount of malondialdehyde produced by the thiobarbituric acid reaction as reported by Li et al. (2010). MDA content was indicated as nmol MDA g^{-1} FW.

5.2.5. Statistical analysis

The experiment used a randomised complete design with three replicates per treatment. The CoStat version 6.311 software was used for statistical analysis. A one and two-way analysis of variance procedure was used. For mean comparisons Tukey's multiple range test was used at a probability level of 5%. Data were given as mean ± standard error (SE).

5.3. Results

The drought treatments affected the total dry biomass and changed with species, but no interaction was found among the experimental factors. The effect of drought on total dry biomass showed a 21% reduction in plants under 40%WC. The species showed a different response to drought stress; in particular, a lower total dry biomass was observed in *D. hybrida* (Table 5.1). The same trend was observed for epigeous dry biomass, with a 23% reduction at 40%WC. The same response was observed in the species studied (Table 5.1). No interaction effects were detected among the experimental factors.

The root-to-shoot ratio differed among species, but no significant changes were found among treatments and interactions at the end of the experimental period (Table 5.1). Higher root/shoot values were observed in *P. hybridum* and *D. hybrida*.

At the end of the experiment, with the increase of stress level (40%WC), a reduction in total leaf area and leaf number was observed. A 29% reduction in leaf area in the 40%WC treatment was detected, and differences were observed among the species (Table 5.1). The decrease in total leaf area was comparable to the decrement in leaf number, with a reduction of approximately 16% at 40% WC (Table 5.1).

Table 5.1. Species (S) and drought stress (D) effects on total (TB) and epigeous (EB) dry biomass, root/shoot ratio (R/S), total leaf area (TLA), and leaf number (LN) on *P. hybridum*, *P. peltatum,* and *D. hybrida*. The plants were subjected to drought stress and recovery period.

Species/Drought	**100%WC**	**60%WC**	**40%WC**	**Mean**	**S × D**
TB (g plant^{-1})					**ns**
P. hybridum	23.70± 0.79	22.73±0.76	18.08±1.27	**21.50±0.94a*****	
P. peltatum	19.83±0.72	15.83±0.68	14.12±1.11	**16.60±0.84 b**	
D. hybrida	12.92±1.90	11.44±0.78	9.78±0.26	**11.38±0.98c**	
Mean	**18.82±1.14A*****	**16.67±0.74A**	**13.99±0.88B**		
EB (g plant^{-1})					**ns**
P. hybridum	16.70± 0.59	15.99±0.90	12.78±1.09	**15.16±0.86a*****	
P. peltatum	16.94±0.72	12.64±0.67	11.47±1.02	**13.68±0.80a**	
D. hybrida	12.93±1.35	11.44±0.39	9.78±0.26	**8.18±0.67b**	
Mean	**14.30±0.89A*****	**12.36±0.65A**	**10.36±0.79B**		
R/S					**ns**
P. hybridum	0.42± 0.04	0.43±0.03	0.42±0.04	**0.43±0.04a*****	
P. peltatum	0.17±0.01	0.25±0.01	0.23±0.01	**0.22±0.01b**	
D. hybrida	0.42±0.04	0.35±0.03	0.43±002	**0.40±0.03a**	
Mean	**0.34±0.03ns**	**0.34±0.02ns**	**0.36±0.02ns**		

TLA (cm²)					**ns**
P. hybridum	941.12±35.00	1025.06±39.19	723.83±32.48	**896.67± 35.56a*** **	
P. peltatum	822.56±6.74	777.82±7.87	602.46±38.12	**734.15±40.91b**	
D. hybrida	767.00±14.04	660.35±12.95	452.49±10.23	**626.61±12.41c**	
Mean	**843.56±18.59A*** **	**821.08±43.34A**	**592.79±26.95 B**		
LN (n°)					**ns**
P. hybridum	72.16±4.00	65.83±3.32	49.33±3.93	**62.44±4.75b*** **	
P. peltatum	62.33±2.89	51.01±3.51	53.67±1.64	**55.64± 2.68b**	
D. hybrida	321.17±16.00	353.33±12.99	286.17±9.45	**320.22± 14.15a**	
Mean	**151.89± 7.63*A**	**156.72±6.60A**	**129.72±7.34B**		

*: $p < 0.05$; ***: $p < 0.001$; ns: $p > 0.05$. Data followed by a different letter were significantly different according to Tukey's test. 100%: control; 60%: light drought stress; 40%: severe drought stress. Data are means ± standard error (n = 3). TB = Total dry biomass; EB = Epigeous dry biomass; R/S = Root to shoot ratio; TLA = Total leaf area; LN = Leaf number.

The effect of Drought Stress-Rewatering (DS-REW) on leaf RWC at different times during the experiment modified among the species. In *P. hybridum*, leaf RWC maintained high values similar to those of the control plants (Figure 5.3A).

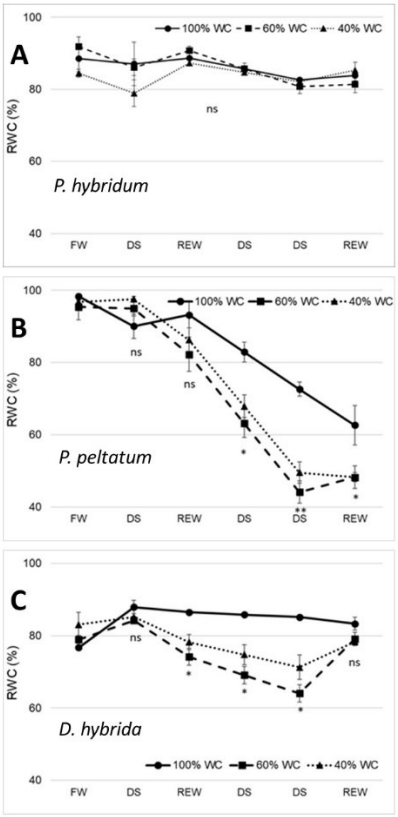

Figure 5.3. Relative Water Content (RWC) in *P. hybridum* (A) *P. peltatum* (B) and *D. hybrida* (C) during the experimental period. Plants were irrigated to container capacity (100% WC) or subjected to water stress treatments (60% WC and 40% WC) and subjected to suspension/rewatering treatment [7 days no water (DS), 7 days irrigation (REW), 14 days no water (DS2), 7 days irrigation (REC)]. Data are mean ± S.E. (n = 6). * denote statistical significance from ANOVA at the 0.05 level; ns = not significant.

Instead, DS-REW treatment decreased leaf RWC in *P. peltatum* during the second drought event, with decreases of 23% and 39 % in 60%WC and 18% and 31% in 40%WC, compared with the control plants (Figure 5.3B). *D. hybrida* (Figure 5.3C) plants showed a trend similar to that of *P. peltatum* plants. During the second drought event, DS-REW treatments had a headless effect on leaf RWC, although they were significant, by 20% and 24% at 60%WC and by 13% and 16% at 40%WC in *P. peltatum* and *D. hybrida*, respectively (Figure 5.3B, C). After the second re-watering period (REW), all plant species showed values similar to those of the control plants (Figure 5.3A, B, C). The net photosynthesis (An) in the 100%WC was at the start of the experiment (FW), on average, 10.4 µmol $CO_2m^{-2}s^{-1}$ (*P. hybridum*), 6.6 µmol $CO_2m^{-2}s^{-1}$ (*P. peltatum*), and 14.1 µmol $CO_2m^{-2}s^{-1}$ (*D. hybrida*). During the first suspension (DS), the treatment reduced An by 41% in *P. hybridum* in both drought stress treatments (60%WC and 40%WC) in relation to the control plants, whereas in *D. hybrida*, a reduction of 38% was observed, but only in 40%WC. No reduction was observed in *P. peltatum*. During the first recovery (REW), *P. peltatum* and *D. hybrida* showed the same values as the control plants; *P. hybridum* showed values below than control plants (by ~50%). During the second drought event (DS), the drought stress diminished photosynthesis by 56% in *P. hybridum* 40%WC, 80% in *P. peltatum* 60%WC and 40%WC, and 70% in *D. hybrida* 60%WC and 40%WC. After the second recovery, *P. peltatum* 60% and *D. hybrida* 60% showed no significant difference in relation to the control plants, whereas the 40%WC treatment showed the lowest value compared to the control plants (by 76% and 64% for *P. peltatum* and *D. hybrida*, respectively). *P. hybridum*, indeed, showed a reduction by 70% in 60%WC and 40%WC compared to control plants (Figure 5.4). Same course was observed for all species for the transpiration rate at all times (Figure 5.4).

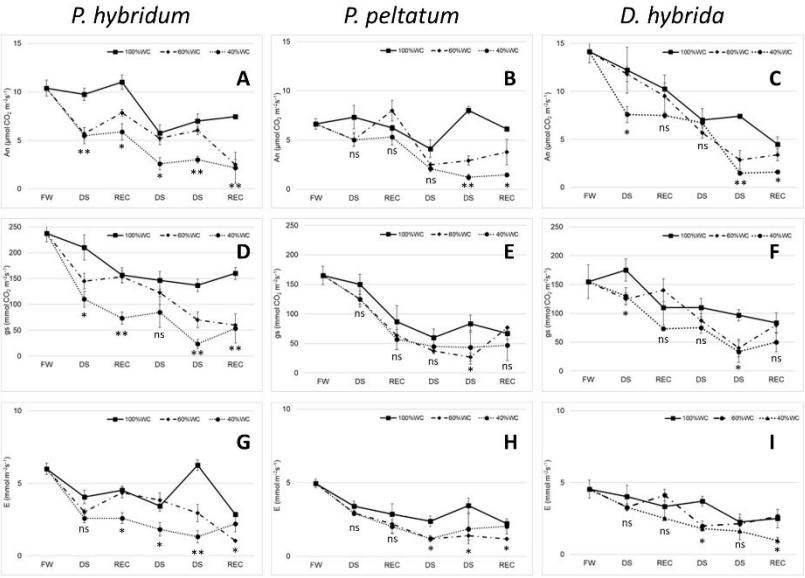

Figure 5.4. Net photosynthesis (A_N), stomatal conductance (gs), and transpiration rate (E) in *P. hybridum* (A, D, and G), *P. peltatum* (B, E, and H) and *D. hybrida* (C, F, and I) during the experimental period. Data are mean ± S.E. (n = 6). *, ** denote statistical significance from ANOVA at the 0.05 and 0.01 levels, respectively; ns = not significant.

Transpiration (E) showed no significant differences in drought-stressed plants during the early water suspension (DS). In the first recovery period, *P. peltatum* and *D. hybrida* plants subjected to drought stress showed no differences compared with the control plants; however, *P. hybridum* showed a significant and progressive reduction in plants with a water content of 40%WC from the first DS until the end of the experiment (Figure 5.4). During the second drought event (DS), drought stress reduced E by 59% and 45% in *P. peltatum* 60% WC and 40% WC, respectively, and by 28% in *D. hybrida* 40% WC. At the end of the second recovery, all species irrigated at 60%WC showed no significant differences respect to the control plants, whereas the 40%WC plants showed lower values in relation to the

control plants (by 23%, 8%, and 61% for *P. hybridum*, *P. peltatum*, and *D. hybrida*, respectively). The stomatal conductance (gs) in the control plants was at the beginning of the experiment (FW), with averages of 240 µmol $CO_2m^{-2}s^{-1}$ (*P. hybridum*), 160 µmol $CO_2m^{-2}s^{-1}$ (*P. peltatum*), and 150 µmol $CO_2m^{-2}s^{-1}$ (*D. hybrida*). During the first suspension (DS), gs was reduced by 41% in *P. hybridum* and by 31% in *P. peltatum* in both drought stress treatments (60%WC and 40%WC) compared to the control plants, whereas in *D. hybrida*, a reduction of 38%, but only in 40%WC, was observed. During the first recovery (REW), *P. peltatum* and *D. hybrida* showed the same values as the control plants; *P. hybridum* preserved the values below those of the 100%WC (by ~50%). In the course of the second drought event (DS), the S-R treatment diminished the photosynthesis activity by 56% in *P. hybridum* 40%WC, 80% in *P. peltatum* 60%WC and 40%WC, and 70% in *D. hybrida* 60%WC and 40%WC. After the second recovery, *P. peltatum* 60% and *D. hybrida* 60% showed no significant difference in relation to control plants, while the 40%WC treatment showed lowest value compared to control plants (by 76% and 64% for *P. peltatum* and *D. hybrida* respectively). *P. peltatum*, indeed, showed a reduction by 70% in 60%WC and 40%WC compared to control plants (Figure 5.4). A similar course was found for the transpiration rate in all times of the experiment in all species (Figure 5.4).

The chlorophyll *a* fluorescence (Fv/Fm ratio) in *P. peltatum* and *D. hybrida* showed significant differences only in the second DS period (Figure 5.5A, C), and at the end of the second re-watering period, the stressed plants (60%WC and 40%WC) showed similar values to the control plants. In *P. hybridum* (Figure 5.5B), D-S treatment had no influence on Fv/Fm during the recovery period (REW).

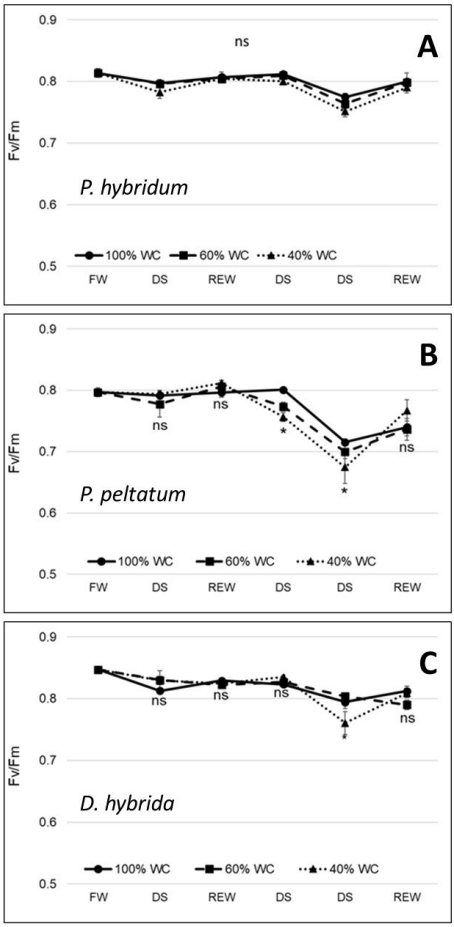

Figure 5.5. Maximum quantum efficiency of the PSII (Fv/Fm) in *P. hybridum* (A), *P. peltatum* (B) and *D. hybridum* (C) during the experimental period. Data are mean ± S.E. (n = 6). * denote statistical significance from ANOVA at the 0.05 level; ns = not significant.

Figures 5.6A, B, and C show the trend of proline content in leaves. Proline content increased during each drought event for all species in stressed plants relative to control plants. (Figure 5.6). The highest values were recorded during the second drought event (DS).

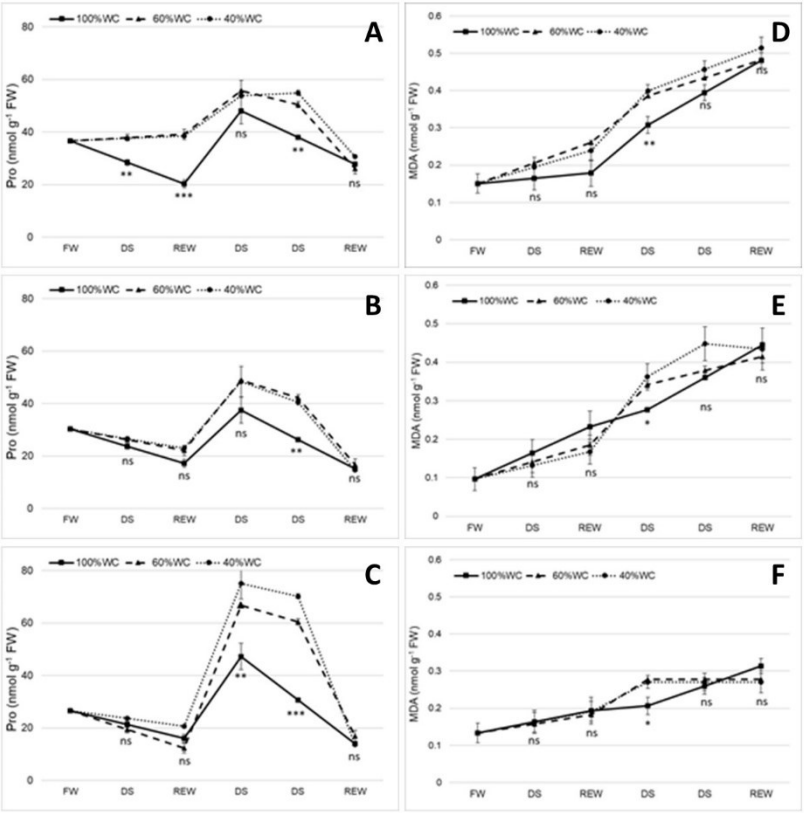

Figure 5.6. Contents of proline and MDA in *P. hybridum* (A,D), *P. peltatum* (B,E) and *D. hybridum* (C,F) during the experimental period. Data are mean ± S.E. (n = 3). *, **, *** denote statistical significance from ANOVA at the 0.05, 0.01 and 0.001 levels, respectively; ns = not significant.

During the first period of water reduction, the proline content increased by 25% in *P. hybridum* (60%WC and 40% WC) (Figure 5.6A) and 10% in *P. peltatum* (60%WC and 40% WC) (Figure 5.6B), whereas no significant differences were observed in *D. hybrida* plants (Figure 5.6C). During the second period of water reduction, the DS treatment increased proline content by 14% in *P. hybridum* (Figure

5.6A), 23% in *P. peltatum* (Figure 5.6B), and 30% in *D. hybrida* (Figure 5.6C), in relation to control plants. After the second recovery period (REW), the DS plants exbited similar values to those of the control plants (Figure 5.6A, B, C).

Overall, malondialdehyde (MDA) tended to increase during the experimental period, but in all treatments (Figure 5.6C, D, E). Its content was significantly higher during the second water-stress period in the water-stressed treatments (60%WC and 40%WC). At the end of the experimental period, all species showed values similar to those of the 100%WC plants.

For all chlorophyll parameters, no interaction effects were observed among days, species, and irrigation treatments (Table 5.2).

Table 5.2. Mean effects of days (D), species (S), and treatments (T) on chlorophyll *a*, *b*, total (Chl*a*, Chl*b*, Chl tot), *a/b* ratio, and carotenoids (Car).

Days	**Species**	**Treatments**	**Chl*a*** **(µg g^{-1})**	**Chl*b*** **(µg g^{-1})**	**Chl tot** **(µg g^{-1})**	***a/b* ratio**	**Car** **(µg g^{-1})**
FW			0.746a	0.248a	0.994a	3.079a	0.156a
DS			0.665b	0.221b	0.887b	3.082a	0.140b
REC			0.592c	0.195c	0.787c	3.096a	0.133b
DS			0.500d	0.168d	0.667d	3.106a	0.119c
DS			0.416e	0.139e	0.555e	3.059a	0.100d
REC			0.334f	0.112f	0.446f	3.119a	0.0818e
	P. hybridum		0.702a	0.234a	0.934a	3.055b	0.168a
	P. peltatum		0.372c	0.114c	0.486c	3.319a	0.105b
	D. hybrida		0.553b	0.193b	0.746b	2.897c	0.092c
		100% WC	0.536a	0.185a	0.721a	3.001a	0.117b
		60% WC	0.535a	0.175a	0.710a	3.134a	0.122ab
		40% WC	0.555a	0.181a	0.736a	3.131a	0.127a
Main effects							
Days			***	***	***	ns	***
Species			***	***	***	***	***
Treatments			ns	ns	ns	ns	***

Interaction					
D x S	***	***	***	*	***
D x T	ns	ns	ns	ns	ns
S x T	**	*	**	ns	**
D x S x T	ns	ns	ns	ns	ns

Values (mean ± se) within each column, followed by the same letter, do not significantly differ at $p < 0.05$ according to Tukey's test; ns = not significant; significant at $p < 0.05$ (*), 0.01 (**), and 0.001 (***). Three biological replicates were used for the analysis (n = 3).

The Chl *a*, *b*, and tot contents exhibited different patterns in relation to days and species (Table 5.2). The chlorophyll contents (Chl*a*, Chl*b*, and Chl tot) did not show significant changes in relation to the water treatments. The effects of interactions were observed between days and species (Figure 5.7). In particular, all species showed a significant reduction in total chlorophyll content as the stress period increased, with the lowest content at the end of the trial experiment. Similarly, interaction effects were observed between species and water treatment (Figure 5.7). *P. hybridum* had the highest Chl tot value compared to the other two species. The *a/b* ratio showed differences in relation to the species. The highest ratio was observed in *P. peltatum*.

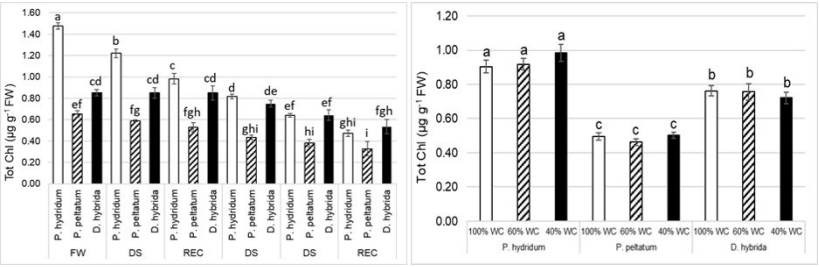

Figure 5.7. Interaction effect of species × days and species × water treatments on total chlorophyll. Data are means ± standard error (n = 3). According to Tukey's test, different letters denote significant differences between irrigation treatments at each sampling time at $P < 0.05$.

Car content (Car) showed a different trend in relation to day, species, and water treatment (Table 5.2). As the number of days of irrigation increased, the carotenoid content decreased. The highest content was observed for *P. hybridum*. The highest car content was found in the 40%WC treatment (Table 5.2).

The first three principal components (PC1, PC2, and PC3) accounted for 37.1, 29.9, and 17.7% of the total variance, respectively. In the principal component analysis, the first two principal components showed a negative correlation with EB, TB, LA, An, gs, Pro, *a/b* ratio,

and MDA, while a positive correlation was detected among R/S, E, RWC, Fv/Fm, Chl a, b, tot, and carotenoids (Figure 5.8). The analysis of PCA showed clear separation among the groups. The lower right quadrant (positive side of PC1) included *P. hybridum* species with physiological adaptation (RWC, Fv/Fm, E) (Figure 5.8). A second group, clustered in the higher left quadrant, included plants of *P. peltatum*, characterised by F0 modification. Finally, the *D. hybrida* plants underwent chlorophyll modification (Figure 5.8).

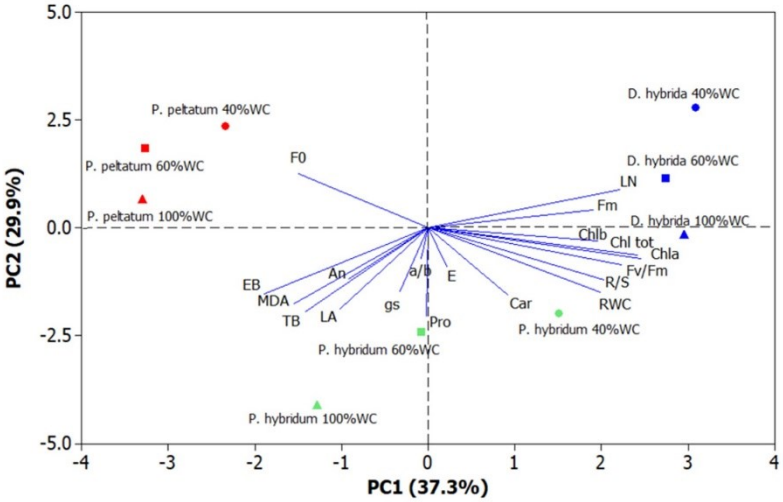

Figure 5.8. Biplot graph and scores based on the first two components of morphometric, physiological, and biochemical traits of *P. hybridum*, *P. peltatum* and *D. hybridum* response to water treatments.

5.4. Discussion

Irrigation and water availability in urban and peri-urban areas can be limited to reduce the management costs. The growth and flowering of herbaceous bedding plants in urban areas are hampered because they are often affected by water deficits, with negative consequences for their aesthetic value (Toscano et al. 2019).

Annual plants that can avoid drought cannot be used extensively in landscapes with little water availability because they quickly lose their ornamental effect. In contrast, drought-tolerant plants, which can maintain a good visual appearance under conditions of water stress, can be used in landscapes where irrigation is sporadic. The choice of plant species is, therefore, strategic for identifying plant species that can maintain their aesthetic appearance, even under conditions of water shortage (Ferrante et al. 2021).

A more efficient approach for selecting drought-tolerant plants is to evaluate the physiological and biochemical responses of the plant species to stress (Cicevan et al. 2016). Differences in the drought responses of different plants to drought emerged even within species belonging to the same genus.

Both the response of plants to drought stress and the possibility of recovery when water availability conditions are restored are complex processes that require plants to spend metabolic energy and activate physiological and biochemical processes. These responses depend on the genotype used, age of the plant and intensity and duration of water stress. (Toscano et al. 2019). To date, choosing the most suitable ornamental plant species for urban green areas or for improving plant tolerance to water stress through breeding is very difficult because little information is available. The development and survival of plants are often measured to evaluate their tolerance to water stress. Therefore, indirect selection of drought tolerance using physiological or biochemical methods may be an easier and more effective approach. In the present study, three herbaceous species were studied to understand their adaptation to drought. These results indicate that significant genotypic variation exists in most of the physiological and biochemical parameters. Response to water stress can be classified as “low”, “moderate” and “severe” based on soil water capacity or water potential (Parkash and Singh 2020); this means that not rather attention is always devoted to the physiological state and the level of stress suffered by plant (Wedeking et al. 2017). In our study,

intermittent drought was applied to understand the potential stress memory of plants in response to the same stress. Abiotic stress memory in plants refers to physiological and molecular changes that enable plants to respond more effectively to recurring stress after initial exposure (Li et al. 2019; Charng et al. 2023). This phenomenon is facilitated by processes such as acclimation, priming, and retention of stress-induced epigenetic modifications (Li and Liu 2016; Sen and Puthur 2020). Interestingly, although the concept of stress memory is increasingly being recognised, the mechanisms that maintain this memory are not fully understood. It has been suggested that the memory of stress exposure is associated with epigenetic changes, accumulation of signalling proteins, and transcription factors (Sen and Puthur 2020; Louis et al. 2023). Moreover, the use of chemical priming agents has been shown to enhance abiotic stress tolerance, suggesting that both natural and synthetic compounds are involved in the establishment of stress memory (Antoniou et al. 2016; Sako et al. 2020).

Based on this information, the responses of the obtained ornamental plants were discussed. During the experiment, as water stress advanced, our plants reduced their ability to assimilate CO_2, and, as a result, photosynthesis was reduced. This is related to the increased resistance in the mesophyll and stomatal layer, which leads to a decrease in organ dry weight (Bahadur et al. 2019).

As an initial response to water shortage, rapid closure of the stomata is observed, which determines a reduction in CO_2 assimilation (Dghim et al. 2018), stomatal conductance (gs), transpiration rate (E), and reduction in net photosynthesis (A_N) (Gurumurthy et al. 2019).

D. hybrida plants subjected to moderate water stress (60%WC), unlike the other two species, showed similar photosynthetic activity as plants with full water content at the end of the second recovery period.

The loss of water content in leaves, observed during the drought stress period, is evidenced by the progressive reduction in RWC. The lowest RWC was found under more severe drought stress than under the other

treatments; this negative difference was more evident in the third week of the trial. After three weeks, the RWC decreased to 78.3, 76.8%, and 69.7% at 80, 60%, and 40% WC, respectively. After re-irrigation, the RWC tried to reach the initial values under the 80% and 60% WC treatments and almost reached the 100% WC value; however, under severe drought stress, the recovery of RWC was slower (Figure 5.2B). The higher leaf water content in *D. hybrida*, especially in *P. hybridum*, can maintain metabolic activities and light dissipation (Pourghayoumi et al. 2017). The ability of *P. hybridum* to maintain its relative water content under drought stress conditions is a good indicator of drought tolerance (Rafi et al. 2019). Some authors have suggested that the RWC is a good index of flower longevity (Fanourakis et al. 2022; Kaur and Jhanji 2023) and leaf dehydration tolerance in ornamental plants (Oraee and Tehranifar 2020; Giordano et al. 2021). Pourghayoumi et al. (2017) found a positive correlation between RWC and total chlorophyll content, suggesting that RWC is responsible for maintaning chlorophyll levels (Rafi et al. 2019).

Through physiological adaptations (reduced stomatal conductance, net photosynthesis, and RWC) our study species were able to adapt to repeated cycles of drought. One of the aims of this trial was to evaluate changes in the accumulation of protective compounds, including amino acids and carbohydrates (Živanović et al. 2020), in response to drought stress and their effects on increasing drought tolerance.

To adapt to water shortage, plants activate biochemical mechanisms such as osmotic adjustment. One mechanism for osmotic adjustment is the accumulation of compatible solutes, such as amino acids and proline (Dien et al. 2019). Under drought stress, our results showed that proline was accumulated in the leaves of stressed plants of different species in relation to the control condition and that more severe drought stress determined an increasing in proline accumulation. Among the studied species, *D. hybrida* showed higher proline content in more stressed plants, as evidenced by a timelier response to water shortage.

According to Chegah et al. (2013), proline can promote cell turgor, maintain osmotic adjustment, and protect cells during dehydration. Pourghayoumi et al. (2017) founded a negative correlation between RWC and proline accumulation; for these authors it was linked to the less effective role of proline on osmotic adjustment.

Liu et al. (2011) observe that while MDA is an indicator of lipid peroxidation under adverse abiotic conditions, proline content accumulates as an osmoprotectant. During drought stress conditions, our species accumulated high amounts of proline, which alleviated cellular hyperosmolarity and ion disequilibrium. At the end of the second recovery period (REW), the DS plants (60% and 40% WC) of all species were similar to the control plants.

Among the studied species, under drought stress conditions, a higher accumulation of proline in *D. hybrida* was recorded. These compatible solutes have been shown to increase stress tolerance in plants by improving osmotic regulation and cell membrane protection (Oraee and Tehranifar 2020; Ozturk et al. 2021; Choudhary et al. 2023). Other researchers (Sun et al. 2012; Rafi et al. 2019) have observed that proline content was not correlated with plant drought stress tolerance because some sensitive plant species accumulated more proline than tolerant plant species.

The photosynthetic pigment content was not affected by drought and recovery stress, whereas a reduction in pigment content was observed in relation to the species and days of the treatments. Abiotic stresses, such as water stress, affect chlorophyll biosynthesis, resulting in a reduction in the chlorophyll content. Our results showed that during the recovery phase, plants delayed chlorophyll biosynthesis following water stress. The lower level of chlorophyll reduced the green colour of the leaves and, hence, was a relevant trait for the ornamental value of the plants.

The overall photosynthetic changes in the three species demonstrate that photosynthetic parameters, along with antioxidant compounds, are good indicators for crop adaptation to drought conditions.

P. peltatum showed fewer changes in photosynthesis and better acclimatisation to stressful conditions.

5.5. Conclusion

The results demonstrate that photosynthetic changes are the most affected by antioxidants and osmolytes, such as proline. The lower leaf gas change variations can be attributed to greater adaptation to drought conditions. Among the three species, *P. peltatum* and *D. hydrida* were the most tolerant.

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6. Screening for physiological plasticity and recovery capacity to drought stress of 15 ornamental shrubs

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Abstract
Drought is the main limiting factor for plants in the Mediterranean region during the summer and water scarcity is greatly felt in the management of green spaces. Identifying plants capable of withstanding this stress is of considerable interest.
In this context, the response of 15 shrub species to severe drought stress (temperatures over 40°C) was analyzed. The trial was conducted in June-July 2023 on a commercial farm in the province of Catania. The plants were grown in 2 L pots on peat-based soil and allowed them to grow under non-limiting water conditions until they reached field capacity. After 20 and 27 days from the start of the trial, irrigation was

suspended for five days. The following treatments were compared: Control, with plants always maintained under non-limiting water regime; Stress, with irrigation suspended during the prescribed period; Biostimulant treatment [Drin by Green Has Italia, dose 1:100 (v/v)] administered to the roots before (T.B.P.) or after (T.B.C.) the suspension period. Measurements were made regarding gas exchange, chlorophyll *a* fluorescence, and chlorophyll concentration, expressed as SPAD values.
The species showed significantly different photosynthetic values as a result of the treatments studied. The biostimulant treatment was more effective when administered before stress was imposed. During the second water suspension, the differences in gas exchange were less significant, and the timing of biostimulant administration—before or after the imposition of the water suspension—was less significant. Overall, the results demonstrate the species-specific response and the effectiveness of biostimulant when the biostimulant is administered before stress is imposed.

Keywords: climate change; biostimulants; chlorophyll *a* fluorescence; gas exchange; SPAD index

6.1. Introduction

Drought stress is regarded as the primary limiting factor for plant growth in the Mediterranean region during the summer months, primarily because of elevated levels of solar radiation and high temperatures (Di Castri 1981; Dai 2011). Water scarcity, coupled with high temperatures and intense radiation, is recognized as one of the most significant environmental stresses that diminish the survival and productivity of plants in arid and semi-arid regions (Chaves et al. 2003; Morales et al. 2013).
Climate change in this region, which is more intense than in other parts of the world, has resulted in a significant increase in water shortages

throughout the year, and this trend is expected to worsen in the future. As noted by Cramer and Guiot (2022), "the Mediterranean is warming 20% faster than the global average, and the region is one of the main climate change hotspots in the world".

Issues related to drought stress are particularly significant for the management of green spaces, where it is not always feasible to continuously meet the water requirements of various species. Identifying plants that can withstand specific abiotic stresses has been the focus of researches (Franco et al. 2006) because this information can enhance the sustainability of green spaces (Caparrós-Martínez et al. 2020; Shehayeb et al. 2025). Given the wide variety of ornamental species utilized in the Mediterranean environment, it is possible to identify genotypes that are better suited to tolerate environmental stresses, particularly drought (Toscano et al. 2019).

There is significant interest in studying the responses of ornamental plants to water deficits in urban and suburban green spaces (Leotta et al. 2023). Plant responses to drought are diverse and interconnected (Efeoğlu et al. 2009), and the ability of plants to adapt to drought stress can vary considerably among different genera and even within the same species (Sánchez-Blanco et al. 2002; Torrecillas et al. 2003).

Drought stress encompasses a range of morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity (Wang and Swail 2001). Numerous studies on ornamental plants, which are often cultivated in small containers with limited water capacity, have demonstrated that plant quality declines as the intensity of water stress increases (Hansen and Petersen 2004; Henson et al. 2006; Álvarez et al. 2009; Bernal et al. 2011; Álvarez and Sánchez-Blanco 2013).

Mediterranean species have developed various physiological and morphological adaptations to cope with drought stress (Dickson and Tomlinson 1996). These adaptations include the regulation of gas exchange (Moriana et al. 2002), osmotic adjustment (Chartzoulakis et

al. 1999), and the development of protective structures on leaves, such as hairs, thick cuticles, and sclerenchymatous cells. Additionally, some leaf modifications occur, including changes in leaf inclination, an increase in thickness, and a reduction in surface area (Castro-Díez and Montserrat-Martí 1998; Karabourniotis 1998; Gratani and Bombelli 2000). Furthermore, these species often exhibit extensive root systems (Malinowski and Belesky 2000).

Numerous morphological adaptations to drought stress involve the aboveground parts of plants. Leaf growth is the plant process that is most sensitive to water deficit (Hsiao 1973; Bradford and Hsiao 1982; Jones 1985). For example, vertically oriented leaves enable plants to minimize the radiant energy they intercept (Pereira and Chaves 1993). Specific leaf area (SLA), commonly used as an indirect indicator of leaf thickness, decreases under drought conditions (Marcelis et al. 1998; Liu and Stützel 2004). This reduction serves as a strategy to enhance water use efficiency (WUE) (Wright et al. 1994; Craufurd et al. 1999), as thicker leaves typically contain higher levels of chlorophyll and protein per unit of leaf area, resulting in greater photosynthetic capacities per unit of leaf area compared to thinner leaves (Liu and Stützel 2004).

For example, it has been demonstrated that the specific leaf area (SLA) is reduced in *Asteriscus maritimus* (L.) Less. due to water stress, which directly results in a decrease in leaf area (Rodríguez et al. 2005). Similar responses have been observed in *Eragrostis curvula* (Schrad.) Nees, *Oryza sativa* L., and *Abelmoschus esculentus* (L.) Moench when subjected to water stress; in all these species, total leaf surface area was significantly diminished following periods of water scarcity (Rucker et al. 1995; Shubhra and Goswami 2003).

Several authors have observed an increase in the root-to-shoot ratio in plants subjected to water stress (Blum et al. 1996; Zwack et al. 1998). This phenomenon represents an effective adaptive strategy (Bargali

and Tewari 2004; Guo et al. 2007) because the root system of a plant leads to more efficient water absorption.

In addition, the morphological changes that occur in response to stress, which may only become apparent over time, certain physiological processes immediately indicate drought damage. One of the primary consequences of water stress is a reduction in net photosynthesis (Hsiao and Acevedo 1975), which is associated with stomatal closure. This response is a mechanism employed by plants to minimize water loss via transpiration (Nayyar and Gupta 2006; Yang et al. 2006). However, the duration and rate of stomatal closure can vary significantly among species (Schulze and Hall 1982).

Woody evergreen plants, including trees and shrubs, have developed various mechanisms to adapt to typical Mediterranean conditions, particularly their ability to endure water scarcity and recover after water is received (Galmés et al. 2007). For instance, lemon plants respond to water stress and subsequent rehydration by activating drought avoidance strategies such as stomatal closure, leaf rolling, and partial defoliation (Ruiz-Sànchez et al. 1997).

Efeoğlu et al. (2009) demonstrated that the relative water content in maize was significantly reduced under drought stress conditions but increased significantly during the recovery period, ultimately reaching the levels observed in the control plants. Other researchers, such as Sánchez-Blanco et al. (2002), showed that *Cistus albidus* L. and *C. monspeliensis* plants that experienced periods of water stress followed by a return of water availability developed distinct avoidance mechanisms. For instance, *C. albidus* limits cell growth and expansion, whereas *C. monspeliensis* L. reduces photosynthetic processes.

A reduction in photosynthetic rates and transpiration has generally been observed in plants under drought stress conditions (Endres 2007; Hessini et al. 2009; Saibo et al. 2009), as well as in environmental

conditions where evapotranspiration demands are exceptionally high (Feng and Cao 2005).

Under these conditions, the limitation of gas exchange due to stomatal closure disrupts the balance between the light response and the Calvin-Benson cycle, primarily because of the reduced availability of CO_2 (Chaves et al. 2009). The photochemical activity of photosystem II (PSII) plays a crucial role in the photosynthetic response to adverse environmental conditions. The photochemistry of PSII has been extensively studied using chlorophyll *a* fluorescence (Colom and Vazzana 2003; Lu et al. 2003), which provides valuable insights into plants responses to environmental stress.

Due to the escalating issue of water scarcity, particularly in the Mediterranean region, research has increasingly focused on analyzing the responses of native shrub species to water stress (Munné-Bosh et al. 2003; Munné-Bosh and Peñuelas 2004; Maraghni et al. 2014). In contrast, there has been less extensive analysis of exotic species that are prevalent in ornamental landscaping (Álvarez et al. 2011). However, a significant knowledge gap remains regarding the responses of certain ornamental species in Mediterranean environments to short-term water stress, which is often exacerbated by extremely high temperatures exceeding 40°C.

Another strategy to enhance drought stress tolerance is the use of biostimulants, which have been shown to improve plant performance under stressful conditions (Ramzan and Younis 2022). Biostimulants serve as agronomic support tools that can amplify the existing tolerance mechanisms in plants, enabling them to withstand abiotic stress. Biostimulants can enhance plant adaptation and tolerance to drought stress by activating or modulating physiological and biochemical processes (Turan et al. 2023). In recent years, there has been a notable increase in the application of biostimulants in agriculture and horticulture to bolster crop resilience to abiotic stress (du Jardin 2015). Mitigation of abiotic stress is perhaps the most

frequently cited benefit of biostimulant formulations (Yakhin et al. 2017).

The mechanisms of action vary and enhance water use efficiency at the leaf level (Bulgari et al. 2019) by improving stomatal regulation, which reduces water loss through transpiration and helps maintain water balance. At the root level, these mechanisms increase the biomass and water absorption capacity. Additionally, at the cellular level, they lower osmotic potential by producing and accumulating of osmolytes (Ouhaddou et al. 2023).

However, drought stress severity is a critical factor influencing plant responses. Although plants typically exhibit responses to mild or moderate water stress, these responses may be absent under conditions of severe stress (Watkinson et al. 2003), which have recently been exacerbated by global change.

In this context, the objective of this study was to analyze the response of 15 shrub species that are widely distributed in the Mediterranean environment and exhibit diverse leaf characteristics to severe drought stress. This stress was induced by the suspension of irrigation and exposure to temperatures exceeding 40°C. Following the stress period, the plants were irrigated to field capacity to assess their recovery potential. To evaluate the possible efficacy of the biostimulant, it was administered at the root level both as a "preventive" measure (prior to the imposition of water stress) and as a "curative" treatment (simultaneously with water replenishment). This approach aimed to determine whether the timing of biostimulant application influenced its effectiveness.

6.2. Materials and methods

The experiment was conducted during June-July 2023 at a commercial nursery near Catania, Italy. Fifteen shrub species were adopted (Table 6.1). Rooted cuttings of the species were transplanted into 14 Ø pots

(one plant per pot) filled with a substrate based on peat and perlite (2/L, v/v).

Table 6.1. Plant species adopted.

Abbreviation	Plant species	Botanical family
Carissa	*Carissa macrocarpa* (Eckl.) A.DC.	*Apocynaceae*
Elaeagnus	*Elaeagnus* ×*submacrophylla* 'Viveleg'[PBR]	*Elaeagnaceae*
Eugenia	*Syzygium australe* (J.C.Wendl. ex Link) B.Hyland	*Myrtaceae*
E. 'Newport'	*Syzygium australe* (J.C.Wendl. ex Link) B.Hyland 'Newport'	*Myrtaceae*
Lantana	*Lantana camara* L.	*Verbenaceae*
Metrosideros	*Metrosideros excelsa* Sol. ex Gaertn.	*Myrtaceae*
Nerium	*Nerium oleander* L.	*Apocynaceae*
Photinia	*Photinia fraseri* Dress 'Red Robin'	*Rosaceae*
Pittosporum	*Pittosporum tobira* W.T.Aiton 'Nanum'	*Pittosporaceae*
R. indica	*Rhaphiolepis indica* (L.) Lindl.	*Rosaceae*
R. umbellata	*Rhaphiolepis indica* var. *umbellata* (Thunb.) H.Ohashi	*Rosaceae*
Salvia	*Salvia officinalis* L.	*Lamiaceae*
Strelitzia	*Strelitzia reginae* Banks	*Strelitziaceae*
V. lucidum	*Viburnum tinus* L. 'Lucidum'	*Viburnaceae*
V. tinus	*Viburnum tinus* L.	*Viburnaceae*

Plants were irrigated regularly for one month at a well-watered (100% WC) level. At the beginning of July, the plants were separated into two groups: control plants (100% WC) and drought-stressed plants (no irrigation for five days). The plants subjected to drought stress, after five days were watered at 100% WC and left in these conditions for 5 days. This stress condition was replied twice (Figure 6.1).

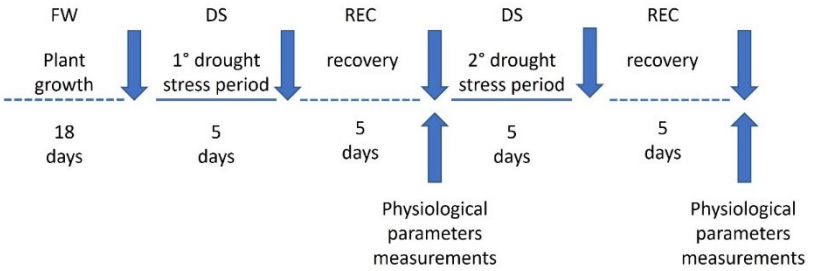

Figure 6.1. Timeline for suspension/rewatering (S-R) treatments used in the experiment. FW = full watering; DS = drought stress event; REC = recovery.

Biostimulant [Drin of Green Has Italia S.p.A., Canale (CN), Italy at the dose of 1:100 (v/v)], was administered at the root level both prior to the imposition of water stress as a preventive measure (T.B.P.) and simultaneously with water replenishment as a "curative" treatment (T.B.C.).
Weather data were registered using a data logger CR1000 (Campbell Scientific Ltd., Loughborough, UK) during the experimental period. Mean temperatures ranged between 30.5 and 35.5 °C, and relative humidity (RH) ranged between 50 and 66%.

6.2.1. Physiological parameters

The chlorophyll content was estimated using SPAD. Before measuring, the SPAD-502 meter (Konica Minolta, Japan) was calibrated using the reading checker supplied by the manufacturer. Each leaf SPAD value is the average of six readings on the upper side of the leaf.
The leaf gas exchange parameters [net photosynthetic rate (AN), stomatal conductance (gs), and transpiration rate (E)] using a CO_2/H_2O infrared gas photosynthesis system (LCi, ADC Bioscientific Ltd., Hoddesdon, UK) were measured for every water treatment. Measurements were taken between 09:00 and 11:00.

Chlorophyll *a* fluorescence was evaluated using a modulated chlorophyll fluorimeter OS1-FL (Opti-Sciences Corporation, Tyngsboro, MA, USA). Chlorophyll *a* fluorescence was expressed as the Fv/Fm ratio, where Fm is the maximal fluorescence in the dark-adapted state.

6.2.2. Statistical analysis

The experiment used a randomised complete design with three replicates per treatment. Data are mean ± population S.E. (n = 6).

6.3. Results

Despite the short duration of stress, drought significantly affected SPAD values (Figure 6.2). The observed differences were primarily related to plant species. Differences due to various treatments within the same species were rarely significant, except for some genotypes. In Eugenia and R. umbellata, SPAD values were notably low in stressed plants. The biostimulant treatment increased these values, making them comparable to those of control plants. In R. umbellata, SPAD values were higher when the biostimulant was applied before the irrigation suspension. In Nerium, the SPAD values were higher under stressed conditions than in control (Figure 6.2).

Net photosynthesis values recorded the day after the first water suspension cycle showed that for several genotypes—E. 'Newport', Elaeagnus, Eugenia, Lantana, Metrosideros, Salvia, Strelitzia, and V. tinus—the values for control plants were more than double those of the stressed plants. In Pittosporum and R. umbellata, the differences, were less pronounced, although substantial. For some species—Carissa, Nerium, Photinia, and V. lucidum—the values were comparable between control and stressed plants (Figure 6.3).

Treatments with biostimulants appeared to be more effective when applied before stress was imposed rather than afterwards. In any case, except for E. 'Newport', Photinia, V. lucidum, and V. tinus, the

application of the biostimulant improved net photosynthesis compared to that of the stressed plants (Figure 6.3).

Stomatal conductance was higher in the control plants than in the stressed plants (Figure 6.4), except for Carissa and Nerium. The application of biostimulants as a preventative measure enhanced stomatal conductance in stressed R. umbellata plants in this study. In Strelitzia, stomatal conductance was higher in plants that received biostimulant for curative purposes (Figure 6.4).

Transpiration rates were generally higher in the control plants. However, in Carissa, Metrosideros, R. umbellata, and Strelitzia, higher values were recorded in stressed plants, especially when they received the biostimulant for preventive or curative purposes (Figure 6.5).

The Fv/Fm ratio consistently showed values below the critical threshold of 0.8, indicating stress (Figure 6.6). Particularly low values were observed for E. 'Newport', Elaeagnus, Eugenia, Metrosideros, Photinia, Pittosporum, R. indica, R. umbellata, and V. tinus. For some species—Carissa, Elaeagnus, Eugenia, Metrosideros, Photinia, R. indica, R. umbellata, and V. tinus—higher values than those of stressed plants were observed following biostimulant treatment. However, no positive effects of biostimulant application were observed in the other species. After the second period of water suspension, the SPAD values in all plant species were lower than those in the first water suspension (Figures 6.2 and 6.7), and the differences between treatments for the same genotypes were smaller and often not significant.

Net photosynthesis was higher in control plants; in some cases – in Carissa, Lantana, Salvia, Strelitzia, and V. tinus – the biostimulant treatment as a preventive measure was able to increase the value of An (Figure 6.8). In Pittosporum and R. indica the biostimulant treatment as a curative measure was more effective.

At the end of the second water withdrawal period, stomatal conductance showed values comparable to those recorded during the first survey; however, the responses among different species varied considerably. Specifically, Lantana, Nerium, Photinia, R. indica, and Salvia exhibited the highest values, occasionally exceeding those observed in the first survey. Treatment with biostimulants never resulted in higher values than the control or stressed plants, except in the case of Carissa, where the preventive application of the biostimulant produced values more than double those of both control and stressed plants (Figure 6.9).

Transpiration rate values at the end of the second water withdrawal period were nearly halved compared with those recorded in the previous survey. Carissa and Metrosideros plants exhibited higher values than the control plants when the biostimulant was applied preventively, whereas R. indica showed higher values than the control plants when the biostimulant was applied preventively and curatively purposes (Figure 6.10).

The maximum quantum efficiency of PSII was comparable to that recorded at the end of the water suspension, although these values were almost always below the threshold of 0.80. The highest absolute values were observed for Carissa, Lantana, Nerium, R. umbellata, Salvia, and Strelitzia. In some cases, plant mortality prevented the detection of chlorophyll *a* in sufficient numbers of specimens (Figure 6.11).

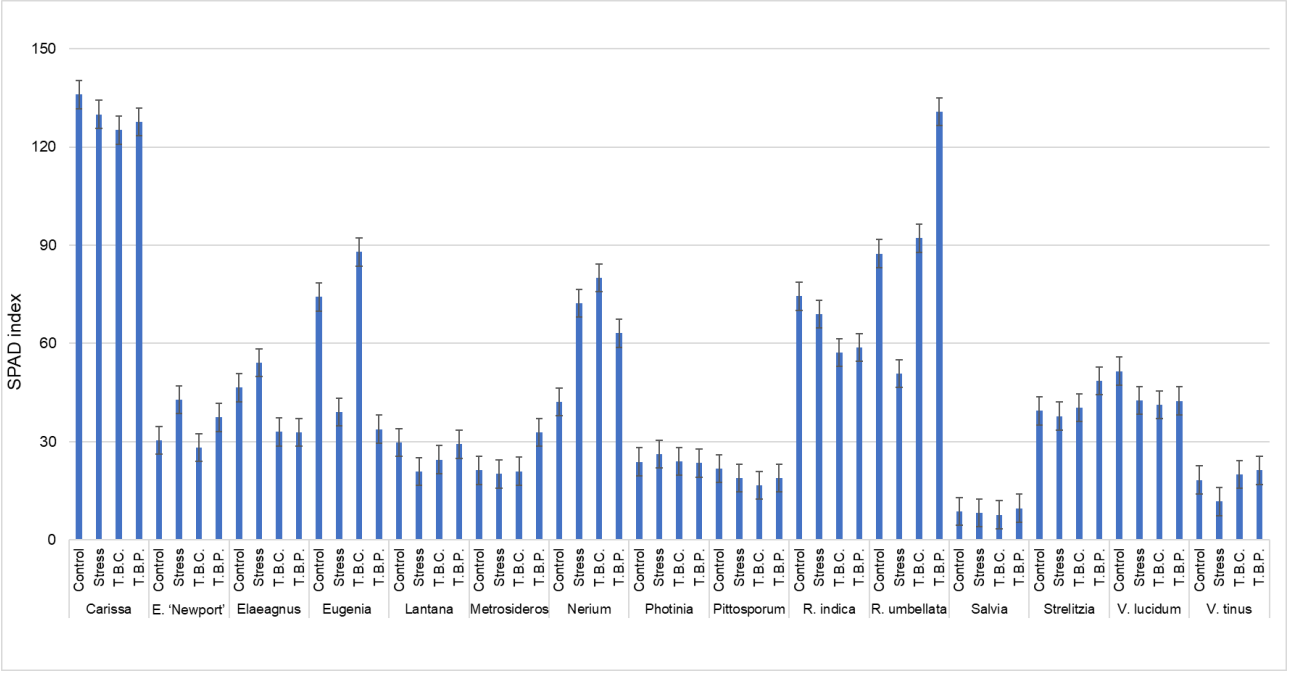

Figure 6.2. SPAD index at the end of the first period of water suspension in relation to different treatments. Data are mean ± population S.E. (n = 6).

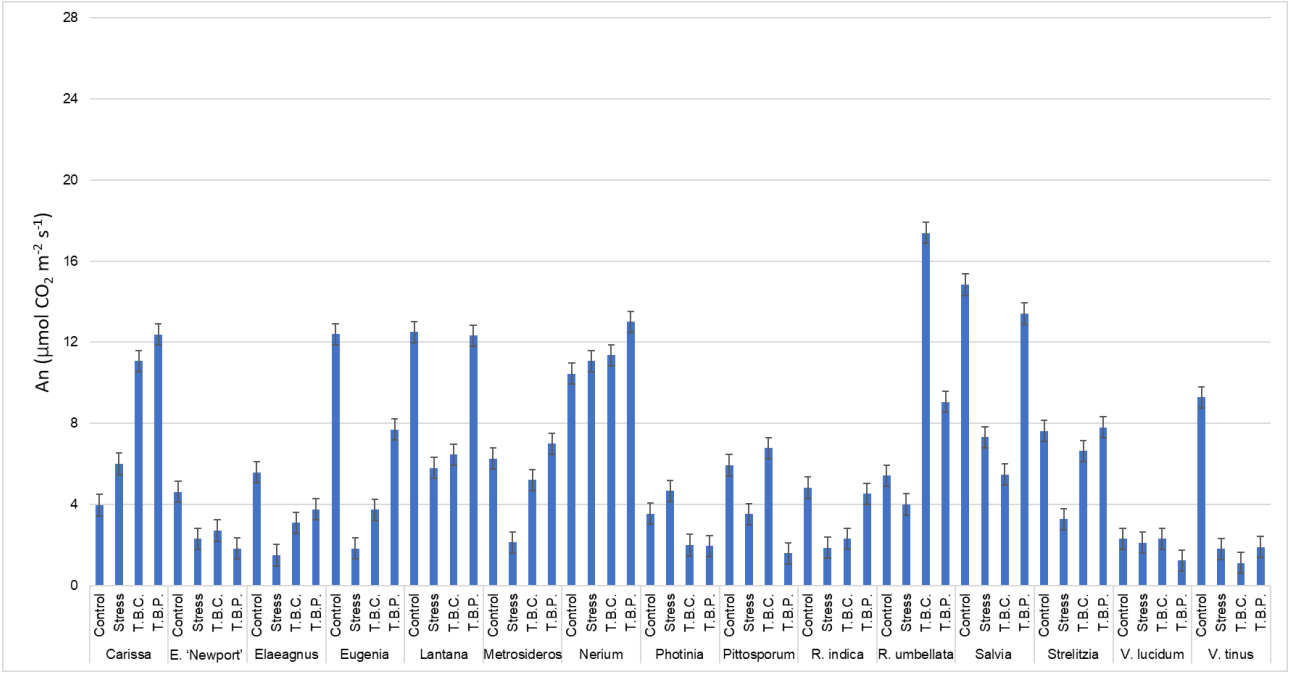

Figure 6.3. Net photosynthesis (An) at the end of the first period of water suspension in relation to different treatments. Data are mean ± population S.E. (n = 6).

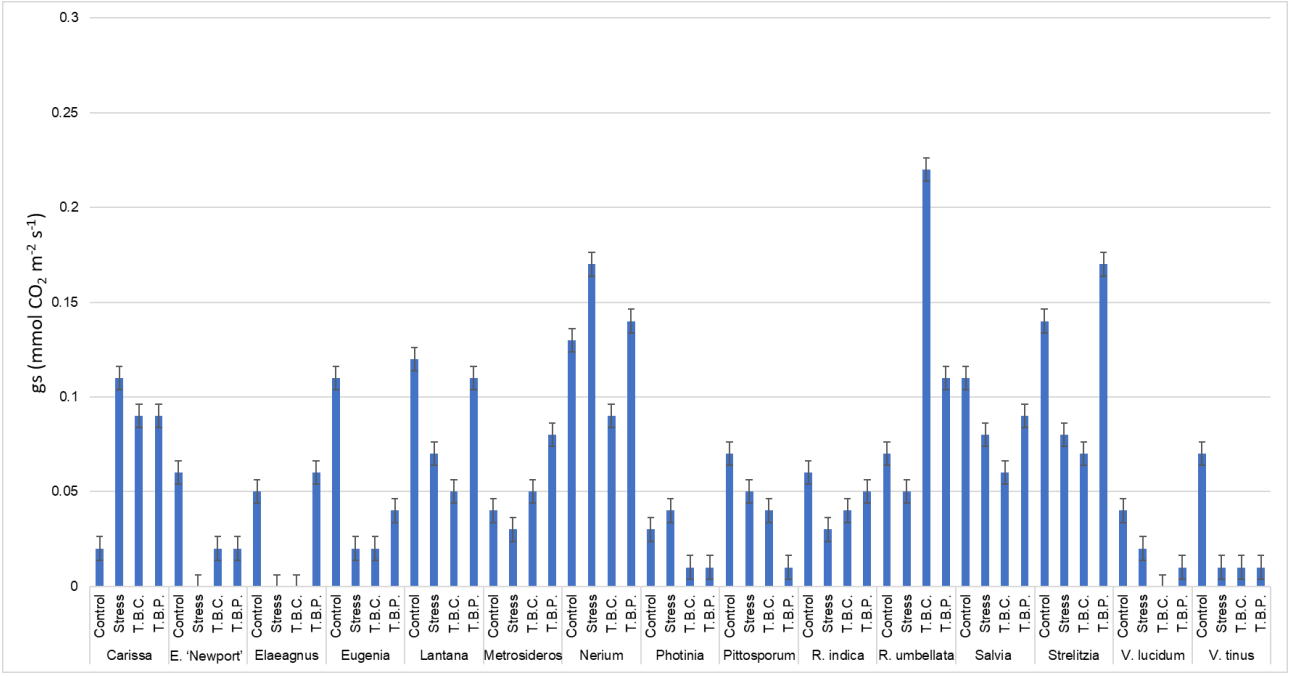

Figure 6.4. Stomatal conductance (gs) at the end of the first period of water suspension in relation to different treatments. Data are mean ± population S.E. (n = 6).

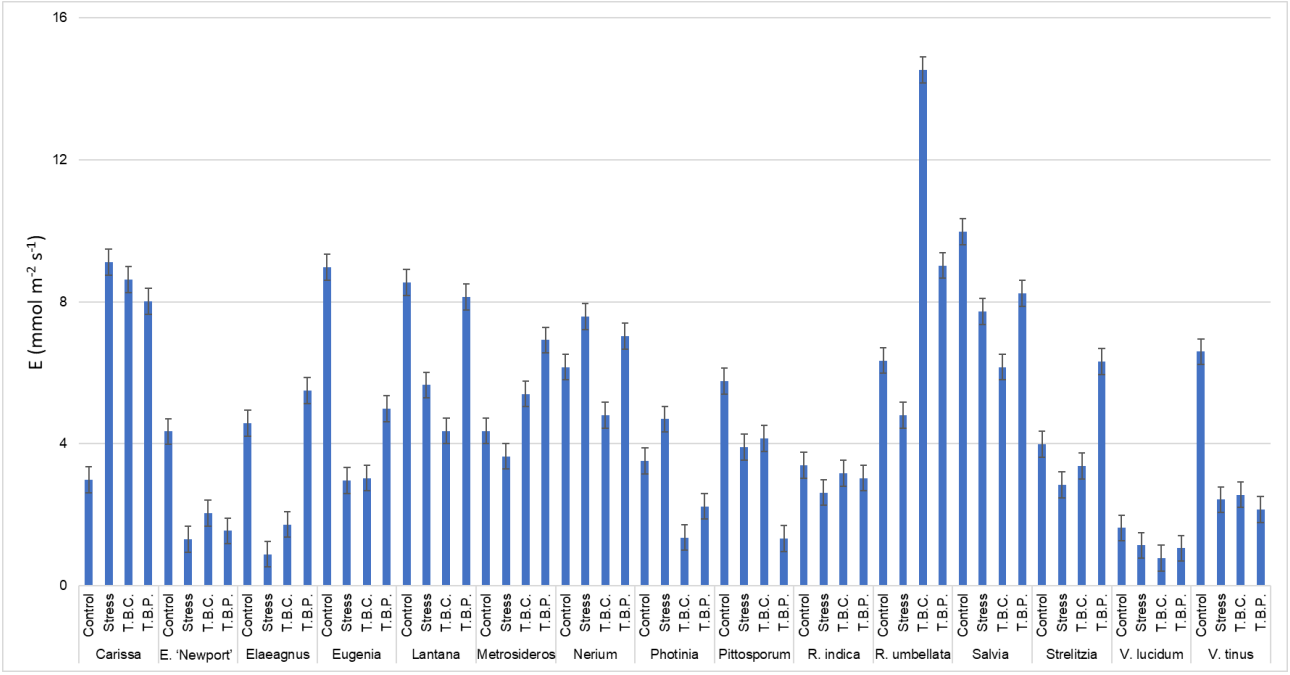

Figure 6.5. Transpiration rate (E) at the end of the first period of water suspension in relation to different treatments. Data are mean ± population S.E. (n = 6).

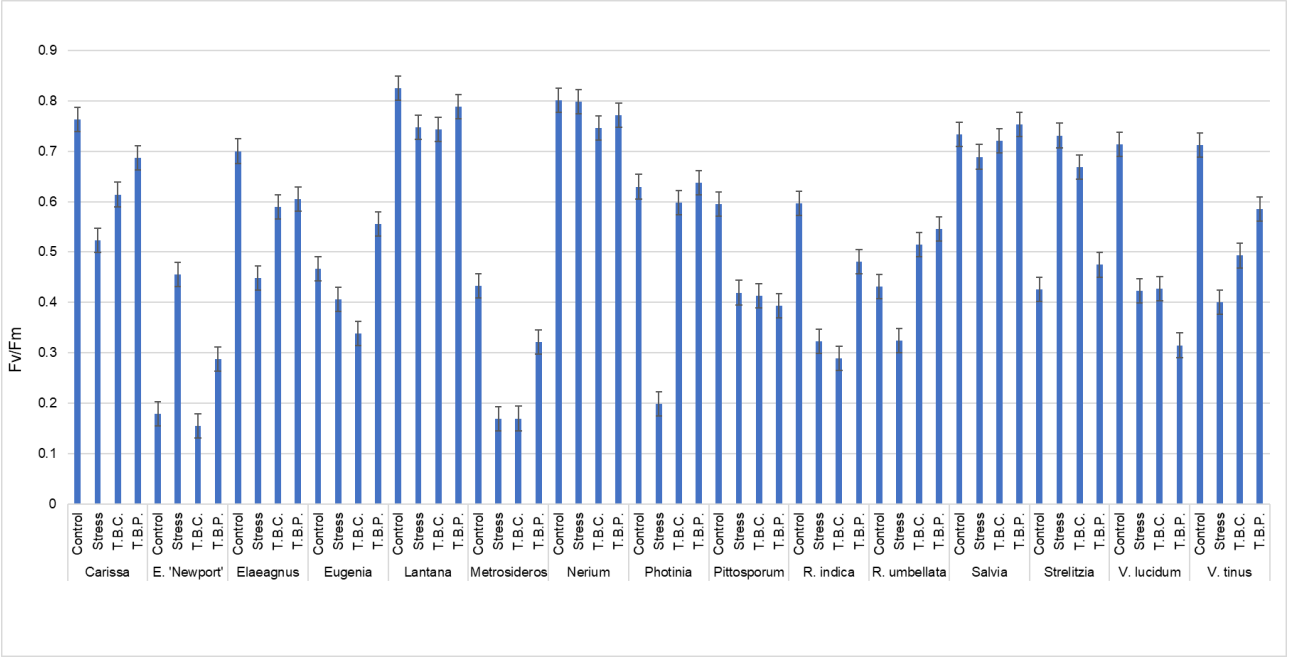

Figure 6.6. Maximum quantum efficiency of the PSII (Fv/Fm) at the end of the first period of water suspension in relation to different treatments. Data are mean ± population S.E. (n = 6).

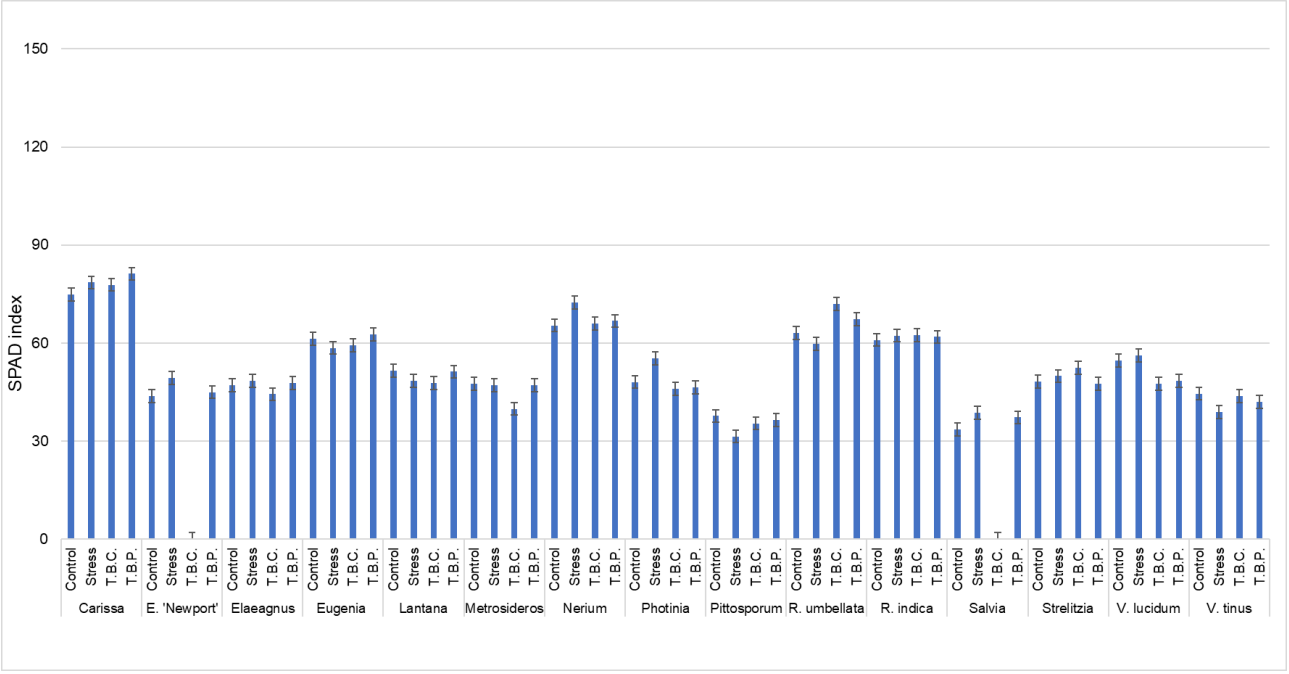

Figure 6.7. SPAD index at the end of the second period of water suspension in relation to different treatments. Data are mean ± population S.E. (n = 6).

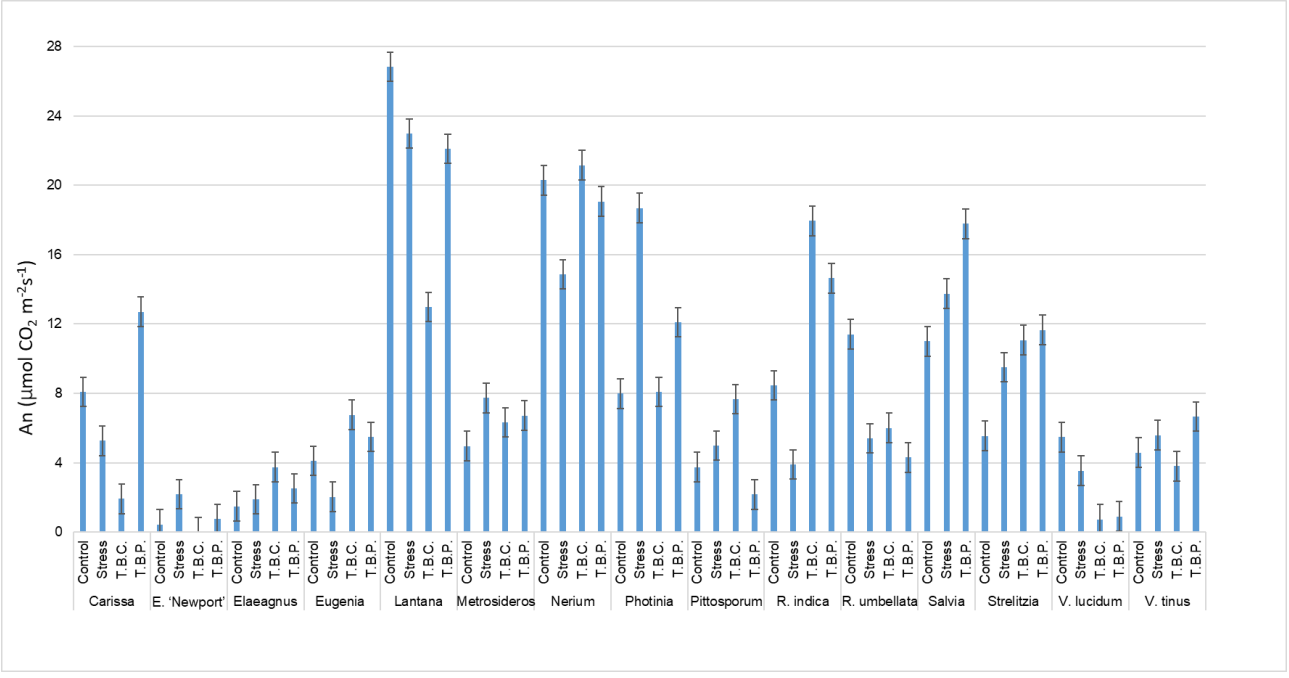

Figure 6.8. Net photosynthesis (An) at the end of the second period of water suspension in relation to different treatments. Data are mean ± population S.E. (n = 6).

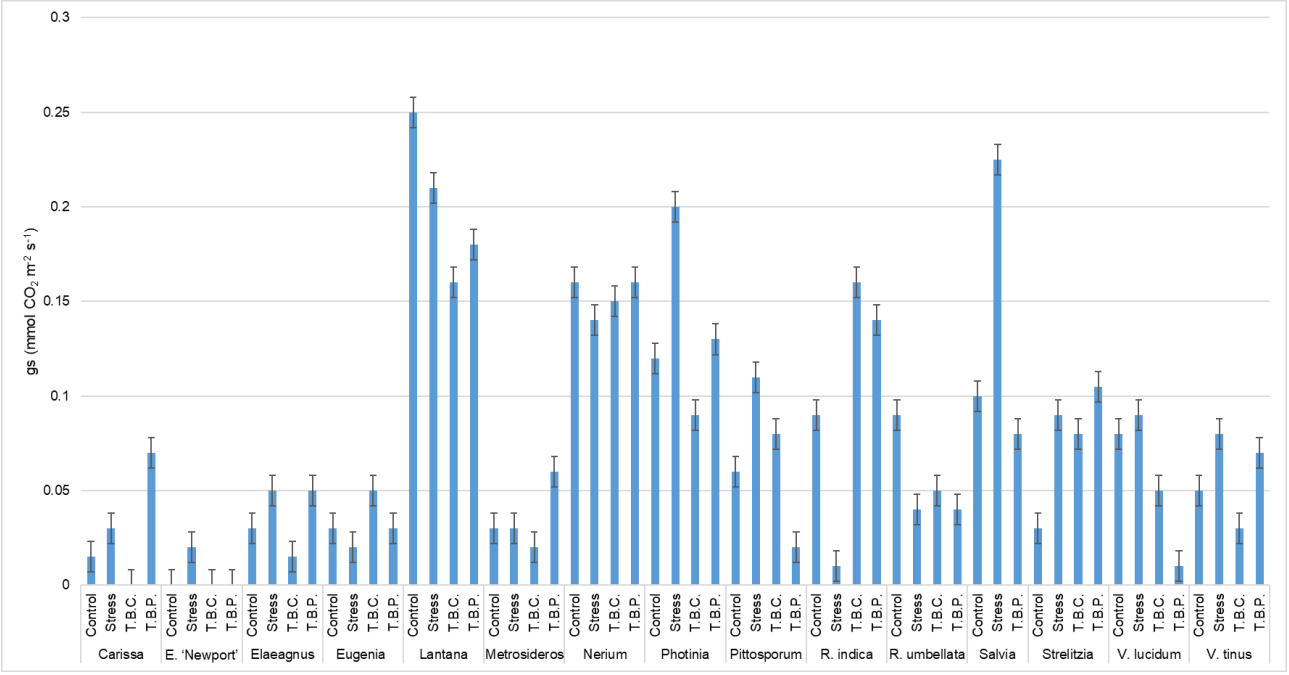

Figure 6.9. Stomatal conductance (gs) at the end of the second period of water suspension in relation to different treatments. Data are mean ± population S.E. (n = 6).

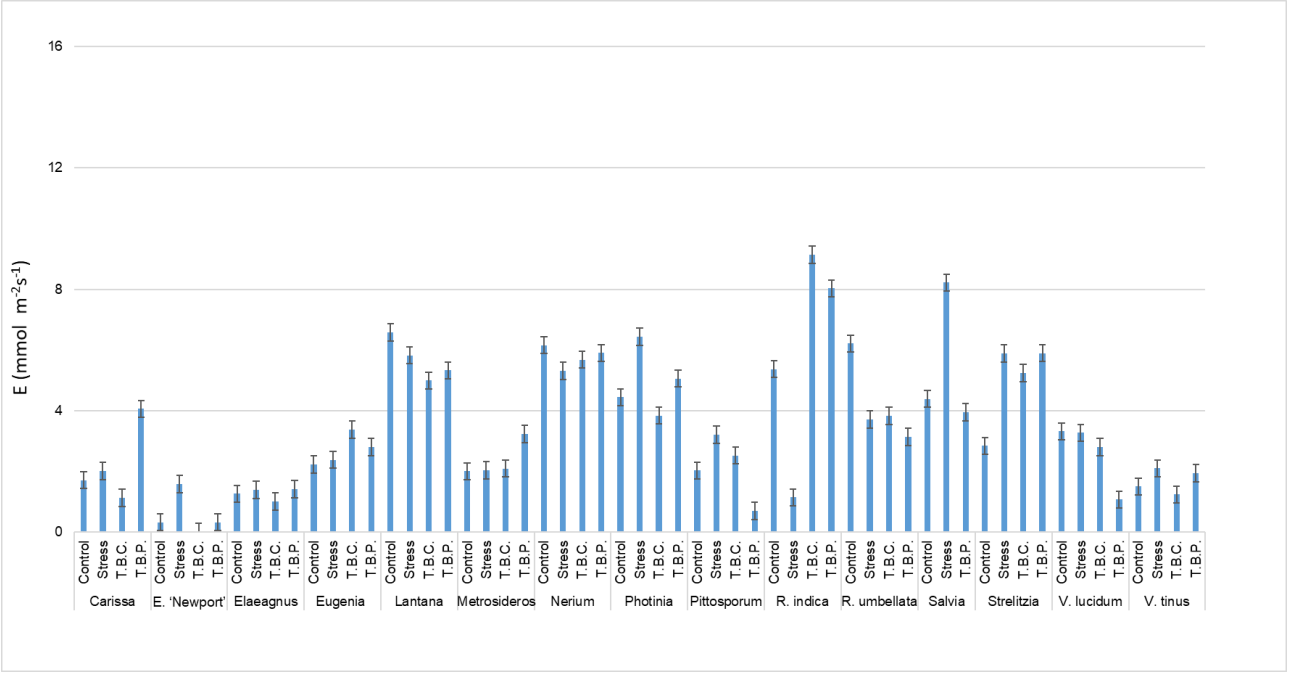

Figure 6.10. Transpiration rate (E) at the end of the second period of water suspension in relation to different treatments. Data are mean ± population S.E. (n = 6).

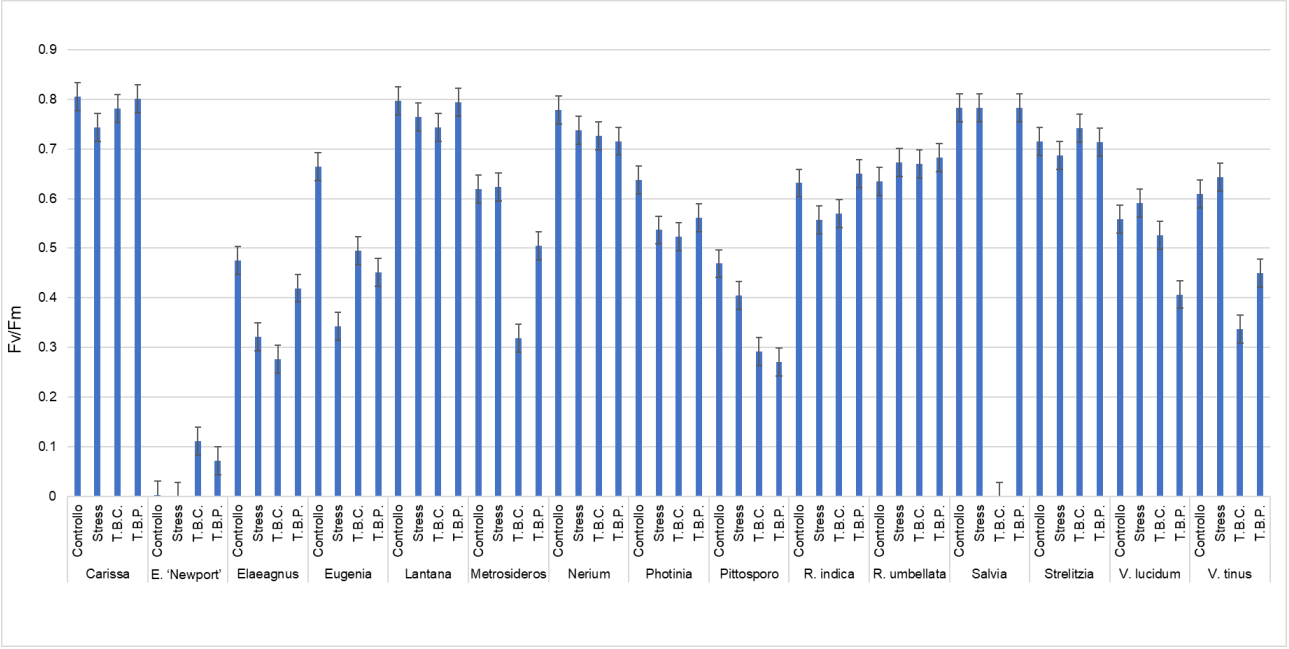

Figure 6.11. Maximum quantum efficiency of the PSII (Fv/Fm) at the end of the second period of water suspension in relation to different treatments. Data are mean ± population S.E. (n = 6).

6.4. Discussion

Urban and peri-urban green spaces often deficiency irrigation systems to reduce their management costs. Consequently, ornamental plants in these areas are frequently exposed to water stress during summer. Many ornamental shrubs and subshrubs demonstrate strong drought tolerance, having adapted their physiology and morphology to survive in arid environments. Identifying the physiological and morpho-anatomical traits associated with drought tolerance is valuable for selecting plant species suited to dry conditions. In urban green space planning, choosing appropriate species is crucial for maintaining the ornamental value of these areas. However, owing to the vast number of species, information on the drought tolerance of many ornamental plants is often limited.

Drought-tolerant plants can be selected using specific physiological, biochemical, or molecular markers that highlight the traits associated with drought tolerance. These markers can be applied at the nursery level or used to identify native wild species that are suitable for urban environments (Ferrante et al. 2021). In particular, physiological markers, such as gas exchange and chlorophyll *a* fluorescence, provide rapid and valuable information. Our trial demonstrated that these measurements are effective for identifying shrubs capable of withstanding drought stress.

Drought stress induces various plant responses, including physiological and metabolic changes (Tian and Lei 2007). Plant responses to drought stress are influenced by climatic, soil, and agronomic factors (Anjum et al. 2011). Water loss occurs primarily at the leaf level through transpiration. Plants experiencing water deficits limit gas exchange because water is a key factor in photosynthesis. Plants under water stress close their stomata to prevent transpiration and water loss (Toscano et al. 2018). Reduced photosynthesis can affect the leaf and plant temperatures. Physiologically, transpiration is

responsible for thermoregulation, and during warmer seasons, temperature regulation is essential for photosynthesis (Álvarez et al. 2018). Reduced transpiration increases tissue respiration, depleting sugar reserves and nutrient supplies. This reduction in energy sources can negatively affect the quality ornamental plants. Decreased carbohydrate levels can induce leaf senescence, leading to abscission or yellowing. In our trial, despite the short period of stress, the plants were not always able to compensate for water loss, resulting in evident yellowing—reflected by a reduction in the SPAD index—as well as the death of some plants among the most sensitive genotypes.

Plants subjected to stress improve water use efficiency by modulating stomatal conductance and light use efficiency. As the energy absorbed by the leaves cannot compensate for water shortages, plants reduce their light use efficiency. This strategy can be monitored by measuring chlorophyll *a* fluorescence, a rapid and non-destructive method for assessing plant responses to abiotic stress (Maxwell and Johnson 2000; Pellegrini et al. 2011). Chlorophyll *a* fluorescence parameters have been used to select plants tolerant to water stress (Percival and Sheriffs 2002) for ornamental use. Plants under stress exhibit a low Fv/Fm ratio, which represents the maximum quantum efficiency of photosystem II. Several studies have reported that the Fv/Fm ratio ranges between 0.78 and 0.85 under non-stress conditions (Toscano et al. 2019), with lower values indicating stressful conditions.

Low Fv/Fm values indicate that PSII has been damaged and that the heat dissipation capacity is reduced in the leaves of both species. Excessive accumulation of light energy in the reaction center causes further damage to the photosynthetic apparatus. This mechanism inhibits the transfer of photosynthetic electrons from the PSII reaction center to the QA, QB, and PQ pools, resulting in a significant reduction in net photosynthesis (Lang et al. 2018). The analysis of chlorophyll *a* fluorescence rates supports the idea that changes in

biochemical processes within the mesophyll (non-stomatal factors) also contribute to the decline in photosynthesis (Álvarez et al. 2018).
Plant susceptibility to drought stress varies depending on the severity of the stress, interactions with other stressors, plant species, and developmental stage (Demirevska et al. 2009). Our results revealed different stress responses among the 15 species studied, which correlated with their varying degrees of tolerance.
Almost all species exhibited a reduction in assimilation under increasing water stress. A significant decrease in stomatal conductance (Gs) suggests efficient adaptive control of transpiration in some species (Hessini et al. 2008). Numerous studies have demonstrated a decline in photosynthetic activity under drought stress owing to stomatal and non-stomatal mechanisms (Samarah et al. 2009). In drought-tolerant species, the reduction in photosynthesis primarily results from stomatal closure, which limits water loss. Conversely, in drought-sensitive plants, a decrease in net photosynthesis is primarily caused by water deficiency, leading to severe damage. Our results revealed a positive correlation between photosynthesis and stomatal conductance in several species, indicating that stomatal closure intensifies with increasing drought stress (Anjum et al. 2011). In most woody species, increased water use efficiency correlates with CO_2 assimilation, which remains proportionally higher than water vapor loss through the stomata, representing an additional drought acclimation mechanism (Álvarez et al. 2011).
Drought stress levels compromise photosynthetic efficiency in several genotypes (Bian and Jiang 2009). Chlorophyll, a major component of chloroplasts, plays a crucial role in photosynthesis, and the relative chlorophyll content is positively correlated with the photosynthesis rate (Guo and Li 1996). Flexas and Medrano (2002) reported that water stress reduces leaf greenness in C3 plants because of chlorophyll degradation. In our study, water deficit significantly affected the relative chlorophyll concentrations in leaves. The observed variations

in chlorophyll concentrations may be attributed to the challenging conditions under which the experiment was conducted (Toscano et al. 2014, 2018).

Plants grown in Mediterranean climates represent valuable genetic material for a better understanding of the morphological and physiological responses to water stress (Toscano et al. 2016).

These results suggest that certain species can be utilized for gardening purposes in the Mediterranean region, despite the varying mechanisms of action in response to water stress among different genotypes. The ability of plants to withstand water stress is likely species-specific, as highlighted by Davies et al. (2016) in *Forsythia* × *intermedia* Zabel and *Lonicera periclymenum* L. In our trial, several species exhibited significant decreases in leaf gas exchange parameters. Under water stress conditions, plants optimize carbon assimilation and minimize water loss by reducing stomatal conductance (Medrano et al. 2002). Consistent with Toscano et al. (2016), the trends in photosynthetic activity and stomatal conductance corresponded to the soil water content. Our experiment revealed a positive correlation between photosynthesis and stomatal conductance in several species, indicating that stomatal closure intensifies with increasing water stress. This is in agreement with Zhang et al. (2013), who reported that in *Fraxinus* plants subjected to water stress, the primary cause of decreased stomatal conductance (gs) was partial stomatal closure rather than changes in stomatal density, which remained relatively constant throughout the growth period. This study also examined the influence of water deficit on plant physiological processes. In the short term, plants exhibit temporary tolerance to water stress through avoidance mechanisms, such as stomatal closure, thereby preventing water loss. However, this response leads to a reduction in CO_2 assimilation (Souza et al. 2018). The decrease in gs observed in our study suggests an adaptive and efficient control of transpiration by some species, representing a mechanism to cope with water stress, especially during

periods of high transpiration, by limiting water loss (Hessini et al. 2008). Severe water deficit negatively affected the photosynthetic rate and stomatal conductance in numerous species, as evidenced by the very low gs values. This response may indicate the non-stomatal limitations of photosynthesis during stress (Álvarez et al. 2018).

The use of biostimulants in sustainable agricultural practices is gaining traction as a promising, safe, and environmentally friendly alternative to enhance crop production performance (Martínez-Lorente et al. 2024). Their application is also expanding to urban green spaces. Unlike plant defense elicitors, which protect against biotic stress by inducing acquired systemic resistance, biostimulants enhance tolerance to abiotic stress (Qi et al. 2022).

In the case of substrate application, as used in our experiment, the effectiveness of products depends on adequate hydration. The efficacy of a biostimulant relies on the penetration of its active ingredients into the roots, with assimilation influenced by factors such as the molecular structure, particle size, and solubility (Martínez-Lorente et al. 2024).

Photosynthetic metabolism encompasses the chemical reactions within chloroplasts that reduce CO_2 into carbohydrates, utilizing ATP and reducing power in the form of NADPH, which are generated through photodependent reactions in the chloroplasts. Various biomolecules, including proteins from both photosystems, accessory pigments, and ions that serve as enzymatic cofactors, participate in these processes. Numerous studies have demonstrated that biostimulants can enhance photosynthesis components, improve its efficiency, and specifically mitigate or reverse the effects of abiotic stress on this vital process. For example, the commercial biostimulant Trainer® has been shown to increase the total chlorophyll content in several crops (Martínez-Lorente et al. 2024).

Chlorophylls are components of light-harvesting complexes, complemented by accessory pigments such as carotenoids (carotenes and xanthophylls) and anthocyanins. The levels of these pigments

typically increase in parallel with chlorophyll following the application of biostimulants. Light absorbed by pigments in the reaction centers of photosystem II is essential for the photolysis of water, electron transfer within photosystems, and the overall electron transport chain. Electron transfer and photosynthetic efficiency can be quantified by measuring various parameters, many of which improve with the application of biostimulants. Enhancements in photochemical efficiency (Fv/Fm ratio) have been observed in lettuce (Lucini et al. 2015) and basil (Rouphael et al. 2023), in photosystem II and photochemical efficiency in spinach (Carillo et al. 2019), and in net photosynthesis in olive (Regni et al. 2021).

Carbohydrate production requires CO_2 capture through gas exchange processes, such as transpiration and stomatal opening and closing. Specific biostimulants have been identified as enhancers of these processes in plants. For example, the application of Trainer® increased net CO_2 assimilation in tomato plants (Rouphael et al. 2017) and improved stomatal conductance, leaf transpiration, and net photosynthesis in geranium (Cirillo et al. 2018). In particular, biostimulants can increase or maintain these parameters in plants growing under unfavorable conditions.

Regarding the potential role of biostimulants in enhancing the response to abiotic stress, our experiment involved administering a commercial product both before and after stress was applied.

Our results suggest that the biostimulants based on free amino acids readily utilized by plant tissues, can enhance plant performance by maintaining stomatal opening, preserving photosynthesis and source-sink relationships (growth), thereby protecting against potential photoinhibition and photooxidation effects. The application of biostimulants increased the chlorophyll content. Consequently, the components of biostimulant induced greater biosynthesis of photosynthetic leaf pigments due to its rich amino acid content,

leading to increased chlorophyll levels (Desoky et al. 2018; Toscano et al. 2023).

There is no doubt, as frequently reported, that the effectiveness of biostimulants is species-specific and dose-dependent, as demonstrated by our results. In our experiment, we observed the critical importance of treatment timing; the best results were almost always achieved when the biostimulant was applied before the stress was imposed. This pre-treatment enabled the plants to enhance their physiological responses, thereby increasing their tolerance to water shortages and high temperatures.

6.5. Conclusion

The Mediterranean environment is characterized by prolonged summer droughts, which impose significant stress on plants and threaten their survival. Due to climate change, especially in the Mediterranean Basin, water availability is decreasing, making it an increasingly precious resource subject to rationing. This scarcity particularly affects green spaces, necessitating effective solutions based on careful species selection and the implementation of technical measures to mitigate drought stress. Among these measures, biostimulants have garned increasing interest. Given the wide variety of potentially usable species, it is essential to identify reliable and non-destructive indicators of plant response. In this context, gas exchange measurements, and in particular, chlorophyll *a* fluorescence values, provide dependable parameters for the early diagnosis of the adaptability of different genotypes to abiotic stresses, especially drought.

The trial, which examined 15 species and/or cultivars of plants used for ornamental purposes in the Mediterranean environment, yielded insightful results regarding the tolerance of different genotypes to drought stress. Based on Fv/Fm values, the genotypes that appeared to be best suited to Mediterranean conditions—also because they

exhibited similar values for this parameter during the second measurement, regardless of whether they were treated with a non-limiting water regime or subjected to periods of water withdrawal—were Lantana, Nerium, Salvia, and Strelitzia. Notably, some species showed a relatively better response during the second cycle of water withdrawal, demonstrating that certain plants are more efficient at modifying their physiology and morphology to adapt to suboptimal environmental conditions. Based on these results, it can be argued that biostimulants could be a valuable tool for mitigating the harmful effects of water scarcity associated with high temperatures. Specifically, the findings appear to confirm that the response is species-specific and that treatment is more effective when the biostimulant is administered preventively before stress is applied. Nevertheless, trials should be repeated to better identify the mechanisms underlying drought stress tolerance.

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7. Influence of dose and extraction modality of biostimulants on drought stress tolerance in *Coleus amboinicus* Lour. plants

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Abstract

Water stress poses a significant challenge to ornamental plants. Identifying genotypes resistant to water stress and developing sustainable solutions to mitigate the adverse effects of water scarcity are becoming increasingly important. One promising approach is the use of biostimulants such as plant extracts. In this context, the experiment aimed to analyze the response of *Coleus amboinicus* Lour. to water stress. The study was conducted in a greenhouse from April to June 2025, and two water regimes were compared: a non-limiting regime, in which all water lost through evapotranspiration was replenished (100% WC), and a limited regime, which restored only 50% of the water lost (50% WC). Plants under both water regimes were treated with *Crithmum maritimum* L., a medicinal plant that may

have a biostimulant effect. Two extraction techniques were employed: aqueous maceration (WE) and alcohol extraction (ethanol and methanol) (AE) and three different concentrations of the active ingredient were tested for each extraction method. The results demonstrated that reduced water availability significantly affected morpho-biometric parameters, with differences often reaching approximately 30%. These effects were primarily observed in the above-ground parts and photosynthetic apparatus. Treatment with biostimulants, particularly AE at 2.5 ml/L, significantly enhanced morpho-biometric parameters. The interaction effects were highly significant, and notably, the positive impact was most pronounced under stress conditions. The application of AE at 2.5 ml/L resulted in a 60.6% increase in total dry biomass compared to the control group and an impressive 131.0% increase in leaf area. SPAD values were generally low and tended to decrease as the growth cycle progressed, although they remained slightly higher in biostimulant-treated plants. Gas exchange parameters were typically higher in plants under non-limiting water regimes. Within the same water treatment, the highest values were generally recorded in biostimulant-treated plants, and the benefits of biostimulant application were especially evident in stressed plants. The efficiency of photosystem II (Fv/Fm) confirmed the stress conditions that characterized the trial. A key consideration is the impact of the biostimulant extraction method and its dosage. Although ethanol and methanol extraction methods are more costly, they have proven to be significantly more effective. The most favorable results were generally achieved with the highest concentrations. Further research is needed to clarify both extraction and administration methods and to determine the optimal dosage for biostimulant application.

Keywords: extraction methods; dose effect; ornamental plants; gas exchange; chlorophyll *a* fluorescence.

7.1. Introduction

Climate change is expected to increase the frequency of extreme drought events, thereby reducing the availability of water for plant growth (Change IPCC 2014). The ornamental plant sector currently faces a significant challenge due to global warming, which will affect both global production (Snyder 2017) and the use of these plants in green spaces because of the limited water resources. Global warming poses a formidable challenge, as the ornamental horticulture must adapt to new climatic conditions (Darras 2020; Eftekhari 2022). One potential solution is to identify genotypes that exhibit greater drought tolerance, require less water, or are better suited to extreme weather conditions (Cicevan et al. 2016; Caser et al. 2017). Consequently, native or naturally drought-tolerant plants are essential for the creation of "dry" or xeriscape gardens (Franco et al. 2006).

Another strategy involves understanding the mechanisms by which plants respond and adapt to water stress. Despite extensive research, the underlying mechanisms remain unclear (Luo et al. 2023). Plants exhibit a variety of morphological and physiological responses to drought stress (Larkunthod et al. 2018; Cal et al. 2019), which are essential for selecting varieties tolerant to water scarcity (Wu et al. 2022).

Exposure to drought stress induces morphological changes in the above-ground parts of plants; they produce smaller leaves and shed older ones to minimize transpiration and, consequently, water loss (Nemali et al. 2015). Although reducing leaf area helps maintain a favorable water status in plants, it also decreases photosynthesis and carbon assimilation.

Plant roots play a crucial role in drought stress responses (Guo et al. 2020). The root-to-shoot ratio serves as an alternative measurement method and is frequently used to evaluate plant biomass allocation (Poorter et al. 2012) or to analyze the differential distribution of

photosynthetic products between aboveground and belowground organs (Titlyanova et al. 1999).

An increased root-to-shoot ratio indicates greater allocation of biomass to underground structures. According to the "optimal allocation theory" (Gedroc et al. 1996), plants preferentially allocate biomass and nonstructural carbohydrates to acquire the resources that most limit their growth (Kobe et al. 2010). For example, drought conditions have been shown to increase the root-to-shoot ratio in rice (Xu et al. 2015) and sagebrush (Caser et al. 2017), whereas waterlogging has been found to reduce the root-to-shoot ratio in winter wheat (Shao et al. 2013) and maize (Herzog et al. 2016).

Photosynthetic pigments are essential for the absorption, transmission, and transformation of light energy during photosynthesis (Reinbothe et al. 2010; Arjenaki et al. 2012). Chlorophyll concentration is an effective indicator for assessing photosynthetic activity (Zobayed et al. 2005). A decline in chlorophyll levels is considered a non-stomatal limiting factor and protective mechanism for photosynthetic structures under abiotic stress conditions (Du et al. 2013; Jumrani and Bhatia 2019; Bhusal et al. 2020). Chlorophyll content directly influences photosynthesis and, to some extent, plant growth (Paknejad et al. 2007).

Photosynthetic activity in plant tissues is inhibited by an imbalance between light capture and utilization under drought stress (Foyer and Noctor 2000). The complete or partial closure of stomata to reduce water loss during drought stress results in changes in the gas exchange within leaves. Several parameters are used to assess changes in gas exchange, including stomatal conductance (gs), transpiration rate (E), and relative leaf water content (RWC) (Giordano et al. 2021).

In drought-tolerant plants, the leaf relative water content (RWC) decreases as soil moisture declines (Gao et al. 2020). Stomatal closure and reduced gas exchange result in decreased photosynthetic activity.

Water use efficiency (WUE) is defined as the ratio of photosynthesis to transpiration (Pn/E). An increase in WUE under water-stressed conditions is associated with adaptation to deficit irrigation, whereas a decrease in WUE is linked to more sensitive species (Forner et al. 2018; Jin et al. 2018). However, plants with low WUE can be more competitive in arid environments because they utilize resources more rapidly, thereby suppressing competition. Conversely, plants with high WUE tend to perform better in the absence of competition, regardless of water availability, likely because of their superior water and nitrogen reserves (Campitelli et al. 2016). WUE may increase, decrease, or remain unchanged under water deficit conditions, depending on the genotype and the severity of water stress (Cameron et al. 2006).

Photosynthesis, which is essential for plant growth, significantly decreases in plants under drought conditions because this process is highly sensitive to stress (Tezara et al. 1999).

The physiological state of plants can be evaluated by examining the integrity of the photosynthetic apparatus and the efficiency of the photosystems (Toscano et al. 2019). Adverse environmental conditions, such as water stress, can impair photosystems (Toscano et al. 2019). An indirect method for assessing this damage is the chlorophyll *a* fluorescence. Specifically, the values of this parameter increase when photosystem II does not function efficiently owing to an imbalance between the number of electrons available in the photosystem and their utilization (Reddy et al. 2004). The Fv/Fm ratio measures the maximum quantum yield of PSII reaction centers and is used to assess the degree of stress in plants. An Fv/Fm ratio ranging from 0.78 to 0.85 indicates the absence of stress (Toscano et al. 2019). This information is crucial for enhancing our understanding of plant physiological responses to drought, establishing guidelines to alleviate drought stress, and selecting and developing species that are well-suited to water scarcity (Nemali and van Iersel 2019).

A complementary strategy to genotype selection for developing drought-tolerant plants is the application of biostimulants to plants. These substances can enhance crop resilience to abiotic stress and improve nutrient use efficiency, plant health, and productivity across various growth stages (Bulgari et al. 2015). Organic farming can particularly benefit from biostimulants, as they can enhance plant tolerance to nutrient limitations commonly associated with this production system, thereby narrowing the gap between organic and conventional farming practices (De Pascale et al. 2017).

The use of biostimulants plays a crucial role in enhancing agricultural sustainability. For example, they can improve production in both quantitative and qualitative terms while simultaneously reducing environmental impacts (Ertani et al. 2015).

There is a growing interest in the use of plant-derived biostimulants (PDBs). PDBs are natural products obtained from higher plants that are rich in secondary metabolites and are among the primary types of bioactive compounds known to activate physiological responses in plants (Martínez-Lorente et al. 2024; Zulfiqar et al. 2020). Within this category, natural plant extracts (PEs) can be identified. These are obtained through simple maceration of the plant and can be prepared using any part of the plant, including seeds, roots, stems, leaves, bark, and flowers. Recently, extracts from licorice root, lemongrass, garlic, and particularly, moringa leaves have been evaluated for their biostimulant properties (Zulfiqar et al. 2020). Typically, medicinal plants rich in secondary metabolites are used for this purpose. Numerous species have potential applications, and their biological capabilities remain to be fully explored.

Sea fennel (*Crithmum maritimum* L.) may play a significant role in this regard. This species belongs to the *Apiaceae* family and is native to Mediterranean Europe, where it is found along the Italian coastline. As a halophytic plant, it can tolerate high salinity levels, enabling it to thrive near the sea, often in crevices. Sea fennel is a rhizomatous,

perennial plant characterized by robust, branched stems that are woody at the base and range in height from 30 to 60 cm. The plant has attracted considerable interest in folk medicine because of its diuretic, antiscorbutic, digestive, and purgative properties (Nabet et al. 2017).
Various authors have investigated the essential oil of this plant, highlighting its antioxidant and antibacterial properties (Flamini 1999; Özcan 2000; Ruberto et al. 2000). Some studies have indicated that falcarindiol, a compound found in leaf extracts, may exhibit potent antibacterial and cytotoxic effects (Meot-Duros et al. 2010). Given these intrinsic characteristics, one can hypothesize its potential use as a biostimulant; however, no references to this application currently exist in the literature to support this hypotesis.
Coleus amboinicus Lour., also known as *Plectranthus amboinicus* (Lour.) Spreng., is a perennial herbaceous plant belonging to the *Lamiaceae* family. It is commonly found in tropical and warm regions of Africa, Asia, and Australia. In Italy, it is primarily used for ornamental purposes and is often referred to as Cuban oregano because of its intense aroma. This species is widely used in traditional medicine worldwide to treat various ailments (Bañuelos-Hernández et al. 2020).
Several phytoconstituents from various phytochemical classes, including phenols, terpenoids, phenolic acids, flavonoids, flavones, and tannins, have been identified in *C. amboinicus* (Karthika et al. 2024). Numerous pharmacological properties of crude extracts from *C. amboinicus* have been documented in vitro and in vivo (Al-Elwany et al. 2022). Species of *Coleus* are recognized for their cytotoxic and anticancer properties, making them promising candidates for cancer treatment (Lukhoba et al. 2006).
In this study, we aimed to analyze the response of *C. amboinicus*, a highly attractive ornamental plant with moderate drought resistance, to two water regimes: a control group with a non-limiting water supply and a stressed group that received 50% of the water lost through

evapotranspiration. The experiment also included various treatments with biostimulants derived from *Crithmum maritimum* using different concentrations and extraction methods (aqueous maceration or ethanol extraction). The objective of this study was to determine the influence of biostimulant on plant response to drought stress.

7.2. Materials and methods

7.2.1. Plant material and experimental conditions

The trial was conducted in a greenhouse at the Department of Agriculture, Food and Environment (Di3A) in Catania, Italy (37°410 N 15°110 E 89 m above sea level) from 7 April to 6 June 2025, on Cuban oregano (*Coleus amboinicus* Lour.). On 5 November 2024, top cuttings from plants in the collection of the Vegetable and Floriculture Section of the Department of Agriculture, Food and Environment of Catania University were rooted in a cold greenhouse in 2-liter containers.

The growing medium was those produced by Vigorplant Italia srl (Fombio, LO, Italy), based on acidic sphagnum peat and pumice. To prevent water stagnation, a light layer of pH-controlled expanded clay (Vigorplant, Fombio (LO), Italy) was spread at the bottom of each container.

On January 21 2025, the plants were topped and repotted in 17 cm diameter, 3-liter containers filled with the same substrate described above. No fertilization was applied during the trial period. Temperatures in February and March 2025 were particularly mild, and several days were overcast. Therefore, the plants were regularly irrigated at field capacity (100%) until 7 April 2025 which was the start date of the trial.

Each pot was uniquely identified by a tag, and the plants were divided into two groups: the first group was irrigated by returning 100% of the water lost through evapotranspiration (100% WC) and the second group was irrigated by returning 50% (50% WC).

For each watering regime, the plants were divided into six groups of eight plants. They underwent the following treatments every 10 days using a pressure pump with a maximum capacity of two liters (Matabi, GreenCity2 model):

- *Control*: foliar spraying to runoff with distilled water.

WE 1 ml/L: foliar spraying to runoff with the natural biostimulant obtained by maceration in distilled water of blended *Crithmum maritimum* leaves, applied at a concentration of 1 ml per litre of distilled water.

WE 5 ml/L: foliar spraying to runoff with the natural biostimulant obtained by maceration in distilled water of blended *C. maritimum* leaves, applied at a concentration of 5 ml per litre of distilled water.

WE 10 ml/L: foliar spraying to runoff with the natural biostimulant obtained by maceration in distilled water of blended *C. maritimum* leaves, applied at a concentration of 10 ml per litre of distilled water.

AE 0.25 ml/L: foliar spraying to runoff with the natural biostimulant obtained by ethanol (96%) and methanol (4%) extraction of blended *C. maritimum* leaves, applied at a concentration of 0.25 ml per litre of distilled water.

AE 1.25 ml/L: foliar spraying to runoff with the natural biostimulant obtained by ethanol (96%) and methanol (4%) extraction of blended *C. maritimum* leaves, applied at a concentration of 1.25 ml per litre of distilled water.

AE 2.5 ml/L: foliar spraying to runoff with the natural biostimulant obtained by ethanol (96%) and methanol (4%) extraction of blended *C. maritimum leaves*, applied at a concentration of 2.50 ml per litre of distilled water.

Irrigation treatments were managed using the gravimetric method (Toscano et al. 2018). During the experimental period, differences in pot weight (weight after irrigation at drainage cessation minus weight before irrigation) were calculated, and irrigation was performed three times per week.

The air temperature and relative humidity inside the greenhouse were recorded using a Mini TH data logger (IP30 protection class, equipped with a protective shell and integrated USB port; Tecnafood, Bomporto, MO, Italy). During the trial, the mean temperature was 23.2 °C, with a maximum of 52 °C; particularly in the final phase, maximum temperatures often exceeded 40 °C. The mean relative humidity was 50.2%, with peaks of 88% and a minimum of 8%; in the last phase of the trial, RH frequently dropped below 60% (Figure 7.1).

7.2.2. Biomass and leaf area

Biomass data were collected at the beginning of the trial through destructive sampling of four plants, determining the fresh and dry biomass of the different plant organs (roots were separated from the substrate by careful washing under running water, while shoots were separated into stems and leaves).

Dry biomass was obtained by oven-drying samples at 70 °C in a ventilated oven until a constant weight was achieved. Leaf area and leaf number per plant were also determined.

The leaf number and leaf area were measured by placing leaves on a white background support, covering them with a transparent glass plate to keep them fully extended, and then photographing them. Images were processed using ImageJ software (NIH, USA).

Final destructive measurements were performed on 42 plants (three per treatment and irrigation regime), following the same methodology as at the beginning of the trial.

7.2.3. SPAD index, gas exchange, and chlorophyll a *fluorescence*

Physiological measurements were performed two days after each biostimulant treatment. In the following results, only some measurements of more significative moments, are presented.

Chlorophyll content was indirectly estimated using the SPAD index, measured on ten leaves of four plants per treatment using a portable chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan).
Gas exchange was measured on six plants per treatment (two plants per replicate, two leaves per plant) using a CO_2/H_2O Infrared Gas Analyzer (LCi, ADC Bioscientific Ltd., Hoddesdon, UK). Measurements were performed in the morning under clear sky conditions between 09:00 and 13:00. Net photosynthesis (An), stomatal conductance (gs), and transpiration rate (E) were recorded.
Simultaneously, the PSII quantum yield was determined using a fluorimeter (OS1-FL, Opti-Sciences Corporation, Tyngsboro, MA). Leaves were dark-adapted for 20 minutes using cuvette clips (Opti-Sciences) before measurements. Chlorophyll *a* fluorescence was expressed as Fv/Fm, where Fm = maximal fluorescence of dark-adapted leaves, Fv = variable fluorescence.

7.2.4. Nitrate and reducing sugar content and osmolyte concentration

The nitrate content in plant tissues was determined following Cataldo et al. (1975). Dried samples (100 mg) were ground, placed in 15-ml Falcon tubes, and extracted with 3 ml distilled water. Tubes were shaken for 2 h and centrifuged for 15 min at 4000 rpm. A 20 µl aliquot of supernatant was transferred to a 4-ml Eppendorf tube, mixed with 80 µl salicylic-sulfuric acid (5% w/v), and 3 ml NaOH (1.5 N). After cooling to room temperature, the absorbance was read at 410 nm with a spectrophotometer (Infinite M Nano, Tecan). Calibration curves were prepared using KNO_3 standard solutions (0, 2.5, 5, 7.5, 10 mM).
Reducing sugars were determined using the DNS (3,5-dinitrosalicylic acid) method. Dried samples (0.1 g) were ground, placed in 15-ml Falcon tubes with 3 ml distilled water, shaken for 2 h, and centrifuged at 10 °C for 15 min at 4000 rpm. A 200 µl aliquot of supernatant was transferred into an Eppendorf tube (perforated cap), mixed with 0.2 ml

DNS reagent, vortexed, and boiled for 5 min at 100 °C. After adding 1.5 ml distilled water and cooling to room temperature, absorbance was measured at 530 nm with the Infinite M Nano spectrophotometer (Tecan). Calibration was performed using glucose standards. The DNS reagent was stored in a dark bottle for up to two months.

The osmolyte concentration was determined with a freezing point osmometer (Osmomat 030, Gonotec). The principle is based on freezing point depression, which is directly proportional to the concentration of osmotically active particles in the solution. The instrument was calibrated prior to use with distilled water (zero point) and a standard solution (upper reference point). The results were expressed as osmol/kg.

7.2.5. Statistical analysis

Data were analyzed using two-way ANOVA, with irrigation and biostimulant treatments as factors, and three-way ANOVA, including time, irrigation, and biostimulant treatments as factors, using CoStat 6.311 (CoHort Software, Monterey, CA, USA). Mean differences were evaluated using Tukey's post-hoc test ($p < 0.05$).

Physiological data were expressed as trends during the treatment period, reported as means ± SE ($n = 6$).

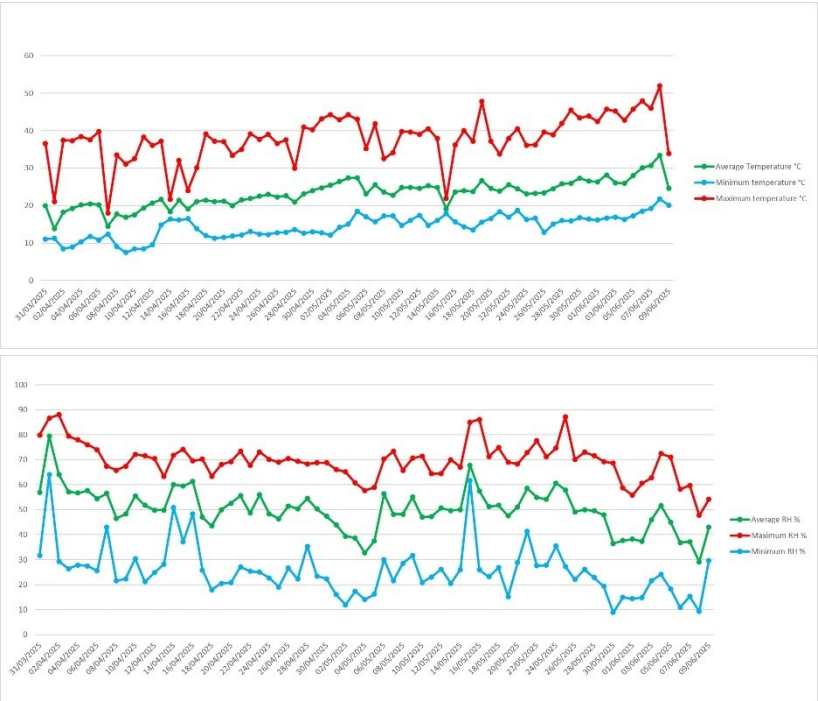

Figure 7.1. Temperature (above) and relative humidity (below) during the trial.

7.3. Results

7.3.1. Biomass and leaf area

The adoption of two water regimes (100% WC and 50% WC) significantly affected all the morphological parameters analyzed. Reductions in the above-ground portion of the plant, caused by decreased water availability, were almost always greater than 35% by the end of the test period. The most substantial reductions occurred in the photosynthetic system: the dry biomass of the leaves, due to receiving only 50% of the water lost through evaporation, decreased

by 39.6% (Table 1). Reductions caused by drought stress were more moderate in the root system, which decreased by 27.1% (Table 7.1).
The ratio of above-ground biomass to total biomass in the two irrigation treatments changed only slightly (0.85 in 100% WC versus 0.83 in 50% WC). However, the ratio of underground biomass to total biomass increased by 13% from the 100% WC treatment to the 50% WC treatment (Table 7.1).
The use of *Crithmum maritimum*-based biostimulant had positive effects that varied depending on the dose and extraction method. Dry stem biomass increased by 24.3% compared to the control when an EA dose of 2.5 ml/L was applied (Table 7.1).
Interaction effects were significant (Figure 7.2). In the 100% WC treatments, the use of the biostimulant never resulted in higher values than the control; at an EA dose of 1.25 ml/L, a lower value was observed. However, when the plants were subjected to the stress regime, a highly variable trend was observed. Notably, the EA dose of 2.5 ml/L resulted in a 75.8% increase compared to the control.
The leaf dry biomass, averaged across the different water treatments, was highest for AE 2.5 ml/L (Table 7.1). The interaction effects between the factors studied were significant. Under the 100% WC treatment, the application of the biostimulant AE 0.25 ml/L resulted in a 25.3% increase compared to the control. At 50% WC, plants treated with AE 2.5% ml/L showed a 51.7% increase compared to the control (Figure 7.2).
Root dry biomass increased by an average of 29.6% across the two water regimes with AE 2.5 ml/L compared to the control (Table 7.1). Interaction effects were significant: under the 50% WC treatment, plants treated with AE 0.25 ml/L showed a 52.5% increase compared to the control. However, no significant changes due to biostimulant treatments were observed under 100% WC conditions (Figure 7.2).
The biomass of the entire above-ground portion showed, on average across the two irrigation treatments, an increase of 21.8% compared

to the control for plants treated with R 2.5 ml/L (Table 7.1). The interaction effects were significant: the use of AE 2.5 ml/L under the 50% WC regime resulted in a 62.5% increase compared to the control. No significant changes were observed under the 100% WC regime (Figure 7.2).

The total dry biomass increased by 23.0% compared to the control with the application of AE 2.5 ml/L (Table 7.1). Interaction effects were significant: under the 100% WC regime, no differences were observed with the use of biostimulants, whereas under the 50% WC regime, the application of AE 2.5 ml/L resulted in a 60.6% increase compared to the control (Figure 7.2).

Biostimulants did not significantly alter the aboveground to total dry biomass or belowground to total biomass ratios. The interaction effects between the factors studied were also not significant (Table 7.1).

Significant changes were observed in leaf characteristics. Leaf area increased by 26.8% when soil water content increased from 50% to 100% (Table 7.2). Drought stress also influenced the number of leaves, which increased by 32.0% as soil water content increased from 50% to 100%. The change in total leaf area was primarily due to the number of leaves produced per individual plant, as the unit leaf area did not show significant variation between the two water regimes (Table 7.2).

The Specific Leaf Area (SLA) was, on average, higher across the various plant biostimulant treatments in stressed plants, showing a 29.1% increase compared to the value recorded in plants maintained at 100% water capacity (WC) (Table 7.2).

Plants treated with EA 2.5 ml/L exhibited a 32.9% increase in leaf area compared to the control, whereas treatment with WE 5 ml/L resulted in an 18.2% increase (Table 7.2). The interaction effects were significant: under 100% WC, the biostimulant treatments produced values comparable to the control, with two exceptions (EA 1.25 ml/L

and WE 10 ml/L), which showed lower values. Under 50% WC, biostimulants generally resulted in higher values, except for WE 1 ml/L and WC 10 ml/L. Notably, the increase compared to the control reached 131% with EA 2.5 ml/L (Figure 7.3).

The average number of leaves per plant, did not differ with the use of biostimulants across the two water regimens (Table 7.2). However, the interaction effects were significant: in plants treated with 100% WC, lower leaf counts were observed in those treated with EA 1.25 ml/L. In contrast, at 50% WC, biostimulants did not affect the number of leaves compared to the control. This suggests that the effect of biostimulant was more pronounced on leaf surface expansion than on the production of new leaves (Figure 7.3).

The unit leaf area increased by 28.2% with the EA 2.5 ml/L treatment (Table 7.2). Interaction effects were also significant: while no changes due to the biostimulant were observed under 100% WC, at 50% WC, the use of biostimulants resulted in higher unit leaf area values than the control, except for the WE 10 ml/L treatment. Specifically, the AE 2.5 ml/L treatment led to an 87.7% increase compared to the control (Figure 7.3).

The specific leaf area (SLA) showed, on average across the different water treatments, the highest absolute values in the AE 2.5 ml/L treatment, with a 17.1% increase compared to the control. Conversely, the use of WE 10 ml/L resulted in an 11.7% reduction compared to the control (Table 7.2). The interaction effects were significant: while under 100% WC the biostimulants did not appear effective, under stress conditions all biostimulants, except for WE 10 ml/L, significantly increased SLA values. Notably, the application of AE 2.5 ml/L led to a 51.9% increase compared to the control (Figure 7.3).

7.3.2. SPAD index, gas exchange, and chlorophyll a *fluorescence*

SPAD values were generally low and decreased as the cycle progressed. Under the 100% WC regime, the highest values were recorded for AE 0.25 ml/L and WE 1.23 ml/L, whereas under the 50% WC regime, higher values were generally observed with WE 1 and 5 ml/L. In the 20 May survey, the highest SPAD value in the non-limiting water regime was recorded with WE 10 ml/L, representing a 24.8% increase compared to that of the control. Even in the stressed treatments, the highest value was observed in plants treated with WE 10 ml/L (Figure 7.4). At the final survey, nearly all biostimulant treatments, except for WE 5 ml/L, showed higher SPAD values than the control under the 100% WC regime. In the stressed treatments, higher values were obtained with water extraction, particularly at the lowest concentration (WE 1 ml/L), and with alcohol extraction at the highest concentration (AE at 2.5 ml/L).

Net photosynthesis in the first May survey showed higher values in the 100% WC treatment with alcohol extraction at lower doses (EA 0.25 and 1.25 ml/L). In the stressed treatments, biostimulants almost always increased photosynthesis values, except in plants treated with EA 1.25 ml/L (Figure 7.5). In the May 20th survey, no improvements were observed in biostimulant treatment plants maintained at 100% WC. However, in the stressed treatments, higher net photosynthesis values were recorded when plants were treated with biostimulants. Notably, the application of WE 1 ml/L resulted in a 393% increase in photosynthesis. In the final measurement on June 3rd, the photosynthesis values were very low. No increase was achieved using biostimulants in treatments involving the restitution of 100% of the water lost through evapotranspiration, whereas in the 50% WC treatments, a clear improvement was observed with the administration of WE 10 ml/L (Figure 7.5).

Stomatal conductance during the first measurement showed values equal to or lower than those of the control across all water regimes (Figure 7.6). In the second measurement, conducted on May 20th, the values were comparable to or lower than the control under 100% WC. However, under 50% WC, biostimulant treatments generally increased the stomatal conductance. In the final measurement, at 100% WC, only the application of EA 1.25 ml/L resulted in values higher than the control, while other biostimulant treatments produced significantly lower values. Under 50% WC, only plants treated with EA 0.25 ml/L exhibited a significantly higher stomatal conductance than the control (Figure 7.6).

Evapotranspiration during the various measurements, particularly in the stressed treatments, was higher in plants treated with biostimulants. However, no consistent pattern was observed across the different measurements, likely due to the varying effects of biostimulant doses and extraction methods (Figure 7.7).

The maximum quantum efficiency of PSII almost always showed values below the threshold of 0.80 (Figure 7.8). On May 8th, plants treated with the biostimulant, applied at various doses and extracted using different methods, generally exhibited higher values. This trend was particularly evident in the most stressed plants during the survey conducted on June 6th.

7.3.3. Nitrate and reducing sugar content and osmolyte concentration

The nitrate content in the leaves of plants at 100%WC was generally lower in those treated with biostimulants. However, in stressed plants, nitrate levels comparable to those of control plants were not observed with WE 10 ml/L and AE 2.5 ml/L. In the roots, the nitrate content was consistently lower in non-stressed plants treated with biostimulants. Conversely, in stressed plants, an increase in nitrate

content was observed, although the values did not differ significantly among the various treatments (Figure 7.9).

The reducing sugar content was higher in the leaves of plants treated with WE 10 ml/L, whereas in stressed plants, higher values were observed in leaves treated with WE 1 ml/L. In the roots, higher values were found in non-stressed plants treated with WE 1 ml/L; however, in stressed plants, no positive effects attributable to the use of biostimulants were observed (Figure 7.10).

The osmolyte content exhibited a nonlinear trend between leaves and roots. Notably, in stressed plants, higher values were observed in both leaves and roots of plants treated with EA 1.25 ml/L (Figure 7.11).

Table 7.1. Effects of different treatments on dry biomass at the end of the trial.

	Dry biomass (g)						
	shoots	**leaves**	**roots**	**epigeous**	**total**	**epigeous/total**	**roots/total**
Water treatments (WT)							
100% WC	15.43±0.48 a	18.60±0.65 a	5.94±0.16 a	34.03±1.08 a	39.98±1.18 a	0.85±0.00 a	0.15±0.00 b
50% WC	9.87±0.39 b	11.23±0.43 b	4.33±0.20 b	21.10±0.78 b	25.42±0.95 b	0.83±0.00 b	0.17±0.00 a
Biostimulant treatments (BT)							
Control	12.20±2.10 bc	13.74±1.94 bc	4.66±0.42 b	25.94±4.04 bcd	30.60±4.42 bc	0.84±0.01 ab	0.16±0.01
AE 0.25 ml/L	13.43±1.81 ab	16.70±2.68 a	4.87±0.55 b	30.13±4.52 ab	35.00±5.04 ab	0.86±0.01 a	0.14±0.01
AE 1.25 ml/L	10.74±0.93 c	13.40±1.29 c	4.99±0.59 ab	24.14±4.40 d	29.13±4.95 c	0.83±0.01 ab	0.17±0.01
AE 2.5 ml/L	15.17±0.84 a	16.43±1.04 a	6.04±0.61 a	31.60±4.48 a	37.63±5.08 a	0.84±0.01 ab	0.16±0.01
WA 1ml/L	12.50±1.51 bc	14.54±2.77 abc	5.23±0.40 ab	27.04±3.49 bcd	32.27±3.83 bc	0.84±0.01 ab	0.16±0.01
WA 5ml/l	12.93±1.37 b	15.96±1.87 ab	5.12±0.24 ab	28.89±2.50 abc	34.01±2.68 abc	0.85±0.01 ab	0.15±0.01
WA 10ml/L	11.61±0.69 bc	13.62±0.47 bc	5.03±0.46 ab	25.23±2.21 cd	30.26±2.66 bc	0.83±0.00 b	0.17±0.00
Significance							
WT	***	***	***	***	***	***	***
BT	***	***	*	***	***	ns	ns
WT x BT	***	***	**	***	***	ns	ns

100% WC = received 100% of water considering the evapotranspiration rate performed using the gravimetric method; 50% WC = received 50% of water considering the evapotranspiration rate performed using the gravimetric method; Control = distilled water treatment; AE = biostimulant extraction from leaves of *Crithmum maritimum* by ethanol (96%) and methanol (4%) of; WA = biostimulant obtained from water maceration of leaves of *C. maritimum*. Data are means ± standard error (n = 3). Data followed by a different letter were significantly different according to Tukey's test at $p < 0.05$; *: $p < 0.05$; ** $p < 0.01$; ***: $p < 0.001$; ns: $p > 0.05$.

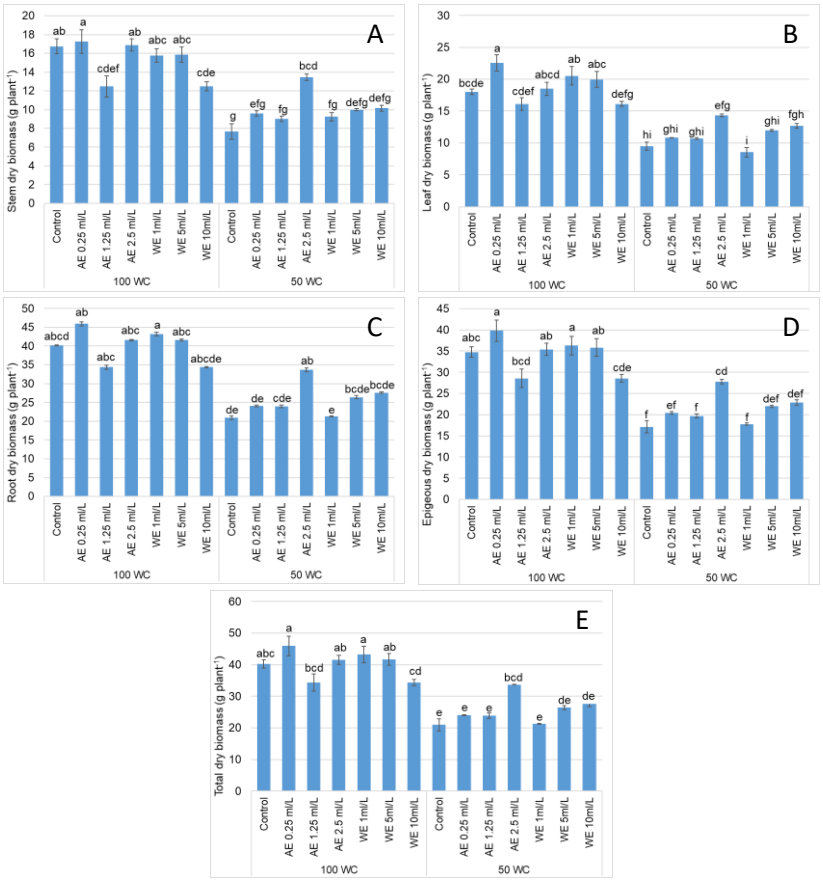

Figure 7.2. Interactive effects of water and biostimulant treatments for stem dry biomass (A), leaf dry biomass (B), root dry biomass (C), epigeous dry biomass (D), and total dry biomass (E). 100% WC = received 100% of water considering the evapotranspiration rate performed using the gravimetric method; 50% WC = received 50% of water considering the evapotranspiration rate performed using the gravimetric method; Control = distilled water treatment; AE = biostimulant extraction from leaves of *Crithmum maritimum* by ethanol (96%) and methanol (4%) of; WA = biostimulant obtained from water maceration of leaves of *C. maritimum*. Columns denoted with the same letters are not significantly different, as determined by Tukey's test at $p<0.05$.

Table 7.2. Effects of different treatments on leaf characteristics at the end of the trial.

	Leaf area	Leaf number	Unit Leaf Area	SLA
Water treatments (WT)				
100% WC	2178.08±90.24 a	874.87±35.14 a	2.51±0.06	117.53±3.67 b
50% WC	1718.31±102.19 b	663.05±22.91 b	2.59±0.11	151.78±5.07 a
Biostimulant treatments (BT)				
Control	1866.15±339.07 cd	778.67±74.54 abc	2.30±0.25 b	132.01±7.55 bcd
AE 0.25 ml/L	2084.33±301.86 bc	872.64±99.06 a	2.44±0.21 b	135.16±10.48 bc
AE 1.25 ml/L	1707.29±247.72 d	681.40±88.74 c	2.53±0.20 ab	134.21±12.95 bc
AE 2.5 ml/L	2479.64±144.22 a	856.90±86.33 ab	2.95±0.11 a	154.61±13.06 a
WE 1ml/L	1684.67±119.77 d	634.17±48.90 c	2.68±0.09 ab	125.55±12.20 cd
WE 5ml/L	2205.21±82.72 ab	852.39±21.25 ab	2.64±0.08 ab	142.83±11.85 ab
WE 10ml/L	1610.10±26.98 d	706.55±21.79 bc	2.29±0.11 b	118.21±14.24 d
Significance				
WT	***	***	ns	***
BT	***	***	**	***
WT x BT	***	**	***	***

100% WC = received 100% of water considering the evapotranspiration rate performed using the gravimetric method; 50% WC = received 50% of water considering the evapotranspiration rate performed using the gravimetric method; Control = distilled water treatment; AE = biostimulant extraction from leaves of *Crithmum maritimum* by ethanol (96%) and methanol (4%) of; WA = biostimulant obtained from water maceration of leaves of *C. maritimum*. Data are means ± standard error (n = 3). Data followed by a different letter were significantly different according to Tukey's test at $p < 0.05$; ** $p < 0.01$; ***: $p < 0.001$; ns: $p > 0.05$.

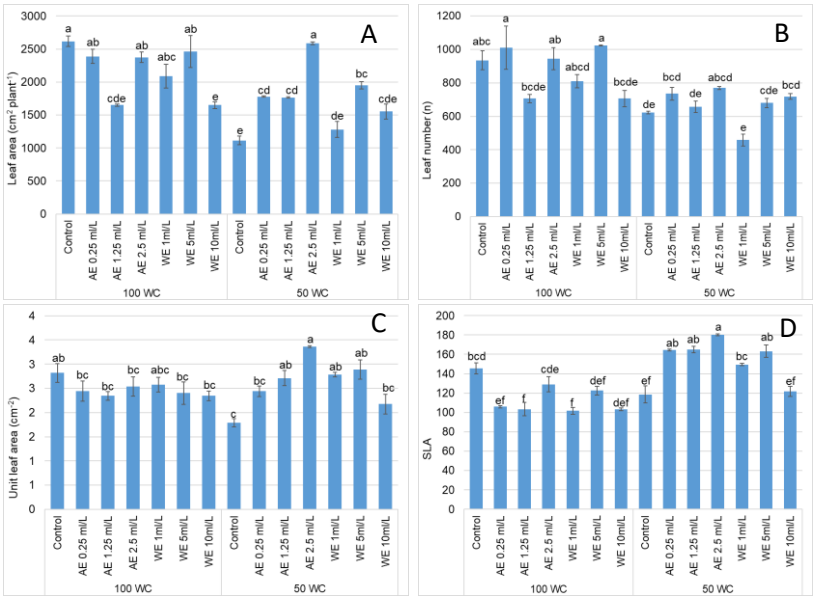

Figure 7.3. Interactive effects of water and biostimulant treatments for leaf area (A), leaf number (B), unit leaf area (C), and SLA (D). 100% WC = received 100% of water considering the evapotranspiration rate performed using the gravimetric method; 50% WC = received 50% of water considering the evapotranspiration rate performed using the gravimetric method; Control = distilled water treatment; AE = biostimulant extraction from leaves of *Crithmum maritimum* by ethanol (96%) and methanol (4%) of; WA = biostimulant obtained from water maceration of leaves of *C. maritimum*. Columns denoted with the same letters are not significantly different, as determined by Tukey's test at $p<0.05$.

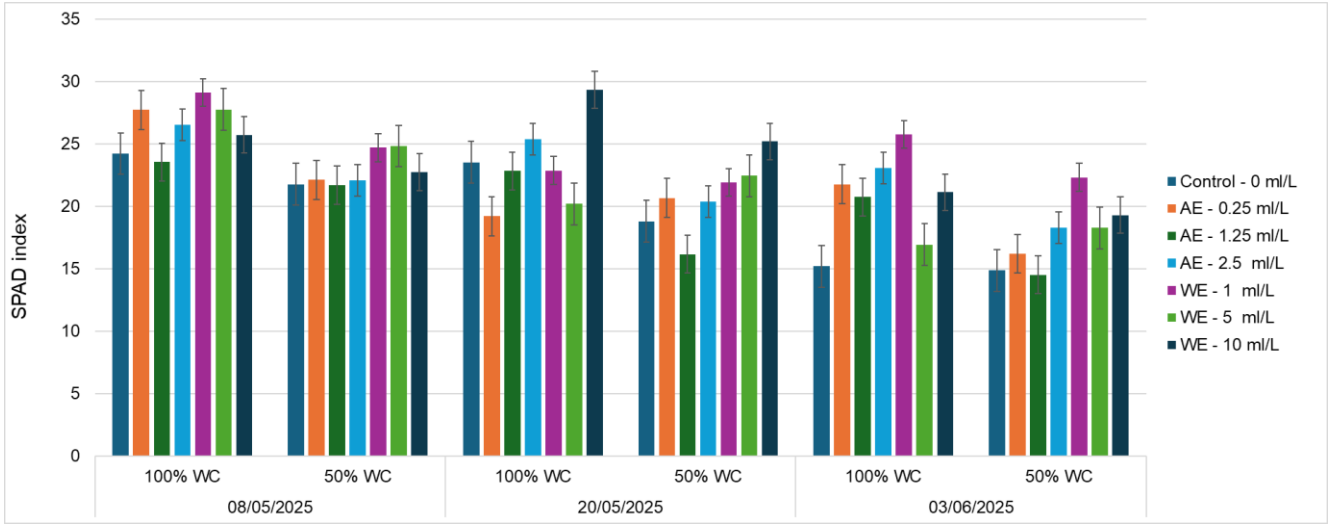

Figure 7.4. SPAD index in *Coleus amboinicus* plants during experimental period for the different biostimulant treatments. 100% WC = received 100% of water considering the evapotranspiration rate performed using the gravimetric method; 50% WC = received 50% of water considering the evapotranspiration rate performed using the gravimetric method; Control = distilled water treatment; AE = biostimulant extraction from leaves of *Crithmum maritimum* by ethanol (96%) and methanol (4%); WA = biostimulant obtained from water maceration of leaves of *C. maritimum*. Data are mean ± S.E. (n = 6).

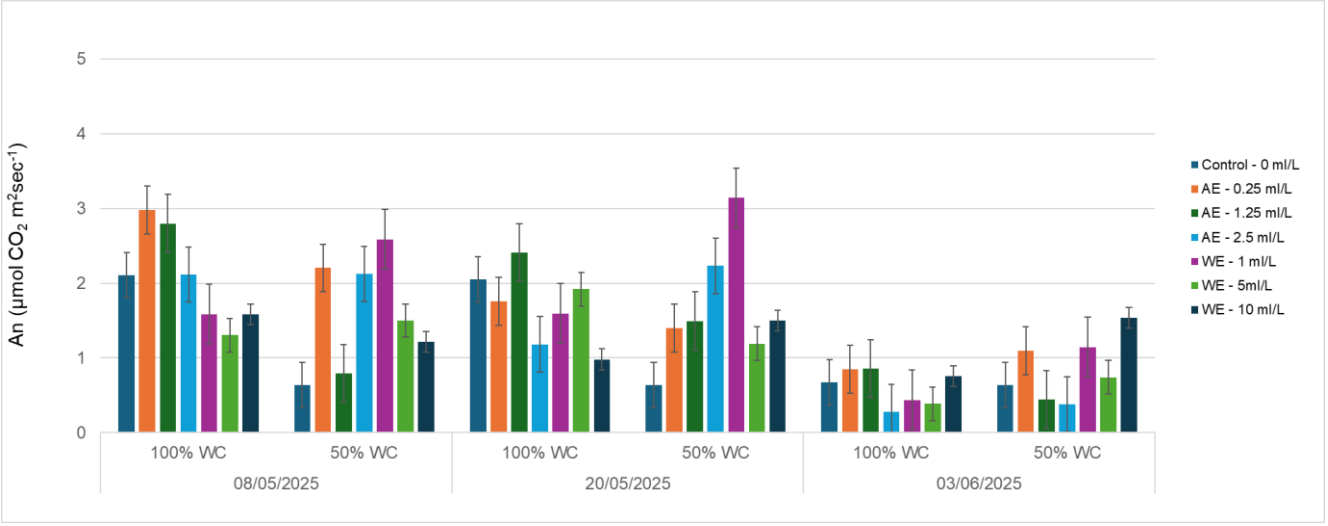

Figure 7.5. Net photosynthesis (An) in *Coleus amboinicus* during experimental period for the different biostimulant treatments. 100% WC = received 100% of water considering the evapotranspiration rate performed using the gravimetric method; 50% WC = received 50% of water considering the evapotranspiration rate performed using the gravimetric method; Control = distilled water treatment; AE = biostimulant extraction from leaves of *Crithmum maritimum* by ethanol (96%) and methanol (4%); WA = biostimulant obtained from water maceration of leaves of *C. maritimum.* Data are mean ± S.E. (n = 6).

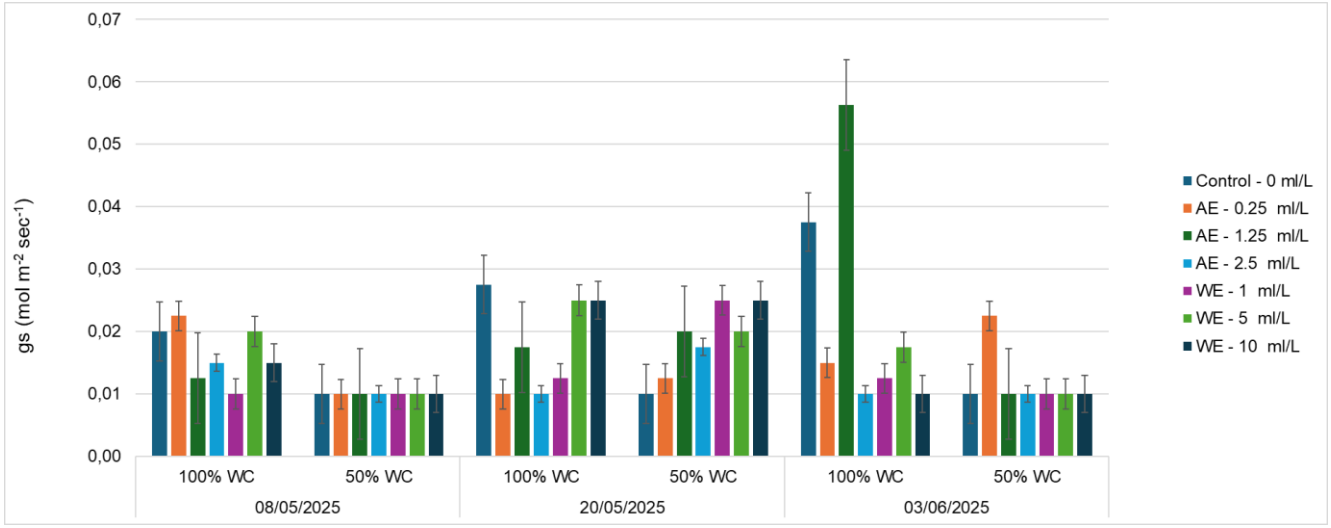

Figure 7.6. Stomatal conductance (gs) in *Coleus amboinicus* during experimental period for the different biostimulant treatments. 100% WC = received 100% of water considering the evapotranspiration rate performed using the gravimetric method; 50% WC = received 50% of water considering the evapotranspiration rate performed using the gravimetric method; Control = distilled water treatment; AE = biostimulant extraction from leaves of *Crithmum maritimum* by ethanol (96%) and methanol (4%); WA = biostimulant obtained from water maceration of leaves of *C. maritimum.* Data are mean ± S.E. (n = 6).

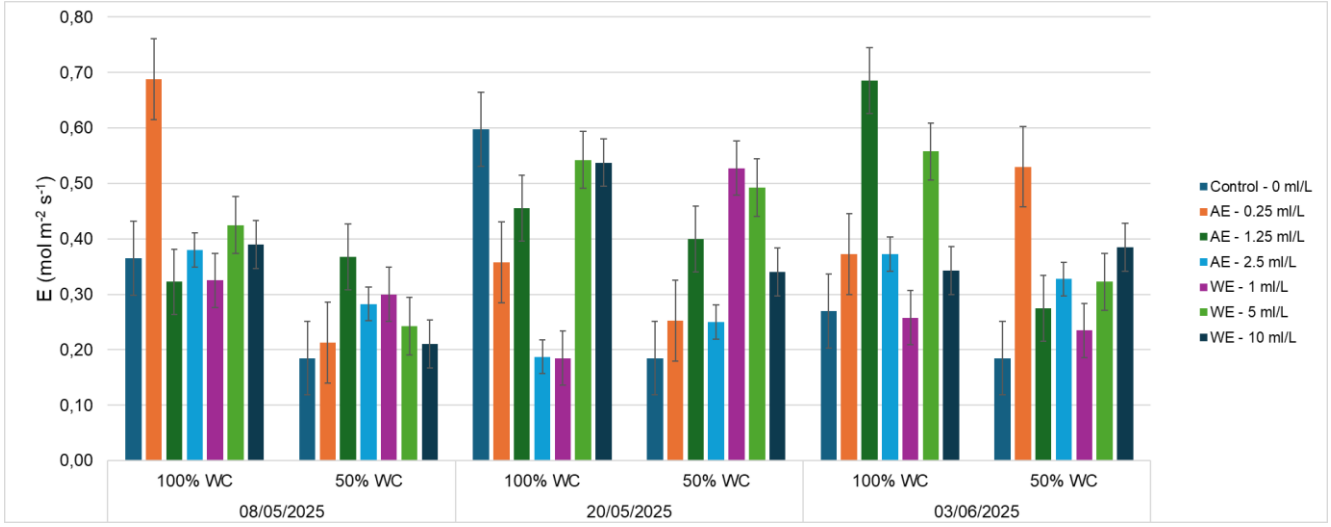

Figure 7.7. Evapotranspiration (E) in *Coleus amboinicus* during experimental period for the different biostimulant treatments. 100% WC = received 100% of water considering the evapotranspiration rate performed using the gravimetric method; 50% WC = received 50% of water considering the evapotranspiration rate performed using the gravimetric method; Control = distilled water treatment; AE = biostimulant extraction from leaves of *Crithmum maritimum* by ethanol (96%) and methanol (4%); WA = biostimulant obtained from water maceration of leaves of *C. maritimum.* Data are mean ± S.E. (n = 6).

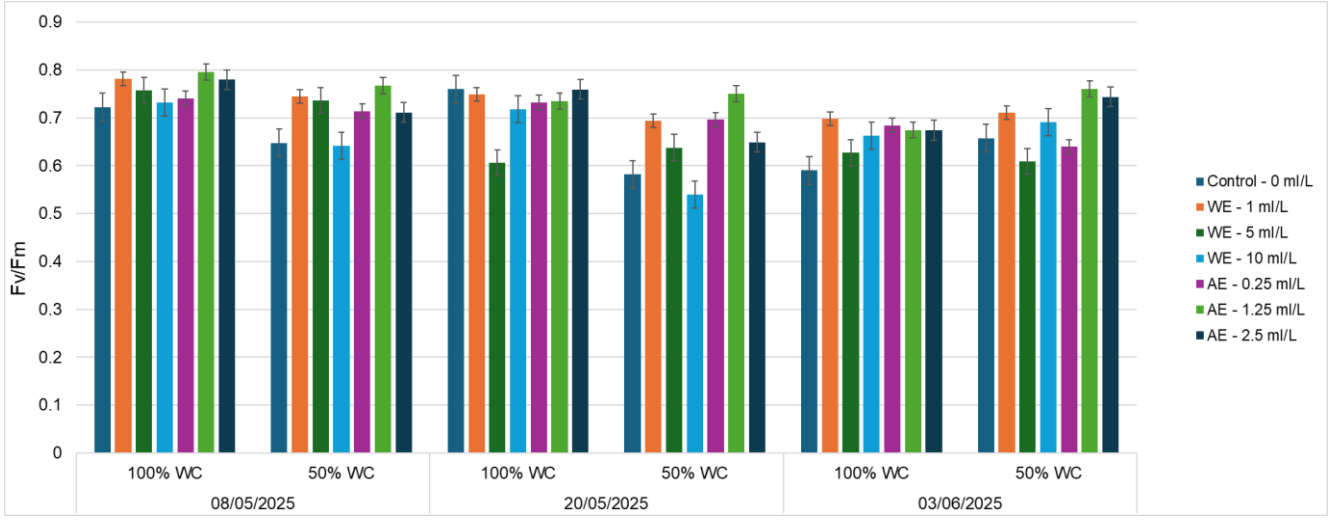

Figure 7.8. Maximum quantum efficiency of the PSII (Fv/Fm) in *Coleus amboinicus* during experimental period for the different biostimulant treatments. 100% WC = received 100% of water considering the evapotranspiration rate performed using the gravimetric method; 50% WC = received 50% of water considering the evapotranspiration rate performed using the gravimetric method; Control = distilled water treatment; AE = biostimulant extraction from leaves of *Crithmum maritimum* by ethanol (96%) and methanol (4%); WA = biostimulant obtained from water maceration of leaves of *C. maritimum*. Data are mean ± S.E. (n = 6).

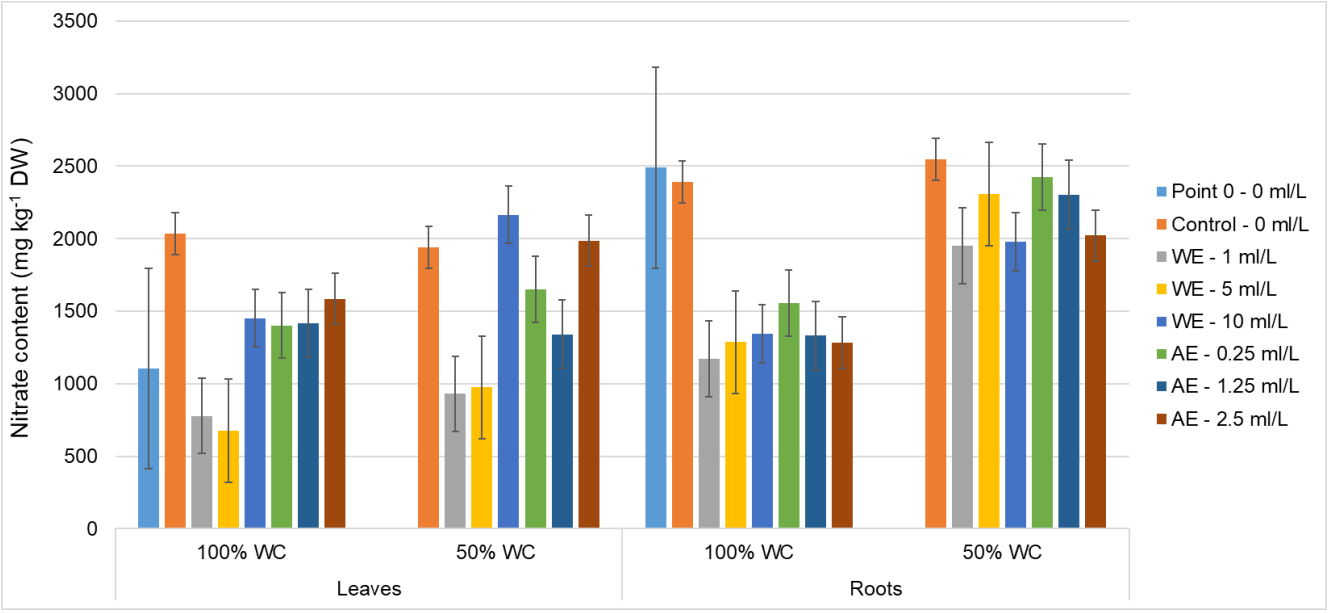

Figure 7.9. Nitrate content (mg kg^{-1} DW) in *Coleus amboinicus* at the point 0 and at the end of the experimental period for the different biostimulant treatments. 100% WC = received 100% of water considering the evapotranspiration rate performed using the gravimetric method; 50% WC = received 50% of water considering the evapotranspiration rate performed using the gravimetric method; Control = distilled water treatment; AE = biostimulant extraction from leaves of *Crithmum maritimum* by ethanol (96%) and methanol (4%); WA = biostimulant obtained from water maceration of leaves of *C. maritimum*. Data are mean ± S.E. (n = 3).

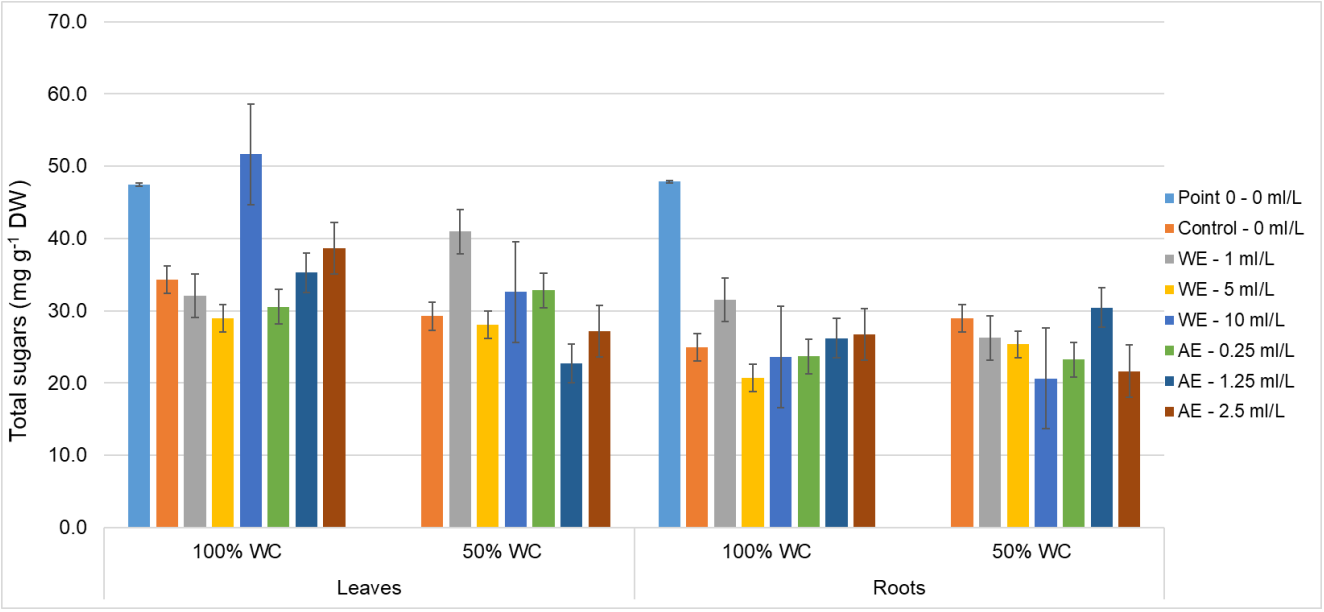

Figure 7.10. Reducing sugars content (mg g^{-1} DW) in *Coleus amboinicus* at the point 0 and at the end of the experimental period for the different biostimulant treatments. 100% WC = received 100% of water considering the evapotranspiration rate performed using the gravimetric method; 50% WC = received 50% of water considering the evapotranspiration rate performed using the gravimetric method; Control = distilled water treatment; AE = biostimulant extraction from leaves of *Crithmum maritimum* by ethanol (96%) and methanol (4%); WA = biostimulant obtained from water maceration of leaves of *C. maritimum*. Data are mean ± S.E. (n = 3).

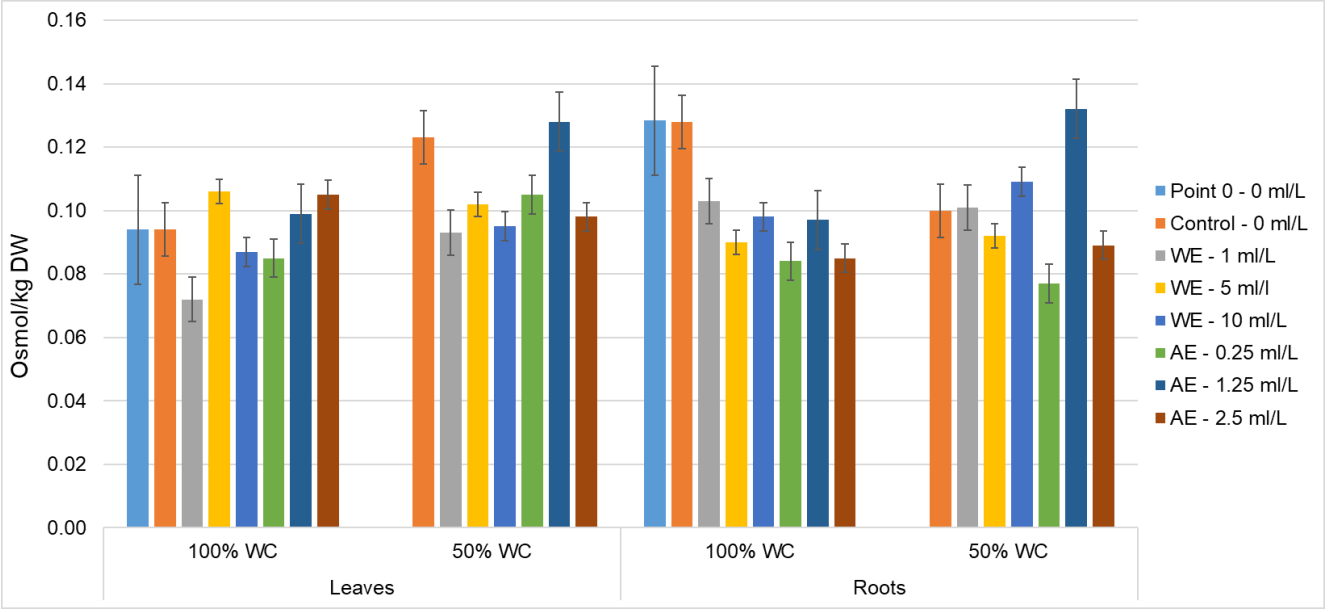

Figure 7.11. Osmolyte content (kg DW) in *Coleus amboinicus* at the point 0 and at the end of the experimental period for the different biostimulant treatments. 100% WC = received 100% of water considering the evapotranspiration rate performed using the gravimetric method; 50% WC = received 50% of water considering the evapotranspiration rate performed using the gravimetric method; Control = distilled water treatment; AE = biostimulant extraction from leaves of *Crithmum maritimum* by ethanol (96%) and methanol (4%); WA = biostimulant obtained from water maceration of leaves of *C. maritimum*. Data are mean ± S.E. (n = 3).

7.4. Discussion

Water availability in urban and peri-urban areas is increasingly limited, partly due to climate change. Consequently, the growth and flowering of herbaceous bedding plants in these areas are hindered by water shortages, negatively affecting their aesthetic value (Toscano et al. 2019). Annual plants, commonly used in flowerbeds, cannot be extensively cultivated because of limited water availability, reducing their ornamental appeal. Therefore, identifying drought-tolerant plants capable of maintaining an attractive appearance under water stress is crucial for selecting species that retain their aesthetic value under water-scarce conditions (Ferrante et al. 2021). This identification often relies on analyzing the physiological and biochemical responses of plant species to drought stress (Cicevan 2016). However, this process is complicated by the fact that drought responses can vary among species within the same genus (Sánchez-Blanco et al. 2002). Currently, there is insufficient information to support the rational selection of ornamental plant species best suited for urban green spaces.

Our results indicate that Cuban oregano, as used in this experiment, employs various mechanisms to resist drought stress, including differential dry matter distribution between roots and shoots, reduced leaf size, and decreased leaf area. These findings are supported by previous studies showing that reduced biomass production results from limited water availability (Shao et al. 2008; Mugnai et al. 2009). The root-to-shoot ratio increases in plants experiencing water scarcity. This response is attributed to the development of larger root systems relative to shoot biomass, which is consistent with earlier studies suggesting that shoot growth is inhibited more than root growth following reduced water availability (Monneveux and Belhassen 1996). An increase in the root-to-shoot ratio has frequently been observed in plants under drought conditions (Blum 1996; Zwack et al.

1998) to reduce water consumption (Wu et al. 2008) and enhance water uptake (Smirnoff 1998). This result was confirmed by the increase in root biomass, which allowed the plants to expand their absorptive surface area. Through this avoidance mechanism, plants adapt to stress without significantly altering their shoot biomass. Nayyar and Gupta (2006) demonstrated that water deficiency affects the shoot parts, particularly the photosynthetic apparatus. Water deficit not only reduced shoot dry weight but also decreased leaf area. These reductions may be due to a decrease in the number or size of the leaves.

According to Mugnai et al. (2009), deficit irrigation reduces the leaf area in *Callistemon citrinus* (Curtis) Skeels. Similar reductions in leaf area have been observed in Pittosporum and Viburnum (Toscano et al. 2014). This finding was also confirmed by Álvarez and Sánchez-Blanco (2013) in *C. citrinus*, where the decrease in leaf area was attributed to a reduction in leaf number, and by Sánchez-Blanco et al. (2002) in *Cistus monspeliensis* L. and *C. albidus* L. In our study, *C. amboinicus* plants subjected to partial restitution of water lost through evapotranspiration exhibited a reduced leaf area at the end of the experiment, resulting from the diminished expansion of individual leaves. This response is attributed to an avoidance mechanism that minimizes water loss through stomatal closure, which reduces whole-plant carbon assimilation and, consequently, plant growth (Bañón et al. 2006).

Plant responses to drought stress are complex processes that require the expenditure of metabolic energy and activation of various physiological and biochemical mechanisms. These responses depend on factors such as genotype, plant age, and drought stress intensity and duration (Toscano et al. 2019).

Plant development and survival are commonly measured to assess drought stress tolerance. Therefore, indirect selection for drought tolerance, through the analysis of immediate physiological responses,

may offer a simpler and more effective approach. In this study, we aimed to analyze the drought response of Cuban oregano, a species that, based on previous evidence (Prathyusha and Chaitanya 2019), appears to exhibit strong tolerance to water stress. Under water-deficient conditions, the plant behaves as a facultative CAM species (Winter et al. 2020).

In our experiment, as water stress increased, the plants exhibited a reduced capacity to assimilate CO_2, resulting in decreased photosynthesis. This reduction is associated with increased resistance in the mesophyll and stomatal layers, which subsequently leads to a decrease in the organ dry weight (Bahadur et al. 2019).

As an initial response to water scarcity, rapid stomatal closure was observed, resulting in reduced CO_2 assimilation (Dghim et al. 2018), stomatal conductance (gs), transpiration rate (E), and net photosynthesis (An) (Gurumurthy et al. 2019).

Through physiological adaptations, such as reduced stomatal conductance and net photosynthesis, the study species was able to adapt to drought conditions. One objective of this study was to evaluate the changes in the accumulation of protective compounds, including carbohydrates (Živanović et al. 2020), in response to drought stress and their effects on enhancing drought tolerance. The reducing sugar content tended to be higher in the roots of stressed plants, whereas it was lower in the leaves.

Extensive experimental evidence indicates that photosynthetic activity is inhibited during the development of drought stress, which may explain the decrease in soluble sugar accumulation under drought conditions, particularly severe drought, compared to control conditions (Dien et al. 2019). The slight reduction observed in this study demonstrates the tolerance of *C. amboinicus* to drought stress.

Plants activate biochemical mechanisms, such as osmotic adjustment, to adapt to water scarcity. One key mechanism involves the accumulation of compatible solutes known as osmolytes. Osmolytes

help maintain homeostasis by creating a water absorption gradient, preserving cellular turgor through osmotic adjustment, and supporting redox metabolism to eliminate excess reactive oxygen species (ROS) and restore cellular redox balance. Additionally, they protect cellular processes from osmotic stress and oxidative damage (Ghosh et al. 2021).

Under drought stress, our results showed that osmolytes accumulated in the leaves of affected plants.

Chlorophyll content, measured using the SPAD index, consistently decreased in control plants subjected to stress compared to those maintained at 100% water capacity (WC). Abiotic stresses, such as water deficit, impair chlorophyll biosynthesis and lead to reduced chlorophyll levels. Our results indicate that plants delay chlorophyll biosynthesis in response to water stress. Maintaining or increasing SPAD values has been associated with greater drought tolerance in cultivars (Puangbut et al. 2017). Reduced chlorophyll content diminishes the green coloration of leaves, partially compromising the ornamental value of plants.

Overall, the changes in photosynthesis demonstrated that photosynthetic parameters, along with certain metabolites, serve as reliable indicators of crop adaptation to drought conditions.

The use of biostimulants is an effective approach to enhance drought stress tolerance. In our trial, the application of a biostimulant derived from *Crithmum maritimum* plants partially modified plant responses, often positively. Plant extracts used as biostimulants provide a natural and sustainable method for stimulating plant growth, improving stress tolerance, and increasing yield, especially in low-input farming systems. The use of these extracts not only supports sustainable agriculture but also promotes health and environmental safety (Bulgari et al. 2019). Recently, there has been growing interest in plant extracts such as licorice root extract, legume-derived protein hydrolysate, lemongrass, garlic extract, and particularly moringa leaf extracts, for

maintaining yield and promoting plant growth (Yadav et al. 2024). Some medicinal plants also serve as potential biostimulants due to their potent secondary metabolites (Mrid et al. 2021; Bhowmick et al. 2024; Mishra et al. 2025). Additionally, *C. maritimum* exhibits notable medicinal properties (Flamini et al. 1999; Özcan 2000; Ruberto et al. 2000).

Plant-derived biostimulants (PdBs) are compounds that stimulate biochemical, physiological, and molecular responses, thereby promoting growth and enhancing plant yield (Drobek et al. 2019; Bhowmick et al. 2024). Often sourced from natural materials, biostimulants play a crucial role in agriculture, particularly in mitigating the effects of stress on plants (Nephali et al. 2020; Rouphael and Colla 2020). Drought stress, characterized by water scarcity, is a significant factor that limitis the growth of many ornamental plants.

Sugars and carbohydrates are essential for plant growth and development, serving as both energy sources and fundamental building blocks for the synthesis of structural molecules. The application of biostimulants has been shown to increase soluble sugar levels in plants, as demonstrated in maize (Ertani et al. 2016), rocket (Abdalla 2013), habanero pepper (Ertani et al. 2014), and tomato (Rouphael et al. 2017). The use of PdBs on crops subjected to stress conditions generally results in elevated soluble sugar concentrations, providing osmoprotective benefits in both dicots (Desoky et al. 2019) and monocots (Abdel Latef et al. 2019).

Photosynthetic metabolism encompasses the chemical reactions within chloroplasts that reduce CO_2 into carbohydrates by utilizing ATP and reducing power in the form of NADPH, which are generated through light-dependent reactions. Various biomolecules, including proteins from both photosystems, accessory pigments, and ions that serve as enzymatic cofactors, participate in these processes. Numerous studies have demonstrated that PdBs can enhance photosynthetic components, improve photosynthetic function, and, more specifically,

mitigate or reverse the effects of abiotic stress on this vital process. For example, the commercial biostimulant Trainer® has been shown to increase total chlorophyll content in lettuce (Colla et al. 2013), pea (Colla et al. 2014), spinach (Rouphael et al. 2018), tomato (Sestili et al. 2018), and lamb's lettuce (Di Mola et al. 2020). Similarly, other PdBs have produced comparable effects on corn (Ertani et al. 2016), perennial wall rocket (Giordano et al. 2020), cabbage (Godlewska et al. 2020), and common bean (Mkindi et al. 2020). Chlorophylls are integral components of light-harvesting complexes, complemented by accessory pigments such as carotenoids (carotenes and xanthophylls) and anthocyanins. The levels of these pigments typically increase in parallel with chlorophyll following PdB application. Light absorbed by pigments in photosystem II reaction centers is essential for water photolysis, electron transfer to photosystems, and the overall electron transport chain. Electron transfer and photosynthetic efficiency can be quantified by measuring several parameters, many of which were enhanced by PdB application. For instance, improvements in photochemical efficiency (Fv/Fm ratio) have been observed in lettuce (Lucini et al. 2015) and basil (Rouphael et al. 2023), in photosystem II and photochemical efficiency in spinach (Rouphael et al. 2018), and in net photosynthesis in olive trees (Regni et al. 2021).

Carbon reduction from CO_2 to carbohydrates occurs in the Calvin-Benson cycle, which follows the generation of reducing power molecules (NADPH) and energy (ATP) during the preceding light-dependent reactions. Carbohydrate production requires CO_2 capture through gas exchange processes, such as transpiration and stomatal opening and closing. Specific Plant-derived Biostimulants (PdBs) have been identified as enhancers of these processes. The application of Trainer® increased net CO_2 assimilation in tomato plants (Rouphael et al. 2017) and improved stomatal conductance, leaf transpiration, and net photosynthesis in geranium (Cirillo et al. 2018).

Notably, PdBs can increase or maintain these parameters in plants growing under unfavorable conditions.

Shoot growth parameters are often used as indicators of future crop yields. In addition to increasing plant height and stem diameter, various biostimulants can positively influence the shoot dry weight and length. For example, Trainer® has been reported to enhance plant height and shoot dry biomass in maize (Colla et al. 2013), as well as shoot dry weight and length in tomato and dwarf pea (Colla et al. 2014), and plant height in geranium (Cirillo et al. 2018). Similarly, biostimulants derived from plant by-products elicit comparable responses, as demonstrated by carob germ extracts, which increased stem diameter and plant height in tomato (Rady and ur Rehman 2016). Leaf area, a key determinant of photosynthetic capacity, can also be increased by biostimulants, as reported in geranium (Cirillo et al. 2018), tomato (Rady and ur Rehman 2016), and maize (Ertani et al. 2009). In maize and olive, the application of protein hydrolysates derived from different sources increased leaf biomass, which is another important plant growth parameter (Ertani et al. 2016). In gladiolus, moringa leaf extracts have been shown to enhance plant height, bulb biomass, bulb diameter, and bulb production (Younis et al. 2018). Similarly, in lily plants, the application of an alfalfa-based biostimulant increased bulb size and produced greener and more expanded leaves (De Lucia and Vecchietti 2012).

PdBs have been reported to increase the height and biomass of cabbage (Godlewska et al. 2020) and rocket (Abdalla 2013), thereby enhancing their total yield. In wild mint, extracts from large-leaf beautyberry improved leaf growth, area, and number (Haider et al. 2009). Similarly, in rose-scented geranium, moringa leaf extracts increased plant height, branch number, and leaf area, while also improving overall yield and volatile oil content (Ali et al. 2018). In our trial, positive effects on biomass and leaf area were observed; however, these effects were dependent on both the dose and the

extraction method. It is also well established that the dose and method of administration significantly influence the efficacy of biostimulant treatment (Li et al. 2022).

Biostimulant treatments can mitigate the negative effects of abiotic stress on plant growth. In tomato and pumpkin plants, the application of Trainer® alleviated the adverse effects of water scarcity, resulting in increased biomass of the treated plants (Abd El-Mageed et al. 2017; Paul et al. 2019). In petunia, the application of *Moringa oleifera* Lam. leaf extract promoted nearly all growth parameters under both control and drought-stressed conditions (Toscano et al. 2023).

When treated with a foliar application of *Chondrus crispus* extract (2 g/L), tomato plants exhibited a significant increase in growth compared to that in untreated controls. Under well-irrigated conditions, growth increases exceeded 12% at all three time points. In contrast, under water deficit conditions, growth increases of 4%, 16%, and 13% were observed after the first water stress, following two days of recovery, and after the second water stress, respectively (Domingo et al. 2023). In our trial, the biomass increases were notably substantial, with dry leaf biomass increasing by approximately 39.6% and the total leaf area by 131%.

7.5. Conclusion

Because of ongoing climate change, water scarcity is rapidly increasing, creating stressful conditions that hinder plant growth. This issue also affects the ornamental plant sector, which is currently experiencing the consequences of global warming. The anticipated reduction in water availability threatens the use of these plants in public and private green spaces. Identifying more drought-tolerant genotypes and biostimulant treatments to enhance plant performance under water-stressed conditions may be an effective strategy.

Trial results have shown that the leaf extract of common sea fennel (*Crithmum maritimum*) can improve the performance of *Coleus*

amboinicus plants, especially when subjected to water stress. The results indicated that the response was primarily dose-dependent and that the extraction method also had a significant impact. Extraction using 96% ethanol and 4% methanol proved to be significantly more efficient, although it is more costly in terms of application.

This intriguing result warrants further investigation. Moreover, there is currently no information in the literature regarding the use of sea fennel as a biostimulant, despite its well-known medicinal property.

Investigations must continue to better define extraction methods, dosages, and optimal treatment timing, as well as to understand the role of *C. maritimum* as a biostimulant compared to other plant-derived and commercial biostimulants, to more accurately assess their efficacy.

The trials need to be repeated with other genotypes, as the response to biostimulant treatment is often species-specific. Therefore, it is necessary to determine whether other species can benefit from the use of these substances. Understanding the mechanisms of action could be key to developing broadly applicable strategies for administering the biostimulant, beyond the variability of species-specific responses.

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8. General discussion and conclusions

Drought stress is particularly acute in Mediterranean environment (Toscano et al. 2019), partly as a result of climate change, and affects ornamental plants, especially those used in green spaces. These plants are often considered an effective means of improving the quality of life and human well-being in urban areas.

The mitigation of drought stress, like all abiotic stress, can be achieved by a multi-action approach (Mikiciuk et al. 2024), such as agronomic management and suitable ornamental genotypes (Leotta et al. 2023a). In a short period, the application of biostimulants, the use of mulching, or the choice of the appropriate species combination could have positive effects on abiotic stress mitigation (Bulgari et al. 2019; Anuar et al. 2023), especially in urban and peri-urban areas. In addition, to agronomic strategies, the selection of tolerant species against various abiotic stresses can have a positive impact on urban environments and human welfare (Kisvarga et al. 2023; Mircea et al. 2024). Wild or underutilised species, in particular, can be opportunely selected to improve or preserve ornamental quality (Gupta et al. 2024). However, further studies are required to understand the interactions and responses of ornamental plants to abiotic stresses in urban environments.

The degree of drought damage also depends on the type of green space present. Some green infrastructures, particularly those whith limited substrate availability to root systems, are particularly susceptible to drought stress (Catalano de Sousa et al. 2016; Shehayeb et al. 2024). A good example of this is the green roofs. However, these latter, are increasingly considered for their contribution to ecosystem services, which are essential for the quality of life in urban areas (Patnaik et al. 2018; Leotta et al. 2023b). Most of these functions are connected to the installed vegetation, making the choice of species one of the most

strategic aspects of design. Even in the Mediterranean environment, sustainable solutions are possible for creating green roofs, which should be based on reducing substrate height to allow installation on a greater number of structures and promote more rational use of water resources. To identify species that can tolerate drought stress, analyzing both plant species present in habitat analogues and certain morpho-functional characteristics can be a useful strategy (Ciccarelli and Bona 2022; Leotta et al. 2023b). However, only experimental trials in representative contexts can provide conclusive information on whether these choices are effective. Another strategy is to increase biodiversity by replacing a single species with a plant community to maximize the positive interactions established between different plant species (Hooper et al. 2005; Marquard et al. 2009). Nevertheless, even in this case, only specific experimental trials can provide the essential knowledge framework needed to promote the widespread adoption of green roofs in the Mediterranean environment. Plant species selection remains one of the most effective approaches for mitigating the negative effects of water scarcity (Espeland and Kettenring 2018). These genotypes, which can tolerate drought stress, are widespread in environments such as the Mediterranean region, where water shortages are frequent. The Mediterranean area, at the same time, is a large source of biodiversity for the ornamental sector, with a high number of plant species that can be used in urban and peri-urban landscapes (Romano and Scariot 2021; Leotta et al. 2025). Morphological and physiological traits are conserved across botanical families. The ornamental contribution is provided by the leaves, flowers, and fruits. All these organs can provide ornamental quality in different periods or seasons. Therefore, the combination of various ornamental species is possible to extend the aesthetic quality of an area (Veinberga et al. 2019). In the study carried out in this PhD thesis, the most important native genera and species were listed and used to select plants that could adapt to urban environments. This review allowed us

to identify 1,137 taxa belonging to 271 genera and 74 botanical families. Morphological traits, such as leaf size, presence of trichomes, wax, etc. allowed us to identify 544 taxa belonging to 199 genera and 68 different botanical families of potential ornamental use (Leotta et al. 2025).

Another experimental trial was conducted to evaluate on three ornamental herbaceous species, *Dimorphotheca hybrida* DC. 'Dalina® Special Sunshine Beauty', *Pelargonium peltatum* (L.) L'Hér. 'Moonflair® Red', and *Pelargonium hybridum* (L.) L'Hér. 'Galaxy™ Red', the ability to recover from water stress, following periodic suspension and restoration of the water supply, and study the mechanisms that support this ability, showed that photosynthetic changes are the most affected by antioxidants and osmolytes, such as proline. The lower leaf gas exchange variations can be attributed to greater adaptation to drought conditions. Among the three species, *P. peltatum* and *D. hydrida* were the most tolerant.

Biostimulants can be a valuable tool for reducing the negative effects of abiotic stress (Franzoni et al. 2022), particularly drought stress. Interest in these topics has significantly increased in recent years. In addition to commercial products, plant-derived biostimulants have attracted attention, even through the aqueous maceration of various plant parts (leaves, roots, and flowers). It has been observed from various quarters that the effect is species-specific and dose-dependent. Trials conducted as part of this doctoral thesis have demonstrated this species-specific effect on fifteen ornamental shrub species subjected to drought stress, while also highlighting the importance of timing of treatment. Specifically, applying the treatment before stress was imposed appeared to be more effective than applying it as a curative treatment after the period of drought stress.

The topic of biostimulants warrants further attention. In another trial conducted on the herbaceous plant Cuban oregano (*Coleus amboinicus* Lour.), it was found that, in addition to the dose effect, the

biostimulant extraction method plays a key role. Ethanol and methanol extraction, in fact, appear to be more effective—although more expensive—than aqueous maceration of plant parts.

Therefore, future research should focus on analyzing the morphobiological and functional responses of various ornamental plant genotypes to identify those best suited for urban green spaces in the Mediterranean environment, where the negative impacts of global change will exacerbate drought stress. Additionally, it is essential to develop agronomic strategies to mitigate such stress. Relying on plant communities rather than single species appears to be a promising approach. The use of biostimulants, particularly botanical extracts derived from plants, represents a significant strategy aligned with the goals of the 2030 Agenda for Sustainable Development. Understanding the mechanisms of action may be crucial for developing broadly applicable methods of biostimulant application, transcending the variability of species-specific responses.

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9. Annexes

9.1. Other manuscript related to the PhD topics

9.1.1. How can plants used for ornamental purposes contribute to urban biodiversity?

Toscano S., Romano D., Lazzeri V., **Leotta L.** and F. Bretzel. Sustainability 2025, 17, 4061. https://doi.org/10.3390/su17094061

How can plants used for ornamental purposes contribute to urban biodiversity?

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Abstract
Sustainability urgently needs to be achieved in urban green infrastructure. Maintaining and restoring biodiversity are critical for developing an urban ecosystem more resilient to abiotic and biotic stresses. The biodiversity of urban green spaces is vital as it reduces the risks associated with climate change (diseases and pests), improves the resilience of the urban ecosystem, and enhances citizens' well-being. Urban green areas can provide important ecosystem services necessary for achieving prosperity, urban well-being, and the One Health paradigm at various scales. Urban green areas can serve as corridors and stepping stones between the rural environments surrounding cities, increasing their connections and reducing the risk of ecological traps. The conservation and restoration of biodiversity are strategies to increase ecosystem services. In this context, this

review aims to analyze the possible contribution of ornamental plants to urban biodiversity, investigating the available knowledge and the gaps that need to be filled. Plants chosen for their esthetic functions are often allogamous species, characterized by showy flowers that attract fauna for pollination, thus helping insects and other fauna survive. If not invasive, these plants can actively contribute to biodiversity in the urban environment and to human well-being. Choosing suitable species and methods that favor plant communities and sustainable maintenance practices improves biodiversity and the ecosystem services that ornamental plants provide.

9.2. Scientific curriculum

9.2.1. Research and professional experience

- 10/2022 – 10/2025: PhD Course in Agricultural, Food and Environmental Science (International), University of Catania. Research project: "Strateges to overcome drought stress in Mediterranean enviroment: the case of ornamental plants". Under the supervision of Prof. Daniela Romano and Prof. Stefania Toscano.

9.2.2. Education and professional qualifications

- February 2020, awarded the ESB Level 3 Certificate in ESOL International All Modes – (C2).
- July 2017, qualification in a public competition for teaching (D:D:G 105/ 2016).
- February 2001 qualification for the freelance profession of agronomist.
- November 2000: Master's Degree in Agricultural Sciences, University of Catania supervisor Prof. Daniela Romano. Degree thesis: "Lineamenti del paesaggio agrario etneo".

9.2.3. Memberships and IDs

- Member of the Italian Society for Horticultural science (SOI)
- ORCID ID: 0009-0005-9794-4298
- Scopus ID: 58293790000
- Web of Science ResearcherID: FII-2910-2022

9.2.4. Published articles

- **Leotta, L.**, S. Toscano, A. Ferrante, D. Romano, and A. Francini. 2023. New strategies to increase the abiotic stress

tolerance in woody ornamental plants in Mediterranean climate. *Plants*, 12(10), 2022. https://doi.org/10.3390/plants12102022

- **Leotta, L.**, S. Toscano, and D. Romano. 2023. Which plant species for green roofs in the Mediterranean environment?. *Plants*, 12(23), 3985. https://doi.org/10.3390/plants12233985
- Toscano, S., D. Romano, V. Lazzeri, **L. Leotta,** and F. Bretzel. 2025. How can plants used for ornamental purposes contribute to urban biodiversity?. *Sustainability*, 17(9), 4061. https://doi.org/10.3390/su17094061
- **Leotta L.**, S. Toscano, A. Ferrante, and D. Romano. 2025. The use of Mediterranean native shrubs for improving the sustainability of urban environments. *Frontiers in Horticulture*, 4:1652517. https://doi.org/10.3389/fhort.2025.1652517

9.2.5. Under review articles

- **Leotta L**., S. Toscano, A. Carchiolo, A. Ferrante, and D. Romano. Morphological, physiological, and biochemical changes of herbaceous ornamental plants subjected to drought stress and recovery. *Helyon*

9.2.6. Conference proceedings

- Carchiolo, A., D. Arena, **L**. **Leotta,** S. Toscano, and D. Romano. 2024. 1 Influenza dell'adozione di lampade LED a diversa lunghezza d'onda e di biostimolanti su microgreens di broccolo. *II Convegno Nazionale di Orticoltura e Floricoltura*, Padova - 19-21 giugno 2024, 63.
- **Leotta, L**., A. Carchiolo, S. Toscano, and D. Romano. 2024. Meccanismi di tolleranza di specie erbacee ornamentali alla siccità. *II Convegno Nazionale di Orticoltura e Floricoltura*, Padova - 19-21 giugno 2024, 64.

- **Leotta, L**., A. Carchiolo, F. Scotto Di Covella, S. Toscano, A. Ferrante, and D. Romano. 2025. Influenza della dose e della modalità di estrazione del biostimolante sulla tolleranza al secco di *Coleus amboinicus* Lour. *Giornate Tecniche SOI sul Florovivaismo - Innovazioni al servizio della filiera*, Pescia - 6-7 novembre 2025.
- **Leotta, L**., A. Carchiolo, S. Toscano, and D. Romano. 2025. L'impiego di biostimolanti per ridurre gli effetti dello stress idrico su lantana e viburno. *Atti XV Giornate Scientifiche SOI,* Pisa, 25-27 giugno 2025.
- **Leotta, L**., A. Ferrante, A. Trivellini, D. Romano, and S. Toscano. 2025. Screening della risposta di arbusti ornamentali alla siccità. *Atti XV Giornate Scientifiche SOI,* Pisa, 25-27 giugno 2025.
- Romano, D., A. Currò, **L**. **Leotta**, and S. Toscano. 2025. La biodiversità degli spazi a verde minimali della Sicilia interna. *Atti XV Giornate Scientifiche SOI,* Pisa, 25-27 giugno 2025.
- Romano, D., S. Giuffrida, S. Del Lesto, **L**. **Leotta**, and S. Toscano. 2025. Ipotesi per il restauro di un giardino storico: il Parco Paternò del Toscano. *Atti XV Giornate Scientifiche SOI,* Pisa, 25-27 giugno 2025.
- Scotto di Covella, F., **L**., **Leotta** A., Mensuali D., Romano, and A. Ferrante. 2025. Effetto di trattamenti con estratti di borragine e finocchio marino sulle piante di salvia ananas. *Giornate Tecniche SOI sul Florovivaismo - Innovazioni al servizio della filiera*, Pescia - 6-7 novembre 2025.

9.2.7. Other conferences/meetings/workshops

- Participation to the National Conference of Horticulture ed floriculture organized by the Italian Society for Horticultural science (SOI), Padova June from 19th until 21th 2024. Oral presentation (Presenting Author): **Luca Leotta**, Agnese

Carchiolo, Stefania Toscano, Daniela Romano. Meccanismi di tolleranza di specie erbacee alla siccità. Full contribution: 'Book of abstract', p. 64. https://www.soihs.it/ortofloro2024/book_of_abstracts.aspx https://www.soihs.it/public/03/02/BOOK%20OF%20ABSTRACTS%2013-6-24_compressed.pdf

- Participation at the 5th Joint Meeting of Agriculture-oriented PhD Programs, Catania 25-28 September 2023, Oral presentation (Presenting Author): **Luca Leotta**, Daniela Romano, Stefania Toscano. Strategies to overcome drought stress in Mediterranean environment: the case of ornamental plants. Full contribution: 'Book of abstract', p. 61. https://www.di3a.unict.it/sites/default/files/files/book-of-abstracts-5th-joint-meeting-of-agriculture-unict-unifg-uniud_0.pdf
- Participation at the 6th Joint Meeting of Agriculture-oriented PhD Programs, Catania 30 September 2024 – 04 Ottobre 2024, Oral presentation (Presenting Author): **Luca Leotta**, Agnese Carchiolo, Stefania Toscano, Daniela Romano. Mechanisms of tolerance of ornamental herbaceous species to drought https://www.di3a.unict.it/sites/default/files/files/BoA_2024_Lesina.pdf
- Partecipation to 15th Scientific days SOI 25-27 giugno 2025 Pisa organized by the Department of Agricuture Food and Environmental Sciences of the University of Pisa and the Institute of Crop Production of the Sant'Anna University High School.
- English course organized by the CLA of the University of Catania, lasting 40 hours to achieve the B2 level. On line from March 8th until June 1st (teacher Prof. Giustino Alessi).

Courses organized by the Department of Agriculture, Food and Environment of the University of Catania (UniCT) according to the Educational Project of the PhD Course:

- "Writing a scientific review article" (teachers Prof. Daniela Spina and Prof. Gaetano Chinnici). June 6th and 9th (4 hours)
- "Scientific publishing in the peer-review era" (teachers Prof. A. Biondi and Prof. Michele Recupero). June 23th and 27th (8 hours)
- "CAD and GIS for sustainable agriculture" (teachers Prof. Provvidenza Rita D'Urso and Prof. Claudia Arcidiacono). July 4th, 5th and 6th (15 hours);
- "ICT for participatory methods, citizen science, and disseminating research" (teacher prof. Teresa Graziano). July 10th, 11th and 12th (15 hours).
- "Agricultural Policies in the UE: from the past to the future" (Teacher Prof. Giovanni La Via). 18th, 19th and 22nd December 2023 (10 hours).
- "Introduction to literature review process: overview and guidelines" (teacher prof. Giuseppe Antonio Di Vita). 12th, 19th February and 4th March 2024 (10 hours).
- "General and multivariate statistical analysis: application with the R software" Teacher Prof. Maria Raimondo. March 7th, 11th, 14th, and 18th 2024 (20 hours)
- "Managing biological data with R: applications for plants and fruit tree species" Teacher Prof. Mario Di Guardo. January 22nd, 23rd, 24th, 25th, and 26th 2024 (20 hours).
- "Introduction and recent trends on experimental approaches for plant science" Teacher Prof. Chiara Catalano March 15th and 22nd, April 5th 2024 (10 hours).

Other courses, seminars and webinars attended:

- Final Conference of the "Bresov EU Horizon Project" BRESOV Consortium Agrigento Valle dei Dioscuri Hotel. From March 29th until March 30th (16 hours).
- Biostimolanti Conference ARPTRA – Fruit Communication Catania - International Airport Hotel. From March 1st until March 2th (16 hours).
- Webinar Presentation Focus: 22/23 Libro Bianco del Verde ASSOVERDE. On line. January 20th (6 hours).
- Workshop: Social Farming Orti del Mediterraneo. Misterbianco CT Via Pascoli, 9/A June 7th (5 hours).
- Libro Bianco del Verde. La salute è verde, il verde è salute: Macchia Mediterranea 4.0. Nuovi modelli per la progettazione, la produzione, la gestione e la cura del verde. ASSOVERDE. Sicilia Fiera exhibition meeting hub Via Bologna 76 Misterbianco October 28th 2023 (4 hours).
- Final Conference Progetto GIFLUID - Green Infrastructures to mitigate flood risks in Urban and suburban areas and to improve the quality of rainwater discharges. CSEI Catania. November 17th 2023 (4 hours).
- Il florovivaismo di fronte alle nuove sfide: tre opinioni a confronto Confagricoltura Ragusa. Nuova città Emaia. Ragusa November 23th 2023 (2 hours).
- Agri School Expo Orti del Mediterraneo. Catania December 14th and 15th 2023 (8 hours).
- Prevalence and distribution of antimicrobial-resistant bacteria in Brazilian food animal production. Catania December 11th 2023 (3 hours).
- Verde & Clima. Impatto ambientale, impronta ecologica e greenwashing energetici, economici e sociali. Assform - Fad - Bologna (BO). On line December 12th 2023 (4 hours).

- Tips for writing a paper for publication. University of Catania Department of Biological Sciences. February 28th 2024 (3 hours)
- I gemelli digitali nella gestione del verde urbano. CREA on line. February 28th 2024 (2 hours).
- Seminar on EFSA (mission activities and opportunities). Department of Agriculture, Food and Environment, Catania, April 15th 2024 (2 hours).
- Analisi e prospettive del verde a Sciacca. Association: "Con i piedi per terra". Sciacca (AG). April 12th 2024 (4 hours).
- Fame Lab Master Class. PSIQUADRO. Città della Scienza, Catania. April 8th 2024 (6 hours).
- Il paradigma agroecologico valori, principi e azioni per la transizione verso sistemi agroalimentari sostenibili Department of Agriculture, Food and Environment, Catania April 22th 2024 (8 hours).
- Research advanced in wood-boring beetle. Department of Agriculture, Food and Environment, Catania May 31th 2024 (2 hours).
- Sustainability Day Ambiente e Agricoltura: I processi di transizione TAOMODA. Taormina, July 18th 2024 (4 hours).
- TA SPRINGER NATURE & CARE – CRUI Webinar October 7th 2024 "How to write a scientific paper" (1 hour).
- TA SPRINGER NATURE & CARE – CRUI Webinar October 8th 2024 "Research integrity" (1 hour).
- Author Journey – Transforative Agreement SPRINGER NATURE & CARE – CRUI Webinar October 9th 2024 (1 hour)
- "Il giardino del Re" sinergie - valorizzazione del Sistema locale, il caso della Villa Comunale di Caltagirone, recupero riqualificazione e rigenerazione del verde storico. Museo della ceramica di Caltagirone, March 25th 2025 (5 hours).

- "Fiori di Sicilia: il giardino botanico di Giusepe Riggio ad Acireale". Palazzo di Città, Acireale May 2nd 2025 (2 hours).
- "Paesaggio agrario siciliano e cultura del cibo: quali connessioni? Department of Agriculture, Food and Environment (UniCT), Catania May 19th 2025 (4 hours).
- "Il castagno come elemento per lo sviluppo locale per le terre dell'Etna: sfide e prospettive" Parco dell'Etna, July 9th 2025 (4 hours).

9.2.8. Training in national or international universities and/or research institutions

As part of the activities of the Ph. D I had the opportunity to visit the Department of Crop Sciences of the "Sant'Anna Superior School" in Pisa. I have been there two times: the first in April 2025 and the second in July 2025 during which I had the possibility to analyze samples of plants subjected to drought stress under the esteemed supervision of Prof. Antonio Ferrante.

9.2.9. Participation to research projects

- Misura 16.1 Programma di Sviluppo Rurale Sicilia 2014-2020 "Produzione di aromi naturali per la conservazione degli alimenti" (A.NA.CO.AL).
- Misura 16.2 Programma di Sviluppo Rurale Sicilia 2014-2020 "Progetto pilota per lo sviluppo sostenibile di nuovi processi produttivi, nel comparto florovivaistico mediterraneo, ad elevata specializzazione tecnologica, attraverso l'impiego di lampade innovative, ad alto rendimento energetico" (Lightflowers).

9.2.10. Teaching activities

- Teaching activities within the scope of the NATIONAL RECOVERY AND RESILIENCE PLAN (PNRR) D.D.G.

No. 475 of 25/05/2023 professional training courses for "Artistic gardeners for historic gardens and parks". For a total of six hours of teaching. The topics covered were "Carrying out maintenance work: flower beds and borders, flowering and foliage shrubs"; "Carrying out maintenance work: lawns".

9.2.11. Thesis co-mentoring

- Irene Spitaleri, MS thesis on the "Risposta di *Coleus amboinicus* Lour. allo stress idrico", master's degree in Agricultural Sciences and Technologies, Department of Agriculture, Food and Environment, University of Catania, Italy.