Biochemical, Physiological, and Molecular Aspects of Ornamental Plants Adaptation to Deficit Irrigation

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Abstract: There is increasing concern regarding global warming and its severe impact on the farming sector and food security. Incidences of extreme weather conditions are becoming more and more frequent, posing plants to stressful conditions, such as flooding, drought, heat, or frost etc. Especially for arid lands, there is a tug-of-war between keeping high crop yields and increasing water use efficiency of limited water resources. This difficult task can be achieved through the selection of tolerant water stress species or by increasing the tolerance of sensitive species. In this scenario, it is important to understand the response of plants to water stress. So far, the response of staple foods and vegetable crops to deficit irrigation is well studied. However, there is lack of literature regarding the responses of ornamental plants to water stress conditions. Considering the importance of this ever-growing sector for the agricultural sector, this review aims to reveal the defense mechanisms and the involved morpho-physiological, biochemical, and molecular changes in ornamental plant's responses to deficit irrigation.

Keywords: ornamental species; water deficit; water stress; defense mechanisms; climate change; stress responsive genes; stress adaptation

1. Introduction

Climate change refers to anomalous atmospheric conditions, as well as sudden unexpected climatic events, such as floods, hurricanes, intense and/or prolonged drought, extreme temperatures, etc. Drought is among the environmental stressors that has the most severe impact on crops throughout the world [1–3]. One-third of arable lands are already defined as arid or semi-arid ones [4], and the severity of drought shows increasing trends [5] since a 5 °C increase in mean air temperature is expected in the following years [6–10]. According to experts, the drylands on Earth will increase by 30% and the drier summers and reduced rainfall are expected to affect mostly Asian mid-continental regions, southern Europe, Northern and South Africa [11]. The reduction of usable water sources and the continuous demographic growth make it necessary to improve water use efficiency in the farming sector in order to ensure food security for the years to come. A big step towards this goal has been made by the introduction of soilless cropping systems, where the use of irrigation water is under continuous control [12]. However, the appropriate supply of water to crops, even in soilless conditions, requires the monitoring of various parameters, such as the growth substrate humidity, the climatic and microclimatic conditions, and most importantly, the water status of plants [13], which is more complex to quantify than climatic and growth substrate related parameters [14]. Furthermore, there may be differences between species or even cultivars of the same species in terms of water stress, especially under deficit irrigation conditions where a genotype dependent response is observed. Scientists are looking for mechanisms that regulate the response of plants to water stress, aiming to either identify the most tolerant species or increase tolerance in the sensitive ones. For this

purpose, genetic studies are based on breeding and genetic engineering of model plants, such as *Arabidopsis thaliana* [1,15,16], so that the obtained responses could be extrapolated to other crops such as staple food, medicinal, aromatic, and fiber plants. The efficient use of water is a crucial point in cultivating ornamental plants which have to respond to different needs, e.g., moderate use of natural resources, climate change, environmental pollution, increasing production costs, and maximizing profits [17,18]. Unfortunately, there is still no standard protocol for the irrigation of ornamental species, and water requirements of plants are covered based on growers' personal experience [14,19].

Knowing the response of different species to water stress conditions would allow the identification of morphological indices and biochemical markers useful for distinguishing sensitive and tolerant species to water deficit stress [20–22]. Therefore, in this review, the morphological, biochemical, physiological, and molecular responses of the main ornamental plants cultivated throughout the world have been studied. Moreover, a literature update regarding the genes involved in ornamental plants' response to water stress is also presented and discussed.

2. The Effect of Deficit Irrigation on Morphology, Growth, and Quality of Ornamental Plants

The growth and morphology of ornamental plants have an aesthetic value and are very important parameters which guide the consumer's choice. The effects of deficit irrigation on the leaf are related to orientation changes, to reduction of leaf area and leaves number, to reduction of trichomes and canopy area, and to increase in leaf thickness as plant responses to avoid water losses [23–26] (Figure 1, Table 1).
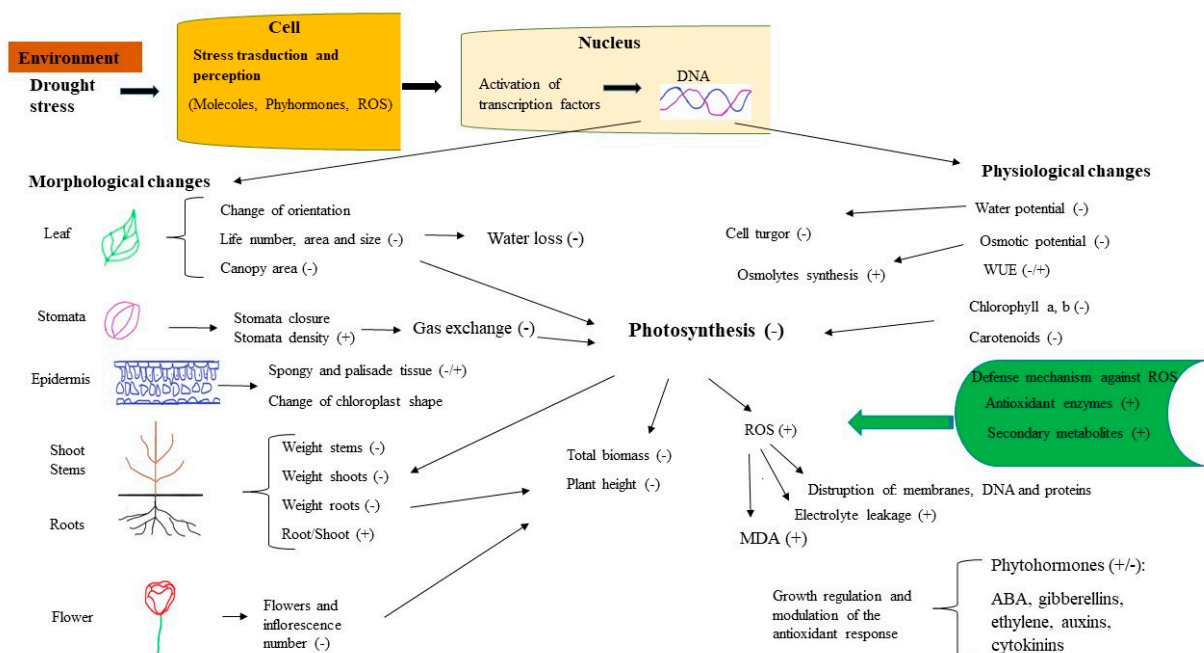

Figure 1. Water stress-induced morphological and physiological changes. (−) reduction due to water stress; (+) increase due to water stress. abscisic acid (ABA); malondialdehyde (MDA); reactive oxygen species (ROS); water use efficiency (WUE).

Lantana and *Ligustrum*, two important ornamental plants of the Mediterranean area, showed an increase in spongy and palisade tissue, following severe water stress [24]. The change in the leaf anatomy serves to increased diffusion of CO_2 from the external atmosphere to the spaces between cells [25,27], while thicker leaves presented higher chlorophyll content and photosynthetic activity [27]. Therefore, these responses related to leaf anatomy constitute an avoidance mechanism to reduce water losses.

Water stress has an impact on the morphology of *Chrysanthemum morifolium* Ramat cv. Hj inflorescences, an ornamental plant characterized by ray and disc florets [28]. The

reduction of soil moisture reduces the number and shape of ray florets, while the number of disc florets increases. In *Callistemon citrinus*, the number of inflorescences did not change under moderate stress but reduced when severe stress was implemented [14]. Avoidance mechanisms are also evident in *Viburnum opulus* L. and *Photinia × fraseri*, two Mediterranean species which show alterations of leaf parameters under both moderate (60% evapotranspiration [ET]) and severe (30% ET) water stress conditions [27]. The changes in leaf parameters depend on the intensity of water deficit as well as on the genotype.

Reduction in leaf thickness in terms of epidermal thickness, palisade, and spongy tissue, and higher stomatal density have been associated with greater water stress sensitivity in *Passiflora alata* plants [29], whereas *Passiflora setacea* has shown fewer leaf anatomical and is considered more tolerant to deficit irrigation. Moreover, deficit irrigation may change the shape of chloroplasts in *Paeonia ostii* plants, e.g., from an oval shape in control plants to a more rounded shape in stressed plants [30]. All the above-mentioned examples reveal the diversity in plants' responses to water stress related to leaf parameters and highlight the complexity of the defense mechanisms against water stress.

Growth reduction is one of the first manifestations that plants are subjected to with water stress. For example, the application of water stress for one, two, or three weeks decreased the growth of poinsettia (*Euphorbia pulcherrima*) in terms of plant height (67.4, 57.0, and 49.0 cm, respectively) and leaf area (2.91, 1.22, and 0.93 cm^2, respectively) [18]. In addition, *Rosa damascena* Mill., a rose from Damascus which is widespread all over the world for its perfume and use in cosmetics and medicine, was subjected for 90 days to 100% of field capacity (FC), moderate water stress (50% FC), and severe water stress (25% FC) [10]. On the other hand, the number of leaves was not reduced by stress, so the reduction in aerial biomass was mainly attributed to a reduction in leaf area [31].

Antirhinum majus cv. Butterfly is an ornamental plant widely used to beautify urban areas and gardens, which also responds to water stress with a reduction of plant growth parameters (leaves, shoots, flowers), as well as with changes in plant nutritional status (the content of N, P, K, Mg, and Ca) [32]. Similarly, two cultivars of *Matthiola incana* L., an ornamental plant of the *Brassicaceae* family widely appreciated for its beautiful and colorful flowers, was subjected to 5 levels of water stress, namely 90%, 80%, 70%, 60% of field capacity [33].

Adonis amurensis and *Adonis pseudoamurensis*, two species belonging to the *Ranunculaceae* family [7] (Table 1), exhibited reduced growth only in the last days of deficit irrigation treatment, indicating that they can tolerate water deficit conditions. Moreover, water stress reduced shoot dry mass in purple coneflower plants (*Echinacea purpurea* L.) by 51.5% [34], while five species of *Passiflora* spp. (*P. edulis*, *P. gibertii*, *P. cincinnata*, *P. alata*, *P. setacea*) showed a reduction in growth within the range of 50–75%, following water deficit conditions [29].

Water stress may also increase the root-to-shoot ratio. This is an adaptive response to deficit irrigation as a result of the increase in the root system growth and the concomitant reduction in the aerial part of the plant [14]. In this way, the plant tries to cope with reduced water availability by increasing water absorption though roots and reducing water loss from leaves at the same time [25,35,36]. Water stress may also cause changes in roots architecture. For example, in *Callistemon citrinus* plants subjected to water stress, the main roots were longer, whereas the growth of small roots, lateral and thinner ones, was eliminated [37]. Similar results were reported for *Nerium oleander* L., *Pittosporum tobira* Thunb., and *Ligustrum japonicum* Thunb. 'Texanum' (Mediterranean ornamental shrubs) plants [12], subjected to four levels of water stress (90%, 80%, 70%, and 60% of container capacity).

Rafi et al. [26] examined the morphological response to water stress in two native, and therefore already adapted to the local climate conditions, ornamental species, namely *Althea rosea* and *Malva sylvestris*, and two exotic ones, namely *Rudbeckia hirta* and *Callistephus chinensis*. The results showed that, concerning roots length, volume, and density, a decreasing trend was observed with increasing water stress severity in the case of *C. chinensis* and *M. sylvestris*. In contrast, in *A. rosea*, the length of the roots increased as

the deficit irrigation levels increased, while roots density decreased in *R. hirta* plants when water stress was more severe.

Three potted Bougainvillea genotypes (*B. glabra* var. Sanderiana, *B.* × *buttiana* 'Rosenka', *B.* '*Lindleyana*' (=*B.* '*Aurantiaca*') were grown on three irrigation levels (100%, 50%, and 25% of substrate moisture) and two canopy shapes (globe and pyramid), aiming to identify the most tolerant genotype and the most useful shape [38]. Moreover, the results showed that total dry biomass was reduced as water stress increased, with the *B.* '*Lindleyana*' genotype recording the highest reduction (33%), followed by *B. glabra* var. Sanderiana (20%) and *B.* × *buttiana* 'Rosenka' (5.5%). The effect of water stress on leaves number was the highest in the case of *B.* '*Lindleyana*' plants (reduced by 43%), followed by *B. glabra* var. Sanderiana (reduced by 33%) and *B.* × *buttiana* 'Rosenka' (reduced by 19%). The authors also suggested that the leaf area was reduced (by 43%) by water stress when canopy shape was pyramidal compared to the global one, while water deficit also reduced the content of N, P, and K in the three genotypes examined [38]. Moreover, according to Rouphael et al. [39], water stress is responsible for the reduction in leaf macronutrient contents in plants, probably because of the lower solubilization due to the water deficit, and therefore the lower absorption and translocation of nutrients [40].

Tolerance mechanisms have also been recorded in *Nerium oleander* L., an evergreen shrub belonging to the *Apocynaceae* family which is widespread in dry and semi-arid regions, such as the Mediterranean ones. In the work of Kumar et al. [1], 1-year-old *Oleander* plants were pot grown in a greenhouse and were normally irrigated until acclimatized. Subsequently, they were subjected to water stress and plants were analyzed after 15 and 30 days of stress initiation. The results showed that there were no effects on stem elongation (cm) and fresh weight of leaves (g) after 15 days of stress, whereas the effects became significant after 30 days of stress.

Four species belonging to the genus *Sedum* L. (*Crassulaceae* family), namely *Sedum spurium*, *S. ochroleucum*, *S. album*, and *S. sediforme*, also called "Green roofs" and being used to adorn the urban area and mitigate area pollution, showed different tolerance to water stress implemented with interruption of irrigation for 4 weeks [22]. All species showed a reduction in plant growth, and changes in morphological parameters (stem length, fresh weight) which allowed to establish a gradual tolerance to deficit irrigation.

Table 1. The effect of water stress on ornamental plants growth and morphology. (−) reduction due to water stress and compared to the control (C); (+) increase due to water stress and compared to the control.

Species	Plant Habit	Deficit Irrigation Treatment	Plant Growth Stage at the Beginning of Treatment	Modulation of Growth and Morphology by Water Stress	References
Lantana camara, Ligustrum lucidum	Shrub	C = 100% of water container capacity; Stress: 75%, 50%, and 25% of C	Two month old rooted cuttings	Dry weight (−) Leaf number (−) Leaf area (−) Leaf thickness (−) Thickness of the spongy and palisade tissue (+)	[24]
Polygala and *Viburnum*		10%, 20%, 30%, 40% of water content of the pot volume		Thickness of the spongy and palisade tissue (+)	[25]
Malva sylvestris, Althea rosea, Callistephus chinensis and *Rudbeckia hirta*	Herbaceous plants	C = 100% of ET_0 (local reference evapotranspiration) Stress: 25%, 50%, 75% of ET_0	1-month-old seedlings grown in the field and acclimatized for one month before treatment begun	Root length, root volume, root density: (−) in *C. chinensis*, and *M. sylvestris*) Root length: (+) in *Althea rosea*) Root density: (+) in *R. hirta*	[26]
Bougainvillea glabra var. Sanderiana, *Bougainvillea buttiana* 'Rosenka', Bougainvillea 'Lindleyana' (=B. 'Aurantiaca')	Rooted cuttings	C = 100% of substrate moisture Stress: 50% and 25% of control	Plants grown in greenhouse into pots filled with 3 L of peat-moss, irrigated with water and nutrient solution	Total dry biomass (−) Leaves number and leaf area (−) Number and flower index (no. dm^{-2} leaf area) (+) N, P, K (−)	[38]
Geranium macrorrhizum L. (Bevan variety from UK, and wild type from Hungary)	Cuttings from rhizome division	Interruption of irrigation for six weeks	Plants grown in greenhouse for 5 months and then a lath house for 7 months, into pots filled with 90% turf, 10% clay, irrigated manually with water	Different leaf area ratio (ratio between the leaf area and total weight of the plant, LAR m^2 kg^{-1}). Different leaf mass fraction (LMF, leaf biomass/total biomass; kg kg^{-1}) and root mass fraction (RMF, root biomass/total biomass; kg kg^{-1})	[41]
Nerium oleander L.	Seeds sampled in the wild	Interruption of irrigation for 15 and 30 days	One-year-old seedlings grown in greenhouse, into pots filled with peat-perlite-vermiculite (50%, 25%, 25%), irrigated with nutrient solution for a week before treatment begun	Stem elongation (−), Leaf fresh weight (−), Leaf water content percentage (−) K^+/Na^+ in roots (−)	[1]
Chrysanthemum morifolium Ramat. cv. Hj	Germplasm	35–40%, 65–70%, 95–100% of soil water holding capacity (WHC), for 62 days	Four-month-old seedlings grown in greenhouse, into plastic pots	Ray florets (−) Disc floret (+)	[28]

Table 1. *Cont.*

Species	Plant Habit	Deficit Irrigation Treatment	Plant Growth Stage at the Beginning of Treatment	Modulation of Growth and Morphology by Water Stress	References
Viburnum opulus L. and *Photinia × fraseri* 'Red robin'	Shrubs	C = 100% ET (Evapotraspiration) Moderate water deficit = 60% ET Severe water deficit = 30% ET, for 5 months	Plants grown in open air and grenhouse, into pots filled with peat, pumice, and osmocote.	Stem elongation (−) Leaf area (−) Number of leaves (−) Foliar biomass (−) Spongy tissue thickness (+) Shoot/root (+)	[27]
Sedum spurium, S. ochroleucum, S. album, and *S. sediforme*	Herbs, and sub-shrubs	C = irrigation twice a week Stress: interruption of irrigation per 4 weeks	Two-month-old seedlings grown in growth chamber, into pots filled with peat, perlite, and vermiculite, irrigated with nutrient solution	Total stem length (−) Leaves fresh weight (−)	[22]
Antirhinum majus cv. butterfly	Seeds	C = 80% of soil water content Stress = 60%, 40%, 20% of soil water content, for 10 weeks	Seedlings grown in greenhouse, into pots filled with sandy loamy soil, irrigated with tap water for three weeks before treatment begun	Shoot height and diameter (−) Number and leaf area (−) Fresh and dry weight of flowers (−) N, P, K, Mg and Ca content (−)	[32]
Passiflora spp. (*P. edulis, P. gibertii, P. cincinnata, P. alata, P. setacea*)	Germplasm	C = 100% of field capacity Stress = interuption of irrigation until apparent wilting (about 96 days)	Seedling grown in greenhouse, into pots.	Plant height (−) Plants dry weight (−) Leaf area (−) Leaves number (−) Different variation of leaf anatomy Stomatal density (+)	[29]
Paeonia ostii (*Paeonia* section Moutan DC)		C = plants watered daily Stress = interruption of irrigation for 4, 8, 12 days	3-year-old plants grown into pots and watered daily	Change of chloroplasts shape	[30]

3. Effect of Water Stress on Physiological Parameters, Hormonal Activity, and Biochemical Changes

3.1. Gaseous Exchange

The complete or partial closure of stomata to reduce water losses in the instance of water stress involves variations in gaseous exchange in leaves (Figure 1, Table 2). Several parameters are considered to measure the changes in gaseous exchange, e.g., stomatal conductance (gs), transpiration rate (E), and leaf relative water content (RWC) [26]. In Damask rose, the stomatal conductance was reduced by 19% in mild stress (50% of field capacity) and by 36% in severe water stress (25% of field capacity) compared to the control treatment (100% of field capacity) [10]. The transpiration rate increased twofold in mild stress (0.88 mmol H_2O m^{-2} s^{-1}) and remained unchanged under severe stress conditions (0.43 mmol H_2O m^{-2} s^{-1}), compared to the control (0.44 mmol m^{-2} s^{-1}). In the same context, stomatal conductance was reduced with increasing water stress in *Nerium oleander* L., *Pittosporum tobira* Thunb., and *Ligustrum japonicum* Thunb. '*Texanum*', while the values for the same parameter were higher in *N. oleander* than in *P. tobira* and *L. japonicum*. [12]. In addition, *N. oleander* had a larger leaf area than the other two species. These results showed that *N. oleander* was more tolerant to water stress than the other two Mediterranean shrubs. In another study, stomatal conductance was reduced in all five species of *Passiflora* spp. which were subjected to water stress until stomatal closure and rehydrated when plants exhibited wilting symptoms [29]. Moreover, at the time of rehydration, the five species exhibited different conductance recovery rates, demonstrating different adaptation to deficit irrigation as well as different adaptation strategies [29].

In tolerant plants, leaf RWC decreases as soil moisture is reduced [7]. In four species examined by Rafi et al. [26] (*Althea rosea*, *Malva sylvestris*, and two exotic *Rudbeckia hirta* and *Callistephus chinensis*), there was a reduction trend for the RWC parameter as water stress increased, while the most sensitive species were *C. chinensis* and *M. sylvestris*, recording lower relative water content by 59.0% and 52.5% compared to untreated plants, respectively. A reduction in relative leaf water content relative water content was also observed in *Adonis amurensis* and *Adonis pseudoaumernsis* [7] (Table 1), while for both species, the relative water content decreased slowly at the onset of stress, and then decreased rapidly.

Leaf water potential (Ψw) and osmotic potential ($\Psi \pi$) are two physiological parameters related to leaf water content and cell turgor. They reduce with increasing stress, as shown in *Bougainvillea* plants subjected to water stress [38]. Moreover, water deficit may reduce evapotranspiration values, stomatal conductance, and water potential, as shown in the case of *Callistemon citrinus* plants [14].

Navarro-Rocha et al. [41] compared the morphological and physiological responses to deficit irrigation in *Geranium macrorrhizum*, a plant widely used for its ornamental characteristics (in particular, for its pink and white flowers), and the presence of germacron sesquiterpene, an important essential oil constituent. The authors examined two varieties of two different origins, namely a variety selected in England (Bevans' (BV)), and a wild Hungarian geranium (GH) [41]. Cuttings of both varieties were grown in greenhouses within pots for 5 months, and after that, some pots were selected and subjected to stress with water holding for six weeks. In both genotypes, water potential did not increase excessively during the deficit irrigation period, and the authors attributed resistance to water stress to the closure of stomata which allowed to regulate water losses. The water potential remained constant for 20 days and then increased, resulting in accelerated water losses from the plants. The greater foliar growth and the better water status of leaves in GH variety were at the expense of root biomass, which was greater in the BV genotype (root mass fraction = root biomass/total biomass = 0.87 kg kg^{-1}). Moreover, both genotypes had similar root water contents which also indicates that GH plants might have a higher transpiration rate. In effect, under adequate water availability conditions, the larger leaf area means higher growth rate, while under water shortage, it results in rapid water losses through increased transpiration. The authors concluded that *G. macrorrhizum* can tolerate water stress for at least one month. Although belonging to the same species, the

two varieties had different morphological and physiological responses to water stress, suggesting that Bevan variety is more suitable for ornamental purposes under water stress conditions [41].

In another experiment, *Viburnum opulus* L. and *Photinia* $\times$ *fraseri* 'Red robin' were grown both in open air and greenhouse conditions and subjected to moderate and severe water deficit. In both species, the water potential of leaves decreased as the water deficit increased, with more negative values being observed in the greenhouse experiment, while the response of *P.* $\times$ *fraseri* plants was delayed compared to *V. opulus*. On the other hand, in the field experiment, severe stress reduced stomatal conductance in *V. opulus* and photosynthetic activity in *P.* $\times$ *fraseri* plants, while under greenhouse conditions, the reduction of stomatal conductance, transpiration, and photosynthesis already occurred even with moderate and severe stress in the case of *V. opulus* and *P.* $\times$ *fraseri*, respectively. The various physiological changes observed under moderate stress suggested that the decidual *V. opulus* was more sensitive to water stress, compared to the evergreen *P.* $\times$ *fraseri* [27].

The closure of the stomata and the reduction of gaseous exchanges imply a reduction in photosynthetic activity. Moreover, water use efficiency defines the relationship between photosynthesis and transpiration (P_n/E). According to the literature, an increase in WUE under water stress conditions is associated with an adaptation to deficit irrigation, while WUE reduction is associated with sensitive species [42–44]. However, plants with low WUE were more competitive in arid environments because they consumed more resources more rapidly thus suppressing competitors. On the other hand, plants with high WUE show a better performance in the absence of competition and regardless of water availability, probably because they had better water and nitrogen reserves [45]. The WUE can increase, decrease, or remain unchanged under water deficit conditions, depending on the genotype and the water stress level [46].

In *Callistemon citrinus*, the water deficit increased the ratio between photosynthesis and stomatal conductance (P_n/gs) [14]. Thus, photosynthesis increased as stomatal conductance decreased up to a stomatal conductance of approximately 100 mmol m^{-2} s^{-1}, whereas for stomatal conductance values less than 100 mmol m^{-2} s^{-1}, photosynthesis was rapidly reduced, suggesting that other parameters (biochemical limitations) may influence photosynthesis. The effect of water deficit on P_n/gs may vary based on many factors, such as the species, variety, and stress intensity [14]. For example, in *Callistemon* plants, photosynthesis remained at acceptable values when stomatal conductance had values between 100 and 200 mmol m^{-2} s^{-1}, which correspond to moderate water stress [14]. Moreover, the moderate water stress in *Callistemon* determined higher P_n/gs and root/shoot ratios, indicating the formation of small plants but of good quality with reduced losses of water and inflorescences similar to the control.

3.2. Chlorophyll Content and Photosynthesis

The physiological status of plants can be assessed via the integrity of the photosynthetic apparatus, and therefore the efficiency of the photosystems [25]. Adverse environmental conditions, such as water stress, can damage the photosystems [25]. For example, in Damask rose, the photosynthetic activity was reduced by 31% with moderate water stress (4.5 μmol CO_2 m^{-2} s^{-1}) and by 55% with severe water stress (2.9 μmol CO_2 m^{-2} s^{-1}), compared to the control (7.5 μmol CO_2 m^{-2} s^{-1}) [10]. An indirect measurement to evaluate this damage is the fluorescence of chlorophyll *a*. In particular, the values of this parameter increase when photosystem II does not work efficiently due to an imbalance between the number of electrons present in the photosystem and their use [47]. The F_v/F_m ratio records the maximum quantum yield of PSII reaction centers and it is used to measure the degree of plant stress [25] and an F_v/F_m ratio between 0.78–0.85 indicates the absence of stress [25]. Ornamental plants of the Mediterranean area, such as *Callistemon* [48], were considered tolerant to water stress since, during the treatment with different levels of deficit irrigation, they kept constant optimum values of F_v/F_m (0.8), showing that they have adopted particular strategies to dissipate the reducing power created during the stress

conditions [49]. The tolerance of the species is observed in practice with the recovery of plant when the stress is over or lessened [25]. In contrast, maximum quantum yield of PSII (F_v/F_m) and net photosynthesis were reduced in *Paeonia ostii* plants when subjected to water stress [30].

In other species such as *Althea rosea*, *Malva sylvestris*, *Rudbeckia hirta*, and *Callistephus chinensis*, water stress significantly affected chlorophyll *a* and *b* content in all four species, while total chlorophyll content was reduced by 16%, 18%, 31%, and 55% in *A. rosea*, *R. hirta*, *C. chinensis*, and *M. sylvestris*, respectively [26]. In *Nerium oleander* L. plants, chlorophyll *a* did not show a reduction after 15 days of stress but it was reduced by more than 50% after 30 days of stress. On the other hand, chlorophyll *b* increased in the first 15 days of stress and decreased similarly to chlorophyll *a* at prolonged stress conditions. In contrast, the carotenoids content was reduced even after 15 days of stress.

Oleander appears to be resistant to water stress because the symptoms related to plant growth, water loss, and reduction of chlorophyll *a* and *b* content are visible only after a month of stress [1]. The reduction of photosynthetic pigments in conditions of water deficit is also shown for *Antirhinum majus* cv. Butterfly [32], *Sedum* sp. L. [22], *Matthiola incana* L. [33], and *Paeonia ostii* [30], indicating sensitivity to water stress conditions.

In purple coneflower (*Echinacea purpurea* L.) plants subjected to water deficit conditions, the chlorophyll content was reduced by up to 37.3%, and that of carotenoids increased by up to 83%, compared to control plants. The increase in carotenoids attenuates the oxidative stress caused by deficit irrigation, as carotenoids prevent the production of singlet oxygen, thus mitigating the damage experienced by this radical [34].

In *Rhododendron delavayi*, the application of 9-days of water stress resulted in reduced photosynthetic activity and damage to chloroplasts, along with a reduction in stomatal conductance and transpiration [50]. Moreover, chloroplasts had an oval shape in control plants, whereas under stress, the chloroplasts became swollen. However, when plants were re-watered, the photosynthetic activity and other parameters were recovered, demonstrating a strong tolerance capacity of this species [50].

3.3. Oxidative Stress: ROS Production and Adaptive Responses

Water stress causes an excess of excitation energy due to the slowdown of photosynthetic activity. This energy causes the formation of oxygen free radicals or ROS in chloroplasts, mitochondria, and peroxisomes [25]. ROS include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), singlet oxygen ($^1O_2$), and ozone (O_3). These molecules are very reactive due to the presence of single electrons at their outer orbitals and may convert to other forms either spontaneously or enzymatically, e.g., O_3 decomposes into H_2O_2, O_2^- and $^1O_2$; O_2^- can be transformed into H_2O_2, and H_2O_2 can react with Fe^{2+} to form OH [25]. ROS are produced by plants not only under stress conditions since they are by-products of aerobic metabolism and are also used as signal molecules, while at normal conditions, their level is kept low by antioxidant enzymes activity [22]. Abiotic or biotic stress may raise the content of ROS, including water stress [25]. An excess of ROS indicates a condition of oxidative stress because, being radical, these molecules are very reactive and may damage or cause cell death [51]. Oxygen radicals affect membranes, proteins, and the genome, therefore cellular structures and metabolism are severely altered [52,53].

Various molecules can be used as an index of oxidative stress, such as H_2O_2 and MDA (malondialdehyde), and electrolyte leakage. H_2O_2 at low concentrations is a signal molecule for the development of tolerance to various biotic and abiotic stresses, while when its concentration increases, it may contribute to oxidative stress as it can oxidize the thiol groups of enzymes by inactivating them [7]. For example, a high increase in H_2O_2 and O_2^- with increasing water stress was observed in *Paeonia* section Moutan DC plants subjected to 12 days of water stress [30].

On the other hand, malondialdehyde (MDA) is a marker molecule of lipid peroxidation and it is formed by the oxidation of polyunsaturated fatty acids caused by ROS. In the

case of purple coneflower plants (*Echinacea purpurea* L.), water stress increased the MDA content by up to 75.8% compared to non-stressed plants, highlighting the important information that can be revealed regarding the susceptibility of various species to stressors [34]. Moreover, an increase in H_2O_2 and MDA was recorded and shown for *Adonis amurensis* and *A. pseudoamurensis* plants subjected to water stress [7]. In particular, in the case of water-stressed plants of *A. amurensis*, H_2O_2 increased from 2.07 µmol g^{-1} FW to a maximum of 4.56 µmol g^{-1} FW, while in *A. pseudomurensis*, the increase was greater and up to 9.13 µmol g^{-1} FW in the first 20 days of water stress and then decreased. Concerning MDA content, *A. pseudomurensis* contained higher amounts, demonstrating that it was more susceptible to water stress than *A. amurensis*. Similarly, Koźmińska et al. [22] examined the response to water stress in four species of *Sedum* L. and suggested that the MDA presence may confirm the sensitivity of the species to this stressor. In the same context, the lack of changes in MDA content detected in other species may indicate the presence of effective defense mechanisms against oxidative stress. Finally, electrolyte leakage is another index for stress evaluation which indicates membrane stability under stress conditions. Therefore, water deficit tolerant plants are expected to present low electrolyte leakage values [26].

However, plants have an "innate" defense mechanism which can either block the formation of ROS or block their oxidative activity when they are formed. This innate immunity refers to secondary metabolites and antioxidant enzymes that plants synthesize to protect themselves against stressors [51,54]. Among the detoxifying enzymes, the most commonly measured are superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione peroxidase (GPX), and ascorbate peroxidase (APX). Catalase is found in peroxisomes, while the rest of the enzymes are found in different organelles [55]. The quantity and presence of antioxidant molecules or enzymes can reveal the plant's response to stress.

For example, in *Adonis amurensis* plants, the CAT and POD enzymes reduce their activity within the first 10 days of stress initiation (2.08 and 521.15 U $g^{-1}min^{-1}$, respectively), while after 30 days of stress, both enzymes increase their activity (3.42 and 695.39 U $g^{-1}min^{-1}$, respectively) compared to the control at the same day (2.62 and 554.31 U $g^{-1}min^{-1}$, respectively) [7]. The POD enzyme also showed a similar trend in *A. pseudoamurensis*, examined by the same authors. In both species (*A. amurensis* and *A. pseudoamurensis*), SOD enzyme reached the maximum of its activity in 10 days after stress (7.76 × 106 and 7.02 × 106 U g^{-1} h^{-1} FW, respectively), and then it reduced as stress retained (2.49 × 106 and 4.12 × 106 U g^{-1} h^{-1} FW, respectively). Similarly, APX reaches its maximum activity at 30 days of stress in both species [7]. Moreover, in both species, H_2O_2 and MDA were detected at low concentration at the beginning of deficit irrigation implementation, probably due to the concomitant accumulation of antioxidant molecules and enzymes. Then, the concentration of H_2O_2 and MDA increased with the persistence of stress, a finding which indicates that in conditions of severe stress, both species were unable to reduce oxidative stress, despite the increase of antioxidant enzymes content, probably due to the disruption of the antioxidant defense mechanism [56].

In the case of *Nerium Oleander* L. [1], water stress induced a 6-fold increase in APX (ascorbate peroxidase) content compared to the control treatment after 15 days of stress and 4.5 times after 30 days of stress, while GR (glutathione reductase) increased its activity by 1.6 times after 30 days of stress. The activation of other antioxidant enzymes tested, such as SOD and CAT, was not observed in *Oleander* plants, indicating they were not involved in the plant defense mechanism.

An increase in all enzymes tested, namely (catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), and superoxide dismutase (SOD), especially the activity of CAT, was observed in Purple coneflower plants (*Echinacea purpurea* L.) subjected to water deficit [34]. Moreover, after 12 days of deficit irrigation application in *Paeonia ostii* plants, a significant increase in the activity of peroxidase (POD) and ascorbate peroxidase (APX) was observed [30]. On the other hand, the SOD enzyme activity was increased in the first four days of stress, and then it was reduced in the 8 following days compared to control plants.

Apart from enzymes, secondary metabolites are responsible for plant's tolerance to stressors. The main antioxidant secondary metabolites are tocopherol, ascorbate, glutathione, phenols, alkaloids, flavonoids, and proline [25,47,51,57–60]. Phenolic compounds, including flavonoids, were found to be increased in response to water stress, indicating their important role in the overall defense mechanism of plants [34,61,62].

In *Nerium Oleander* L., the total phenols content was slightly increased within the first 15 days of stress and further increased after 30 days of stress. Flavonoids behave as an inducible defense mechanism and their concentration increases only in conditions of severe stress (e.g., after 30 days of deficit irrigation) [1]. In PanAmerican and Cinderella, two cultivars of *Matthiola incana* L. subjected to water deficiency, the anthocyanin content increased from 0.92 to 1.31 (g FW) and from 0.90 to 1.44 (g FW), respectively, while the phenolic compounds content increased from 0.22 to 0.43 (mg GAE g^{-1} FW) and from 0.27 to 0.38 (mg GAE g^{-1} FW) [33]. Moreover, water stress increased the total phenols content by 17%, 29%, and 38% in plants of *C. chinensis*, *A. rosea*, and *R. hirta* respectively, compared to control plants [26], while an increase in the content of secondary metabolites such as chlorogenic acid, luteoloside, and 3,5-dicaffeoylquinic acid was also observed in the flowers of *Chrysanthemum morifolium* under water stress conditions [28]. Finally, the increase of phenols and flavonoids are an index of sensitivity to deficit irrigation in the case of *S. album* and *S. sediforme*, compared to more tolerant *S. spurium* and *S. ochroleucum* in which phenols or flavonoids are not formed after stress. However, phenolic compounds alone are not a safe index for stress tolerance and other molecules and enzymes have to be measured to evaluate plants response to water stress.

Ascorbic acid is another important antioxidant molecule which regulates the concentration of pro-oxidants, such as H_2O_2 and the closure of stomata and photosynthetic activity. Its action is reflected in leaf growth, flowering, and senescence [63,64]. The content of ascorbic acid, and other antioxidant compounds, such as phenols and flavonoids, is highly affected by various abiotic stressors, such as salinity, high temperature, and water stress [63]. For example, in *Conocarpus lancifolius* Engl., an ornamental species belonging to the *Combretaceae* family and considered as tolerant to semi-arid environments [63], the increase in phenols and flavonoids content in response to water stress was not accompanied by an equal increase of ascorbic acid content. According to the authors, this response to deficit irrigation is the result of a balance between various antioxidant molecules trying to cope with the oxidative stress, or of the faster synthesis of phenols and flavonoids compared to ascorbic acid. The same authors also suggested that phenols, such as caffeic acid and quercetin, have greater antioxidant power than ascorbic acid, hence the higher content detected [63].

3.4. Biochemical Changes

Water stress affects the osmotic balance due to changes in plant water status [65,66]. The main physiological responses of plants trying to adapt to the osmotic stress caused by deficit irrigation are the osmotic adjustment (OA) [3,67], or the accumulation of solutes in cells at levels that allow water uptake [31,68,69]. These solutes are proline, amino acids, glycine betaine, sugars [67,70,71]. However, the energy used and committed for the synthesis of these molecules cannot be used for growth and is called "fitness cost".

Proline has been found to accumulate in plants following numerous abiotic stressors [72,73]. In addition to its osmoprotective activity, proline is also an antioxidant and activator of antioxidant enzymes and is involved in the activation of genes activated by stress [74]. Its accumulation is considered an index of stress tolerance [26]. However, in some species, the higher proline content is associated with stress conditions rather than stress tolerance, meaning that plants with higher proline accumulation are considered sensitive to water stress [26]. This is confirmed by the negative correlation which is usually found between RWC and proline content [75]. In particular, water stress increased proline content by 363%, 115%, 103%, and 83%, in *M. sylvestris*, *C. chinensis*, *R. hirta*, and *A. rosea*, respectively, compared to control plants. However, the proline content was higher in

M. sylvestris and *C. Chinensis* which are considered sensitive to water stress, compared to the other two species (*R. hirta* and *A. rosea*) which are considered tolerant to water stress [75]. Moreover, in the Damask rose, the proline content increases from 14.5 mM (C) to 33.8 mM (50% FC), and 75.5 mM (25% FC), under water deficit [10]. An increase in proline content under severe water conditions (30 days of withholding water) was also found in *Adonis amurensis* and *Adonis pseudoamurensis* [7].

Osmolytes such as soluble sugars and proteins may increase at a certain level of stress and then reduce as stress progresses, denoting the fact that this mechanism is effective at first when the plant tries to defend itself and up to a point where stress interferes too much with plant physiological processes, seriously compromising the synthesis of soluble sugar and proteins. For example, under deficit irrigation conditions, *Oleander* plants accumulated much more sugar than proline and glycine betaine, which only slightly increased their concentration with stress (about 1.3 times, compared to the unstressed control treatment). It could be suggested that in *Oleander* plants, sugars assume a more important role as osmoregulatory compounds compared to proline and glycine betaine, thus demonstrating their importance in plant metabolism and in defense mechanism as well [1].

3.5. Hormonal Activity

Hormones hold a key position in plant defense mechanism against abiotic stresses [7]. Abscisic acid (ABA) plays an important role in resistance to water stress [76] since it regulates stomata closure to and reduces transpiration. Moreover, ABA is also involved in the increase of the antioxidant response of plants against ROS [77]. Some studies showed that adaptation of plants to arid environments is linked to the reduction in gibberellins (GA) and a concomitant increase in ABA content [78,79]. For example, an increase in ABA and a decrease in GA content was recorded in *Adonis amurensis* and *Adonis pseudoamurensis* plants subjected to deficit irrigation. Since GA is a growth-promoting hormone [80], its reduction may indicate a plant strategy of reducing water consumption needed for plant growth and biomass production, while increasing tolerance to stress at the same time.

Ethylene is also important in plants' response to stress and it has been found to induce leaves senescence under deficit irrigation conditions [25]. Moreover, in the work of Gadzinowska et al. [81], an attempt was made to study the biochemical mechanism which regulates the adaptation of sweet briar rose (*Rosa rubiginosa* L.) to arid lands, through analyzing auxin, cytokinin, and gibberellin synthesis. The authors reported that after 30 days of stress, a 3-fold increase (39 μg/g DW) in abscisic acid concentration was observed in stressed sweet briar seedlings compared to control plants (approximately 13 μg/g DW), demonstrating the significant role of abscisic acid in the species response to prolonged stress. Moreover, a series of gibberellins were detected, namely GA1, GA3, GA4, GA5, GA6, GA7, GA8, GA9, among which GA3, GA4, GA5, and GA6 increased with stress, especially GA3 which increased by 329.8% (3-fold compared to the control) [81]. On the other hand, GA9 content was reduced by 65.5% compared to the control. According to the authors, the tolerance of rose plants to water stress was due to the reduction of specific gibberellins (e.g., GA7, GA8, and GA9), since through gibberellins, deficiency plants may reduce their growth and use excessive energy towards the defense mechanisms against water stress, thus confirming the concept of "fitness cost" [81].

The same authors also showed that deficit irrigation resulted to the accumulation of specific auxins, such as indole-3-acetic acid (IAA), indole-3-acetic acid methyl ester (IAA-Met), indole-3-carboxylic acid (IAA-CarbA), indole-3-acetyl-l-aspartic acid (IAA-AsA), indole-3-acetyl-l-glutamic acid (IAA-GluA), and indole-3-butyric acid (IBA). In contrast, the content of other auxins, such as Oxo-IAA (oxindole-3-acetic acid), 4-Cl-IAA (4-chloroindole-3acetic acid), and 5-Cl-IAA (5-chloroindole-3-acetic acid), was reduced under water stress conditions [81]. The role of auxins against water stress consists in the increase of lateral roots and induction of stress genes which allow the synthesis of ABA and the modulation of antioxidant enzymes [82].

Concerning cytokinins, a varied response was observed and 8 cytokinins were increased, whereas 6 others were reduced. In particular, the cytokinin Kinetin riboside increased up to 136.2% compared to the control [81]. According to the authors, the reduction in cytokinins content due to an over-expression of the cytokinin oxidase/dehydrogenase (CKX) enzyme also resulted in reduced growth of roots and the entire plant, allowing the accumulation of bioactive molecules [81]. Besides, some cytokinins may activate transcription factors to increase tolerance to water stress through the stimulation of salicylic acid. Finally, the authors, after comparing the total amount of auxins, cytokinins, and gibberellins, highlighted that stress increased the total content of gibberellins at the expense of auxins and cytokinins [81]. This finding suggests that in the rose plants examined, the overall hormonal balance is more important for plants response to water stress than the changes in specific groups of hormones.

Table 2. Effect of water stress on physiological parameters of ornamentals plant.

Species	Plant Habit	Deficit Irrigation Treatment	Plant Growth Stage at the Beginning of Treatment	Modulation of Physiological Parameters by Water Stress	References
Sweet briar rose (*Rosa rubiginosa* L.)	Shrub	Reduced irrigation for 30 days: 11.2 L of water in control plants and 3.6 L in stressed plants (67.9% less). Plants did not receive water in the last three days of experiment	Seedlings, grown in a garden tunnel, into plastic boxes filled with Klasmann-Deilmann TS1 substrate and sand (*v/v*: 1:2) and irrigated with 11.2 L of water per box	ABA (+3-fold) Gibberellin (+/−). Auxine (+/−). Cytokinin (+/−).	[81]
Adonis amurensis and *Adonis pseudoamurensis*	Middle and lower part of the hillside grassland	C = 32% of soil moisture Stress: interruption of irrigation for 5, 10, 20, and 30 days	Seedlings grown in natural conditions, into polyethylene plastic pots filled with turf and sand, irrigated with water for 4 weeks before experiment begun	RLWC (−); H_2O_2 (+); MDA (+); Pro (+); Total phenols (+); flavonoids (+); CAT, POD, APX, SOD (+/−); ABA (+); GA (+/−)	[7]
Malva sylvestris, Althea rosea, Callistephus chinensis and *Rudbeckia hirta*	Herbaceous plants	C = 100% of ET_0 (local reference evapotranspiration) Stress: 25%, 50%, 75% of ET_0	1-month-old seedlings grown in the field and acclimated for one month before treatment begun	RLWC (−); Chl *a* and Chl *b* (−); Pro (+), Total phenolic compounds (+); EL (+)	[26]
Geranium macrorrhizum L. (Bevan variety from UK, and wild type from Hungary)	Cuttings from rhizome division	Interruption of irrigation for six weeks	Plants grown in greenhouse for 5 months and then a lath house for 7 months, into pots filled with 90% turf, 10% clay, irrigated manually with water	Water potential (Ψ) (+) Different amounts of water that the aerial parts (WSL) and roots (WSR) were able to store	[41]
Chrysanthemum morifolium Ramat. cv. Hj	Germplasm	35–40%, 65–70%, 95–100% of soil water holding capacity (WHC), for 62 days	Four-month-old seedlings grown in greenhouse, into plastic pots	Chlorogenic acid; luteoloside, and 3,5-dicaffeoylquinic acid (−)	[28]
Viburnum opulus L. and *Photinia* × *fraseri* 'Red robin'	Shrubs	C = 100% ET Moderate water deficit = 60% ET Severe water deficit = 30% ET, for 5 months	Plants grown in open air and grenhouse, into pots filled with peat, pumice, and osmocote.	Leaf water potential (−) gs (−); ET (−); P_n (−) WUE (+)	[27]
Sedum spurium, S. ochroleucum, S. album and *S. sediforme*	Herbs and sub-shrubs	C = irrigation twice a week Stress: interruption of irrigation per 4 weeks	Two-month-old seedlings grown in growth chamber, into pots filled with peat, perlite, and vermiculite, irrigated with nutrient solution	Chlorophyll *a, b* and carotenoids (−); MDA (+); Total phenols (+); Total flavonoids (+); Pro (+)	[22]

Table 2. *Cont.*

Species	Plant Habit	Deficit Irrigation Treatment	Plant Growth Stage at the Beginning of Treatment	Modulation of Physiological Parameters by Water Stress	References
Callistemon citrinus cv Firebrand' (*Crimson bottlebrush*)	Rooted cuttings of 2 year-old	C = 100% of container capacity Stress: moderate stress (50% of control) and severe stress (25% of control)	Two-year-old seedlings grown in greenhouse, into pots filled with coconut fiber, peat, and perlite, irrigated with water for three weeks before treatment begun	ET (−); RLWC (−); gs (−); WUE (+)	[14]
Rhododendron delavayi	Shrub	C = daily irrigation Stress = interruption of irrigation for 5 and 9 days	Five-year-old plants grown in greenhouse, into pots filled with peat and coconut coir	A (−); ROS (+); Damage to chloroplast ultrastructures	[50]
Matthiola incana L. (PanAmerican and Cinderella cultivar)	Seeds	C = 100% of field capacity Stress: 90%, 80%, 70% 60% of field capacity	Seedlings grown in greenhouse, into plastic pots filled with loam, decayed leaves, rotten manure, and river sand (50:25:12.5:12.5), irrigated with tap water, until plants reached the eighth true leaf	Chl (−); CAT (+); anthocyanin content (+); phenolic compounds (+) Pro (+)	[33]
Conocarpus lancifolius Engl. (Combretaceae)	Shrub	C = daily irrigation Stress = interruption of irrigation for 12 days	Shoots at the 13-15 leaf growth stage grown in greenhouse, into pots filled with sandy soil and peat, irrigated with distilled water	A (−); Electrons transport (−); Ascorbic acid (−); Flavonoids (+), Phenols (+)	[63]
Purple coneflower (*Echinacea purpurea* L.)		C = 100% of field capacity Stress = 20, 40, 60% of field capacity, until full flowering stage	Seedlings grown in a farm on soil, irrigated until four leaf stage	Chl *a* and *b* (−), Carotenoids (+); Pro (+); MDA (−) Enzymes antioxidant activity (+); Phenols (+); Flavonoids (+)	[34]
Paeonia ostii (*Paeonia* section Moutan DC)		C = plants watered daily Stress = interruption of irrigation for 4, 8, 12 days	3-year-old plants grown into pots and watered daily	H_2O_2 (+); O_2^- (+); RLWC (−); Pro (+); MDA (+); Chl (−); Carotenoids (−); POD, APX activity (+); SOD activity (+/−); F_v/F_m (−) A (−)	[30]

Evapotranspiration rate(ET) = mmol H_2O m^{-2} s^{-1}; gs = stomatal conductance (mmol m^{-2} s^{-1}); photosynthesis rate (A) (P_n, μmol m^{-2} s^{-1}); water use efficiency (WUE) (μmol CO_2/mmol H_2O); leaf water potential (Ψw) = MPa; leaf osmotic potential (Ψπ) = MPa; RLWC (relative leaf water content); malondialdehyde (MDA); electrolyte leakage (EL); abscisic acid (ABA); Chl = chlorophyll; (APX) ascorbate peroxidase; (SOD) superoxide oxidase; (POD) peroxidase; (CAT) catalase; (GR) glutathione reductase; glycine betaine (GB); total soluble sugars (TSS); proline (Pro); glycine betaine (GB); oxygen reactive species (ROS).

4. Stress Genes Involved in Plant Tolerance Mechanism against Water Stress

The first perception of water stress by plants is achieved through the root system. The plant responds to stress with physiological, biochemical, and molecular changes and this response depends on the activation of specific genes. Studies on *Arabidopsis thaliana* revealed the transcription products of these genes and identified transcription factors synthesized during the water stress response [25]. From these studies, it emerged that the intensity of stress activates specific genes involved in the response [83]. A target example of the response to water stress is the synthesis of dehydrin as well as the activation of ABA and ethylene pathways. Among the transcription factors involved in this response are ABRE, AREB, AREB/ABFs, DREB/CBF, ABF/AREB, NAC, WRKY, AP2, ethylene response elements [84], MYB2, and MYC2 [85].

Genes involved in the response to deficit irrigation also encode proteins, such as the late embryogenesis abundant (LEA) [86,87], and membrane proteins, such as aquaporins, i.e., the water channels [25].

Dendrobium catenatum is a species belonging to the *Orchidaceae* family, appreciated not only as an ornamental plant but also for its pharmacological properties [88]. The polysaccharides contained in the stems of the species possess anti-inflammatory and antioxidant properties. The content of these polysaccharides is very sensitive to the amount of light and water available to the plant. Huang et al. [88] performed a genetic analysis of superoxide dismutase (SOD) in *Dendrobium catenatum*. SOD enzymes could be found in different cellular compartments, and were distinguished according to the cofactor they were bound to, e.g., Cu, Fe, and Mn (Cu/ZNSOD, Fe/SOD, and Mn/SOD) [89]. Genetic screening led to the identification of 8 genes that code for the SOD enzyme, namely 4 genes for Cu/ZNSOD: *DcaCSD1*, *DcaCSD2*, *DcaCSD3*, *DcaCSD4*, with probable localization of the gene products being chloroplast and cytoplasm; 3 genes coding for FeSOD: *DcaFSD1*, *DcaFSD2*, *DcaFSD3*, with localization of the gene product being chloroplasts (excluding *DcaFSD3*); and 1 gene coding for MnSOD: *DcaMSD1*, which product was located in the mitochondrion (Table 3). Furthermore, *DcaCSD2*, *DcaCSD3*, *DcaCSD4*, and *DcaMSD1* genes were expressed more in flowers and leaves than in roots and stems. Through phylogenetic analysis, Huang et al. [88] also found that these genes were phylogenetically linked to gene sequences of *Arabidopsis*, *Oryza sativa*, *Phalaenopsis equestris*, and *Apostasia shenzhenica*. The authors then identified the gene regions in these genes involved in the synthesis of hormones (gibberellins, abscisic acid, salicylic acid), and the response to cold, light, and water stress, while they also revealed that all SOD genes were upregulated under severe deficit irrigation conditions [88]. *DcaCSD2* and DcaCSD1 genes were upregulated by up to 6 times and three times under water stress, respectively, compared to control [88]. Finally, the authors highlighted that *FeSOD* and *MnSOD* are usually found in algae and bryophytes, while *Cu/ZnSOD* is present only in higher plants, indicating that this form evolved later, and probably due to environmental stresses which became more complex over time [88].

Table 3. Water stress responsive genes.

Gene	Species	Cellular or Subcellular Localisation	Activity during Water Stress	References
DcaCSD1-2-3-4	*Dendrobium catenatum*	Chloroplast (DcaCSD1), citoplasm	Cu/ZnSOD synthesis	[88]
DcaFSD1-2-3	*Dendrobium catenatum*	Chloroplast	Fe/SOD synthesis	[88]
DcaMSD1	*Dendrobium catenatum*	Mitochondrion		[88]
HjCYC2c	*Chrysanthemum morifolium*	Young inflorescence	Adjusting of shape flowers of *Chrysanthemum morifolium*	[28]
FLS	*Chrysanthemum morifolium*	Young inflorescence	Adjusting of pathways of flavonoids during water stress	[28]
Lhca, *Lhcb* (18 genes), *Psa* (11 genes), *Psb* (15 genes) (all involved in photosynthetic apparatus synthesis), *F3H*, *DFR*, *ANS* (flavonoids biosynthesis) PP2C (abscisic acid synthesis), BAK1 and BRI1 (brassinosteroids synthesis)	*Rhododendron delavayi*	Leaves	Response to stimulus; Biosynthesis of secondary metabolites (flavonoids and brassinosteroids); Synthesis of photosystem I and II proteins, and electron transport chain proteins; Synthesis of ATP synthase	[50]
F3H, *CCOAOMT*, *CYP98A CAD*, *GLU*, *ZEP*, *NCED*, *CCD*, *TKL*, *RPI*, *FBP*, *KCS*, *ECH*, *PPT*, *LOX*, *CYP*, *ORP*	*Paeonia ostii*	Leaves	Increase of proline, flavonoids, stilbenoid, diarylheptanoid, and gingerol. Reduction of chlorophylls, carotenoids, phenylpropane and fatty acids.	[30]

In studies with *Rhododendron delavayi* plants, an evergreen ornamental species, subjected to deficit irrigation for 9 days, it was revealed through transcriptome sequencing analyses the expression of 22,728 differentially expressed genes (DEGs) [50]. DEGs encoding photosystem I and II proteins, electron transport chain proteins, and light-harvesting chlorophyll-protein complex (*Lhca*, *Lhcb*, *Psa*, *Psb* genes) were found to be downregulated in the presence of deficit irrigation treatment, whereas the same DEGs were upregulated in the absence of stress (control or re-watered plants), allowing the recovery of photosynthetic activity. Other DEGs involved in the antioxidant response system (synthesis of flavonoids, anthocyanins, and antioxidant enzymes SOD, CAT, POD, GSH, APX) and in the transduction of the hormonal signaling were also upregulated during stress (Table 3). According to the authors, the presence and expression of these genes allowed *Rhododendron delavayi* plants to protect their photosynthetic activity and to exhibit a strong tolerance to water stress [50]. In fact, *Rhododendron delavayi* was shown to have a high concentration of MDA, SOD activity, and proline, and soluble sugars content during stress, while the values of the same parameters were reduced with re-watering [50].

Zhao et al. [28] sequenced the *HjCYC2c* gene in *Chrysanthemum morifolium* Ramat. cv. Hj, which is downregulated in ray florets but upregulated in disc florets, after water stress. They also identified the *FLS* gene, which is involved in flavonoids biosynthesis and determines the symmetry of *Chrysanthemum* flowers. It was also observed that in the case of water stress, *FLS* was downregulated in ray florets and upregulated in disc florets. According to the authors, these two genes interacted with each other in both the synthesis of flavonoids and the regulation of flower symmetry in *Chrysanthemum morifolium* under water stress conditions [28]. Moreover, the gene expression analysis of water-stressed *Paeonia ostii* plants revealed 22,870 DEGs, of which 12,246 were up-regulated and 10,624

were downregulated. Those upregulated were mostly DEGs involved in the biosynthesis of proline, arginine, flavonoids and stilbenoids (*F3H*, *CCOAOMT*, *CYP98A*), where the down-regulated ones were mainly involved in the biosynthesis of pigments, phenylpropanoids, fatty acids, and in photosynthesis (*CAD*, *GLU*, *ZEP*, *NCED*, *CCD*, *TKL*, *RPI*, *FBP*, *KCS*, *ECH*, *PPT*, *LOX*, *CYP*, *ORP*) [30].

The response of sensitive and tolerant ornamental plants to water stress is shown in Figure 2, where in sensitive plants, morphological and physiological changes appear at low and middle levels of stress above which plants generally fail to survive, whereas in tolerant plants, morphological and physiological changes appear at levels between middle and high stress.
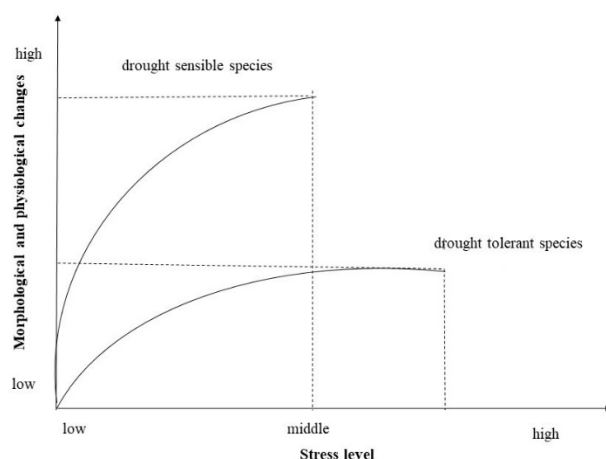

Figure 2. Graphic representation of morphological and physiological changes in drought-sensible and drought-tolerant ornamental plants as water stress level increases.

5. Agricultural Practices to Mitigate Water Stress in Ornamental Plants

A sustainable practice to cope with water stress, with wide spreading use in agriculture, is the application of biostimulants. They are substances of natural origin and microorganisms, such as fungi and bacteria, that are beneficial to plants. Recent studies have revealed that they can mitigate or eliminate the oxidative damage caused by biotic and abiotic stresses on vegetable crops. Furthermore, the use of biostimulants in agriculture can help reduce the excessive use of fertilizers and pesticides [90].

Mycorrhizal fungi have been found to increase the resistance of plants to water stress [25]. They absorb water through their hyphae, which they transfer to the plant. They can also regulate the stomatal opening through hormonal signals. Furthermore, they are involved in osmotic adjustment with greater accumulation of solutes, such as proline, in plants treated with mycorrhiza [25]. Besides, they improve the nutritional status of plants. In the work by Asrar et al. [32], *Glomus deserticola* (AMF) was used to inoculate seeds of *Antirhinum majus* cv. Butterfly. The seedlings were then subjected to various treatments, e.g., 80% (control treatment), 60%, 40%, and 20% of soil water content. The authors showed that *Glomus deserticola* increased tolerance to water stress in *A. majus* since mycorrhiza-treated plants showed increased leaf water potential and leaf water content, and reduced leaf electrolyte leakage, compared to non-mycorrhiza-treated stressed plants [32]. Furthermore, in the presence of fungi plants had a better growth and higher yield of flowers, a better nutritional status (in terms of macro elements content), and a greater accumulation of chlorophyll. The main effect of AMF seems to be the increased surface area, the improved architecture and the higher length of roots which allow the greater absorption of water from the soil. Furthermore, the lower proline accumulation in mycorrhiza-treated stressed plants indicated their higher tolerance compared to the non-mycorrhiza-treated stressed plants.

Another example of the beneficial effect of biostimulant was the better performance of *Petunia* spp., *Viola tricolor*, and *Cosmos* spp. plant grown under water deficit conditions which was achieved through the use of *Ascophyllum nodosum* extracts [91]. Biowaste soluble

hydrolysates also increased the photosynthetic activity and gas exchange of *Hibiscus* spp. subjected to water deficit [92]. According to some authors, the positive effects of the various biostimulants is the higher accumulation of biomass, the increased number of flowers, and finally, the production of hormones, such as gibberellins and cytokinins, which stimulate growth under stressful conditions [93].

Darvizheh et al. [34] showed that the exogenous application of salicylic acid and polyamine spermine in purple coneflower plants (*Echinacea purpurea* L.), an ornamental plant also known in medicine for the anti-inflammatory and antioxidant properties of its extracts, increased the antioxidant defense, the pigment contents (chlorophyll and carotenoids), plant biomass, flavonoid, and proline content, whereas it reduced MDA content when plants were subjected to water stress.

Another way to increase resistance to water stress is to expose plants to irrigation cycles and water stress. In these cases, plants responded with better recovery, meaning they adapted to water stress by modulating their physiology for survival [23]. In fact, the plants reduced gas exchanges to reduce transpiration while maintaining good photosynthetic activity. Moreover, light water stress is used to reduce plant growth in pots, as shown in *Cornus alba*, *Lonicera periclymenum*, and *Forsythia* × *intermedia* plants in the work by Davies et al. [94].

6. Conclusions

Concerning the response of plants to environmental stress, such as drought, the species or even the cultivars within the same species, are divided into sensitive and tolerant. Sensitive genotypes generally cannot sustain their growth under prolonged or severe stress. On the other hand, tolerant genotypes manage to survive severe or prolonged stress, but up to specific limits which vary among the species and varieties. From the different species analyzed in this review, it emerged that both sensitive and tolerant plants have an innate defense mechanism which includes morphological changes, such increase of leaf thickness, and the reduction of stomata density and plant growth, as well as physiological changes, such as the restoration of osmotic balance, the closure of stomata, and the synthesis of antioxidant molecules and enzymes. The response to water stress also includes hormonal activity, transcription factors, and the activation of specific genes. Therefore, in tolerant species, the stress response is greater than in sensitive plants, in terms of the amount of molecules produced and enzymes activity. The better understanding of the defense mechanisms of plants against water stress is of major importance in order to apply targeted practices that will increase tolerance and allow the survival of crops under unfavorable conditions. In this context, the use biostimulants is a novel and eco-sustainable agricultural practice which may ensure not only improved water use efficiency in both sensitive and tolerant ornamental plants, but also high yields under deficit irrigation. Another practical application could be the irrigation management according to species or variety specific requirements that could allow revegetation and landscaping even in regions with limit water resources. Therefore, future studies are needed in order to better understand the synergistic effects of biostimulants and the innate defense system of plants under stress, as well as to establish specific agronomic protocols that allow sustainable cropping of ornamental plants under stressful conditions. Finally, considering the species- or variety-dependent response of plants to stressors and to biostimulant products application, further studies are needed to identify those combinations that allow better crop performance under water limitations.

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