PhD THESIS

Evaluation of ecological and landscape features of ornamental plant genotypes and their response to abiotic stress factors

(SUMMARY OF Ph.D. THESIS)

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INTRODUCTION

As the world's population continues to grow and global urbanization increases, and the effects of climate change become increasingly visible, the ecosystems and landscapes of the future will be increasingly domesticated and projected or 'anthropized' (MIRCEA *et al.*, 2023a). Developing and maintaining sustainable landscapes is one of the most challenging and imperative challenges for scientists, practitioners and stakeholders in improving the landscape and ensuring sustainability in resource management. To comply with these quantified objectives, landscape ecology and landscape design should and must play a key role (MIRCEA *et al.*, 2023 a, b).

Abiotic stressors include a range of adverse environmental conditions, including drought, salinity and temperature extremes, all of which can negatively impact plant growth and survival (MIRCEA *et al.*, 2023 c). Among the limiting factors acting adversely on plants, water stress, characterised by inadequate water availability, is a milestone because it occurs on a large scale in many parts of the globe, limiting the distribution, landscape potential or impact of ornamental plants in landscaping (MIRCEA *et al.*, 2023 a, c).

Ornamental plants, as key elements in landscape design, need to behave properly to abiotic stressors, especially in regions prone to water scarcity or irregular rainfall (MIRCEA *et al.*, 2023 c, d). While many ornamental plants are native to the regions where they are grown, global horticultural trade facilitates the spread of non-native species into new geographic regions. This phenomenon has recently led to increased scientific debate or controversy about the invasive risk posed by some ornamental plants (MIRCEA *et al.*, 2023 c). Invasive ornamental plants have the potential to disrupt fragile ecosystems, leading to the decline or extinction of native species. By elucidating their response to abiotic stress, their ecological impact can be better predicted and managed, contributing to biodiversity conservation (MIRCEA *et al.*, 2023 b, d). Research on the response of invasive ornamental plants to abiotic stress is of great interest, while water stress is a key factor that can influence the spread in cultivated and spontaneous flora of plants that may have decorative features, but at the same time are difficult to control weeds that can alter the structure of plant associations or the balance of ecosystems.

Understanding how ornamental plants can counteract the effects of water stress and develop resistance or tolerance mechanisms to water scarcity is crucial for predicting their behavior in novel ecosystems and for making objective choices in landscape architecture regarding management and control strategies, helping to mitigate economic and ecological costs in landscape beautification, and the use of sustainable resources (MIRCEA *et al.*, 2023 a, b, c, d).

STRUCTURE OF THE THESIS

The PhD thesis entitled "Evaluation of ecological and landscape features of ornamental plant genotypes and their response to abiotic stressors" is structured in two main parts, totalling 156 pages and contains 8 chapters, 15 tables, 47 figures and 399 references.

The first part of the thesis consists of a bibliographical survey of the research upon the issues addressed in the thesis and is structured in three main chapters. **Chapter 1** summarises the information on the main abiotic stressors acting on ornamental plants in cultivated and wild flora. **Chapter 2** covers the issues related to the defence mechanisms of plants to abiotic stress, namely the protective layer of the epidermis represented by the cuticle, as well as different chemicals such as unsaturated fatty acids and osmoprotectants. **Chapter 3** shows the aspects on the variability of ornamental plant response to different abiotic stress factors, saline, thermal and water stress respectively, the latter with emphasis on invasive ornamental plants.

The second part of the PhD thesis is structured in six chapters that present the personal contribution of the author, through the results of the research and their significance. The results of the research were published in four articles in peer-reviewed (ISI) journals with impact factor (IF), two in Q1 and two in Q2 journals. **Chapter 4** presents the aim and objectives pursued in the research. The research aimed to obtain information of interest on different ornamental genotypes of ecological, landscape, economic and social importance, resistant or tolerant to water stress, suitable for landscaping. The research also looked at their invasive potential, considering the risk of spreading these species in different environments.

In accordance with the proposed aim, the main objectives of the research were the following: to test the behaviour of some ornamental floral species of ecological, landscape, economic and social importance or potential to the action of water stress factors; to evaluate the response of plants under osmotic stress conditions during seed germination and water deficit during vegetative growth and to analyse possible mechanisms of plant tolerance to water stress; assessment of the invasive potential of some plants and the possibilities of extending their range; identification of biochemical markers that can be used to characterise plant response mechanisms to drought; identification of water stress resistant or tolerant genotypes that can be recommended for landscaping.

Chapter 5 presents the materials and methods used to evaluate the ecological and landscape characteristics of ornamental plant genotypes and their response to abiotic stress factors, using specific methodologies, seed germination tests, water stress testing, biochemical parameter analysis, etc.

RESULTS AND DISCUSSIONS

Chapter 6 presents the results of the PhD research in the form of scientific articles published in peer-reviewed journals. The chapter comprises four sub-chapters, each of which is represented by a research problem carried out according to the proposed objectives and valorised in the form of an article published in an ISI journal.

The first theme addressed was the assessment of drought tolerance of some ornamental species of the Asteraceae family and the possibilities of using multivariate analysis for this purpose. The morphological characteristics of plants and their vegetative growth were examined, as well as the main biochemical markers involved in plant resistance or tolerance to stress. Among the six ornamental species, the most drought tolerant were *A. houstonianum*, *G. carinata* and *L. vulgare*, and the most sensitive were *C. officinalis*, *C. chinensis* and *X. bracteatum*. The group of more tolerant species also included two species reported as invasive outside their native range (*A. houstonianum* and L. *vulgare*). The study highlighted the usefulness of multivariate analysis methods, namely the principal component analysis (PCA) and the canonical analysis of variation (CVA), to identify variation in morphological parameters involved in biomass accumulation, as well as biochemical ones in revealing drought tolerance. Plants accumulate proline as a general response to abiotic stressors, but proline involvement in water stress tolerance mechanisms is highly variable among species. A strong positive correlation was identified between proline content and photosynthetic pigments, confirming the active role of this compound in osmoregulation processes and in the elimination of oxidative stress on the photosynthetic system. The research revealed that potentially invasive species are more tolerant to water stress, except for *L. vulgare*, which was more severely affected by one month of water supply suppression. Invasive species *A. houstonianum* and *G. carinata* tolerated severe water stress better, the result supporting the hypothesis that phenotypic plasticity of invasive species may play a significant role in the potential of these plants to invade new habitats.

The results obtained in this research were published in an ISI journal (WoS): **MIRCEA, D. M.,** CALONE, R., SHAKYA, R., SAAVEDRA, M. F., SESTRAS, R. E., BOSCAIU, M., SESTRAS, A. F., VICENTE, O. 2023, Use of Multivariate Analysis in Screening for Drought Tolerance in Ornamental Asteraceae Species, *Agronomy 13(3):687.* [IF 3.949, Q1]. https://doi.org/10.3390/agronomy13030687

The second research theme focused on the effect of water deficit on seed germination, plant growth and biochemical response under water deficit conditions in four potentially invasive ornamental species of the Poaceae family (*Cymbopogon citratus, Cortaderia selloana, Pennisetum alopecuroides, P. setaceum*). Stress-induced changes in different biochemical markers (photosynthetic pigments, osmolytes, antioxidant compounds, Na^+ and K^+ content of root and shoot), showed different responses depending on the species and stress treatments. The results indicated that drought tolerance appears to depend largely on the active transport of Na^+ and K^+

cations to the aerial part of the plants, contributing to osmotic adjustment. There were no significant changes in Na^+ or K^+ concentrations in roots or shoots when monovalent cations were analysed, except for an increase in K^+ concentration in *P. alopecuroides* roots, which ensures a good plant response to drought. The study confirmed that under stress conditions, plants redirect resources from biomass accumulation to activation of defence mechanisms and osmotic adjustment by accumulation of osmolytes, such as proline and total soluble sugars. The results explain the relative geographical and environmental distribution of the species studied. The study indicated that special attention should be paid to *P. alopecuroides* species, which shows a high invasive potential that is not generally recognised.

The results obtained in this research were published in an ISI journal (WoS): **MIRCEA, D. M.,** ESTRELLES, E., AL HASSAN, M., SORIANO, P., SESTRAS, R. E., BOSCAIU, M., SESTRAS, A. F., VICENTE, O. 2023, Effect of Water Deficit on Germination, Growth and Biochemical Responses of Four Potentially Invasive Ornamental Grass Species, *Plants 12(6):1260.* [IF 4.658, Q1]. https://doi.org/10.3390/plants12061260

The third theme focused on the assessment of drought response by morphological and biochemical markers in two *Tropaeolum* species used in landscaping. Under stress conditions, proline, an essential factor in osmotic control, was a good indicator of stress as its concentration increases during osmotic stress. *T. minus*, less drought tolerant than *T. majus*, which showed a higher proline content. MDA was found to be negatively correlated with substrate moisture and plant growth factors. H_2O_2 concentrations did not change in *T. majus* and even decreased in *T. minus*, but the resulting levels were influenced by the amount of photosynthetic pigments, flavonoids and phenols, as well as leaf water content, in both species. Growth and metabolic indicators showed that *T. majus* and *T. minus* plants responded similarly to water deficit, but the less losses of fresh and dry biomass in *T. majus* revealed a better resistance, or at least tolerance, to severe water stress. The study showed a strong relationship between patterns of variation in flavonoid and chlorophyll content obtained by sensor-based (Dualex optical sensor determinations in the greenhouse) and spectrophotometric (spectrophotometric measurements in the laboratory) methods.

The results obtained in this research were published in an ISI journal (WoS): **MIRCEA, D. M.,** CALONE, R., SHAKYA, R., ZUZUNAGA-ROSAS, J., SESTRAS, R. E., BOSCAIU, M., SESTRAS, A. F., VICENTE, O. 2023, Evaluation of Drought Responses in Two *Tropaeolum* Species Used in Landscaping through Morphological and Biochemical Markers, *Life 13(4):960.* [IF 3.253, Q2]. https://doi.org/10.3390/life13040960

The fourth theme was dedicated to the evaluation of water stress response during seed germination and vegetative growth in six ornamental species (*Bidens pilosa, Centaurea cyanus, Echinacea purpurea, Limonium sinuatum, Lobularia maritima, Oenothera biennis*). In the absence of stress, the most competitive species during germination were *B. pilosa*, *O. biennis* and *C. cyanus*, the latter in terms of germination speed. However, in the presence of PEG 6000, *C. cyanus* had the highest ability to withstand osmotic stress of all species examined, followed by *L. sinuatum*. Germination in *Oenothera biennis* was competitive up to -0.75 MPa, but germination in *B. pilosa* was

strongly reduced and MGT increased at this concentration. *B. pilosa* and *C. cyanus* were noted for longer hypocotyl and radicle growth, as well as better overall seedling vigour index. *Echinacea purpurea*, the least prone to invasion hazard of the species studied, had the highest MGT (mean germination time) under control circumstances and took the longest time to complete germination.

Regarding the impact of drought on plant vegetative development, all species tolerated irrigation with half the amount of water used in the control (intermediate water stress). In the absence of irrigation (severe water stress), *L. maritima* and *L. sinuatum* stood out as the species that most successfully mitigated the damaging effects of drought stress in the severe water stress treatment, while *O. biennis* was the most sensitive. According to the study, significant invasiveness characteristics were those related to germination, especially in the absence of stress. Only *L. sinuatum*, a typical halophyte, performed well under water stress in both growth phases, while *L. maritima*, another halophyte, is stress resistant during vegetative growth but has poor germination under osmotic stress. The results indicated that *O. biennis* is susceptible during growth of young plants, to severe water stress, suggesting that its presence as an invasive is more problematic in temperate and wetter regions than in the Mediterranean Sea area. Multivariate analysis separated this species from all others due to higher production of sugars, flavonoids and phenols, even in the absence of stress, but also at the lowest proline concentration. On the contrary, the highest proline concentrations were recorded in plants subjected to water stress in the most tolerant species, *L. sinuatum* and *L. maritima*, demonstrating the importance of this amino acid in drought tolerance.

The results obtained in this research were published in an ISI journal (WoS): **MIRCEA, D. M.,** CALONE, R., ESTRELLES, E., SORIANO, P., SESTRAS, R. E., BOSCAIU, M., SESTRAS, A. F., VICENTE, O. 2023, Responses of different invasive and non-invasive ornamental plants to water stress during seed germination and vegetative growth, *Scientific Reports 13(1):13281* [IF 4.997, Q2]. https://doi.org/10.1038/s41598-023-40517-7

CONCLUSIONS

Chapter 7 is assigned to conclusions based on the results obtained.

The use of ornamental plants in landscaping can ensure not only a beautiful aesthetic environment and a pleasant atmosphere in the created outdoor space, but also an environment that is environmentally friendly, recreational and beneficial to physical and mental health. In the effective use of ornamental plants in landscape design, both the careful planning of the space and the desired design, the ecological requirements of the plants, and the possibility of using genotypes with a suitable response to abiotic and biotic stress factors should be considered, so that the proposed project meets the current requirements for resource sustainability. Among abiotic stressors, drought is one of the most important challenges facing ornamental species. As water resources will be increasingly limited due to global warming, selection and diversification of water stress tolerant genotypes become priorities of contemporary ornamental horticulture.

The identification and use of ornamental plants with an appropriate response to water stress is an important condition for the success and sustainability of any landscape design. The use of genotypes that are resistant or tolerant to stress factors, particularly drought, water scarcity, or the desire to save water, becomes the most effective means for the success of future projects.

The research carried out during the PhD has identified species with a suitable response to water stress, which can be recommended for use in the creation of green spaces of different types, where the maintenance needs of the plants are less costly and maintaining them in a healthy state and with favourable visual impact is easier. The proposed solutions respect the environment, promote the use of native or organic genotypes that can support local biodiversity and reduce maintenance needs, and ensure the saving of important drinking water resources.

RECOMMENDATIONS

The research carried out in the PhD thesis provides useful information for horticultural and landscape practice as well as for plant breeding programmes. Species noted for their favourable water stress response characteristics can be used and promoted in the sustainable design and landscaping of green spaces. As climate change continues to have an impact on landscapes, the research in this thesis offers a forward-looking perspective and aims to strike a balance between the aesthetic appeal of ornamental plants and the ecological integrity of landscapes, creating a harmonious path between nature and design.

Promoting and raising awareness of the benefits of ornamental plants resistant to abiotic stress caused by water scarcity can contribute to good education and environmental awareness among students, landscape gardeners, horticulturists and the general public. At the same time, it can have beneficial effects and positive

consequences in terms of future action to protect and enhance the environment and the sustainable use of natural resources, including drinking water. Identifying the invasive potential of certain plants of landscape interest and knowing the possible risks they pose can help prevent situations that affect the balance of ecosystems.

Good collaboration between experts in landscape design, horticulture, ecology, botany, plant and environmental protection, etc. and a multidisciplinary approach to the sustainable design of green spaces under drought conditions and in the context of global climate change is the key to ensuring environmental beautification and resource sustainability in landscape design.

INNOVATIVE CONTRIBUTIONS OF THE THESIS

The research carried out in the present doctoral research was conducted in a complex, interdisciplinary framework, in which the subject of assessing the ecological and landscape characteristics of ornamental plant genotypes and their response to abiotic stress factors included issues from different fields of study related to horticulture, namely agronomy, environmental science (ecology and environmental protection), biotechnology, biology, etc.

Comprehensive evaluation of ecologically, landscape and economically important plants of ornamental interest provided relevant information on their response to water stress. Through tests analysing both seed germination and plant growth under simulated drought conditions, species with morphological, physiological and biochemical characteristics have been identified that provide them with a remarkable capacity to adapt to water scarcity or environmental conditions with limited water resources.

In addition to assessing the response to abiotic stress and the mechanisms involved in plant resistance or tolerance to environmental stressors, research has also addressed the invasive potential of some species with decorative characteristics. Plant growth variables and biochemical markers revealed significant species differences in response to water stress.

Analysis of the response to water stress revealed that some ornamental species may show adaptability to water stress, and variations in proline and malondialdehyde concentrations are reliable biochemical indicators of water stress effects. The study also revealed the close relationship between patterns of variation in flavonoid and chlorophyll content obtained by sensor-based (direct) and spectrophotometric (laboratory) methods.

Original results were also obtained on plant characteristics associated with invasiveness, including those related to seed germination. Although cultivated in some areas as ornamental or medicinal plants, some species are also 'agricultural' and environmental weeds classified with a high-risk score in the Global Compendium of Weeds. The models used can be particularly useful in identifying and reporting those that may pose a risk to cultivated and wild flora because of their invasive nature.

As climate change continues to have an impact on landscapes, the research summarised in this thesis offers a valuable future perspective seeking to balance the aesthetic appeal of ornamental plants with the ecological integrity of landscapes, creating a harmonious path between nature and design.

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