Water Economy via Oriented Root Elongation of Mediterranean Plants: Physiological Parameters

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Abstract—Plant species grown in the Mediterranean region have been selected for an experimental study regarding plant ability to endure harsh, abiotic conditions such as water deficiency. Young plants of Ceratonia siliqua L., Myrtus communis L., Nerium oleander L., Rosmarinus officinalis L. and Pittosporum tobira (Thunb.) were placed in elongated plastic tubes and rhizotrons, which allow non-destructive observations of plant material, and were grown under controlled light and temperature conditions, with varying irrigation patterns in a growth chamber, for a 30-day experimental period. The results show that Ceratonia siliqua, Myrtus communis and Nerium oleander are more capable of enduring drought conditions, in comparison to Rosmarinus officinalis L. and Pittosporum tobira (Thunb.). The results can be used for planning green urban landscapes with introduction of naturalistic planting, using plant species adapted to the scarcity of water during prolonged, ambient drought conditions.

Index Terms—Drought, Mediterranean, Roots, Urban landscape, Water stress

I. INTRODUCTION

The climate of the Mediterranean ecosystems is characterized by hot, arid summers and wet winters [1]. The common life forms in the Mediterranean region are evergreen trees and shrubs that tolerate the dry period [2-3]. Generally, Mediterranean-type ecosystems cover the majority of the territory of Greece [4]; however, urban landscape is affected by human activities and therefore consists of a variety of native, introduced and alien plant species, including many ornamental species.

Drought is a severe abiotic factor that causes stress to plants [5] and affects plant growth and distribution in wild and cultivated lands [6]. In arid and semiarid habitats, water availability, soil nutrient scarcity, elevated temperature and excess of solar radiation are the main constraints on plants’ survival [7]. Under natural conditions, repeated, episodic drought events might be severe and durable, which affect plants’ acclimation [8]. In the Mediterranean region, problems associated with water scarcity continuously enhance; therefore, initiatives towards solutions including water management are of high priority.

Plants’ responses to drought stress tolerance include biochemical, molecular, morphological and physiological mechanisms [9-11]. When the natural conditions are stressful, the vegetative growth, life cycle and reproduction of plants may be inhibited [12]. The root system is very important for plants to overcome water stress; for example, plants living in arid and semi-arid environments achieve access to water regimes found in deep soil layers by elongating their roots [11], [13]. Although different root lengths exist among plant growth forms, there is plasticity in root response to habitat conditions, depending on the plants species [14]. Also, the soil characteristics affect the root development [15]. Deep roots are important not only for water uptake, but also for the rhizosphere and the soil micro-environment [16]. Plant species have developed different acclimation strategies of water uptake from either deep or shallow water horizons by balancing between (root) growth cost and access to water horizons in the soil [17].

In plants adapted to adverse conditions effective response mechanisms have been developed [6]. Such responses encompass the closure of stomata and the production of metabolites (sugars, amino acids, secondary metabolites etc.) that enable plants to maintain their functions under water scarcity [18-19]. Increased accumulation of compatible solutes—such as soluble sugars and proline—in plant tissues is a strategy of withholding water from dry environmental conditions [20]. Soluble substances are involved in turgor maintenance and membrane stabilization of plant tissues [21]. Accumulated osmolytes contribute to osmotic adjustment and ion homeostasis [22]; in addition, they can stabilize proteins and membrane structures [6], [23].

Proline is usually accumulated in plant tissues in response to water deficit. There are several studies that affirm the correlation of proline levels in plant tissues with water stress [24-27]. Drought regulates proline biosynthesis genes with induction of proline synthesis from glutamate [23]. In some plants there is developmental control of proline accumulation in different organs [28-29]. Also, proline may act as nitrogen storage in stressed plants that concomitantly slow their growth rate [30-31].

The cells’ membranes are destabilized when exposed to prolonged water stress periods e.g. changes in sugar content may cause membrane dysfunction and subsequently cell death [32]. Also, during periods of water stress the chloroplasts’ membranes destabilization can be avoided by the presence of sugars [12], [33]. Sugars contribute to the cells’ osmotic adjustment by decreasing the osmotic potential
and thus promoting the movement of water into the cells, in water deficient ecosystems [12]. The increase of soluble sugars concentration can be deployed as a metabolic signal in response to stress [31]. In fact, many Mediterranean species exhibit non-rapid growth rates [34]. Plant nutrient uptake and photosynthesis may be reduced and/or inhibited, during the dry period [10], [35]; also, chlorophyll content is affected by drought and can be used as physiological trait of plants’ status [36-37].

The study and knowledge of roots’ depth and functionality is important to understand water-energy-carbon exchange, plant productivity and plant acclimation to environmental conditions [14]. Techniques that enable researchers to study root functionality without intervention include tubes and rhizotrons. Rhizotrons can be considered modified human-induced forms that involve in situ root observation; these are non-destructive methods that allow periodical observations of the roots, sampling and visualization of their growth [17].

*Ceratonia siliqua* L. (carob tree), *Myrtus communis* L. (myrtle) and *Nerium oleander* L. (oleander) are evergreen species native to the Mediterranean region, *Rosmarinus officinalis* L. (rosemary) is an aromatic shrub, whereas *Pittosporum tobira* (Thunb.) (Japanese pittosporum) is a native species to the Far East that has been introduced to the Mediterranean region and is often encountered in urban spaces [34], [38].

The aim of the present study is to evaluate and understand different plant species’ response to water stress by comparing physiological parameters.

II. MATERIALS AND METHODS

A. PLANT MATERIAL

Young plants of *Ceratonia siliqua* L., *Myrtus communis* L., *Nerium oleander* L., *Rosmarinus officinalis* L. and *Pittosporum tobira* (Thunb.) were placed in plastic tubes (10 cm in diameter and 100 cm in length) and rhizotrons (3 cm thick x 30 cm wide x 50 cm long) (Fig. 1). The plastic tubes were treated as described by Sharp and Davies (1985) [39], i.e. they were cut along their length, while maintaining their volume and soil columns. This arrangement allows monitoring of root development and water status, as well as sampling of segments throughout the study [40]. In addition, the rhizotrons allow non-intervened observations of root growth. Tubes and rhizotrons with the studied species were kept in a growth chamber, at 25±1 °C, 50% relative humidity and 16 h photoperiod (500 μmol m$^{-2}$ s$^{-1}$ PAR). Plants inserted in the tubes and the rhizotrons remained under well watering conditions for a week. Then, they were exposed to environmental conditions simulating the Mediterranean summer, water deficit conditions. The tubes were carefully opened and samples of roots and shoots were collected at five-day intervals, during the experimental period. Tissue samples were oven-dried at 50 °C for 72 h, weighted, and ground for the subsequent analyses using a Thomas Wiley Model 4 Mill (Thomas Scientific, Swedesboro, NJ, USA).

B. CHLOROPHYLL

The total chlorophyll (Chl) content was spectrophotometrically determined in leaf samples according to a modified acetone method [41]. Chlorophyll was extracted from 0.1 g dried and grinded samples, homogenized with 10 mL acetone (80% v/v) and filtered through Whatman #2 filter paper to become fully transparent [42]. The chlorophyll content was measured in aliquots of the tissue extracts using a Novaspec II (Pharmacia Biotech, Cambridge, England) spectrophotometer; absorbance readings of five replicates have been used for the calculations. Chlorophyll content is expressed as μg g$^{-1}$ of dry weight of the tissue.

C. PROLINE

The concentration of proline was determined spectrophotometrically according to the method described by Bates et al. (1973) [43]. Dried tissue samples were crushed into fine powder and homogenized with aqueous sulphosalicylic acid (20 mL, 3% w/v), and the homogenate filtered through Whatman #2 filter paper. Two mL of the filtrate reacted with acid-ninhydrin solution (2 mL) and glacial acetic acid (2 mL) in triplicate test tubes, which were heated at 100 °C for 1 h in a water bath and the reaction terminated in an ice bath. After cooling, the reaction mixture was extracted with 4 mL toluene, homogenized in a vortex. The chromophore containing the toluene was aspirated from the aqueous phase and the absorbance at 520 nm was measured using toluene as a blank sample. Values of proline content are expressed as μmol g$^{-1}$ of dry weight; L-proline (Serva, Heidelberg, Germany) solutions were used for the standard curve.

D. SOLUBLE SUGARS

The concentration of sugars was determined spectrophotometrically according to the sulfuric acid method described by Albalasmeh et al. (2013) [44]; dried and grinded tissues (0.1 g) were homogenized in 20 mL of double distilled water. 1 mL of sample solution was mixed with 3 mL of sulfuric acid in a test tube and agitated for 30 s. Then, the solution was cooled in ice for 2 min to bring it to room temperature. Finally, light absorption was read using UV-vis spectrophotometer at 315 nm. The results are expressed as mg g$^{-1}$ of dry weight of the tissue.
E. Statistical analysis

The results are presented in charts as mean values ± Standard Error (S.E.). In order to determine the differences in ecophysiological response of the studied species during the experimental conditions, a one-way analysis of variance (ANOVA) was performed at \( p \leq 0.05 \) and the Tukey test was applied to compare the means. All statistical tests were performed using the SPSS statistical v. 23.0 (SPSS Inc., Chicago, IL, USA).

III. RESULTS AND DISCUSSION

A. Chlorophyll

The measurements of leaf chlorophyll content are shown in Fig. 2. The chlorophyll content declined in the considered species during the water stress period. *C. siliqua* and *M. communis* present rather stable values, whereas in *R. officinalis* fluctuations have been observed during the experiment. By the end of the water stress treatment some *P. tobira* and *R. officinalis* plants exhibited yellow-colored leaves- i.e. a phenotypic result indicating loss of chlorophyll. Under severe water stress conditions pigment depletion might lead to cell membrane destruction [37]. Under natural conditions, *N. oleander* is capable of photosynthetic acclimation, providing a stable photosynthetic mechanism during the dry period [35]. In the current study, unwatered *N. oleander* plants showed decreased chlorophyll content; however, along with *C. siliqua* and *M. communis*, they showed smaller chlorophyll losses compared to *R. officinalis* and *P. tobira*.

B. Proline

The results of root proline content in the considered plant species are presented in Fig. 3. *C. siliqua*, *M. communis* and *N. oleander* show a stable increase in root proline content, whereas *P. tobira* and *R. officinalis* present a more abrupt increase in proline content after 15 days of treatment; by the end of the study (30 days), proline content was substantially increased in most of the considered species, especially *R. officinalis* and *P. tobira*. Proline content is useful as an indicator of drought stressed plants [25], [45-46] and it affects stress tolerance of plants in multiple ways [23]. The results indicate that *P. tobira* and *R. officinalis* undergo more stress than *C. siliqua*, *M. communis* and *N. oleander*.

C. Soluble sugars

Both shoot (Fig. 4) and root (Fig. 5) sugar content increases in the studied species during the water withholding treatment. Shoot measurements appear higher than those of the root samples; in considering both tissues, *P. tobira* shows the highest sugar content, followed by the shoots of *R. officinalis* and the roots of *M. communis*. Other studies have shown that the highest amounts of shoot soluble sugars were found in species with shallow root system [47]. Deep rooted plants like *C. siliqua* possess survival tactics under water-withholding conditions [48].
IV. CONCLUSION

Abiotic stress affects plant growth and productivity; plant metabolism is perturbed and the network needs to be reconfigured [19]. It is noteworthy to mention that tissues’ water potential of plants placed in pots and exposed to controlled watering conditions, might change faster than those of plant exposed to the same conditions in the field [49]. Knowledge of plant species that use effective mechanisms to cope with water scarcity will help us to develop green urban landscapes irrigated with declining water consumption. Ecophysiological characteristics of native and cultivated plants that grow under the Mediterranean climatic conditions indicate resistance to drought stress [34], [50]. It seems likely that drought-tolerant species should be taken into consideration for planting areas with inadequate irrigation conditions.

REFERENCES

[1] H. Aschmann, “Distribution and peculiarity of Mediterranean ecosystems”, in Mediterranean type ecosystems, F. di Castri, H. A. Mooney, Eds. Springer Berlin Heidelberg, 1973, pp. 11–19.

[2] H. A. Mooney and E. L. Dunn, “Convergent evolution of Mediterranean- climate evergreen sclerophyll shrubs”, Evolution, 24(2), 1970, pp. 292–303.

[3] S. Rhizopoulou and K. Mitراسkos, “Water relations of evergreen sclerophylls. I. Seasonal changes in the water relations of eleven species from the same environment”, Annals of Botany, 65(2), 1990, pp. 171–178.

[4] K. Georgiou and P. D. Hare, “Patterns and traits of the endemic plants of Greece”, Botanical Journal of the Linnean Society, 162(2), 2010, pp. 130–422.

[5] E. A. Bray, “Responses to abiotic stresses”, in Biochemistry and Molecular Biology of Plants, 2nd ed., B. Buchanan, W. Grussem and R. L. Jones, Eds. Wiley Blackwell, 2000, pp.1158–1203.

[6] O. Vicente, M. Al Hassan, and M. Boscau, “Contribution of osmolyte accumulation to abiotic stress tolerance in wild plants adapted to different stressful environments”, in Osmolytes and Plants Acclimation to Changing Environment: Emerging Omics Technologies, N. Iqbal, R. Nazar and N. A. Khan, Eds. Springer India, 2016, pp. 13–25.

[7] F. Valladare, “Licht heterogeneity and plants: from ecophysiology to species coexistence and biodiversity”, in Progress in Botany, K. Esser, U. Lu¨nger, W. Beyerlach, F. Hellwig, Eds. Springer Berlin Heidelberg, 2003, pp. 439–471.

[8] P. E. Menez-Silva, L. M. Sanglard, R. T. Avila, L. E. Morais, S. C. Martins, P. Nobres, C. M. Patreze, M. A. Ferreira, W. A. Araujo, A. R. Ferne and F. M. DaMatt, “Photosynthetic and metabolic acclimation to repeated drought events play key roles in drought tolerance in cactice”, Journal of Experimental Botany, 68(15), 2017, pp. 4309–4322.

[9] J. B. Passiouh, “Drought and drought tolerance”, in Drought Tolerance in Higher Plants: Genetical, Physiological and Molecular Biological Analysis, E. Belhassen, Ed. Springer Netherlands, 1996, pp. 1–5.

[10] P. Villar-Salvador, L. Ocaña, J. Petiuelas and L. Carrasco, “Effect of water stress conditions on the water relations, root growth capacity, and the nitrogen and non-structural carbohydrate concentration of Pinus halepensis Mill. (Aleppo pine) seedlings”. Annals of Forest Science, 56(6), 1999, pp. 459–465.

[11] D. Gui, F. Zeng, Z. Liu and B. Zhang, “Root characteristics of Alliagi sparrostelus seedlings in response to water supplemental in an arid region, northwestern China”, Journal of Arid Land, 5(4), 2013, pp. 542–551.

[12] S. Mahajan and N. Tuteja, “Cold, salinity and drought stresses: an overview”, Archives of Biochemistry and Biophysics, 444(2), 2005, pp. 139–158.

[13] P. E. Villagra and J. B. Cavagnaro, “Water stress effects on the seedling growth of Prosopis argentina and Prosopis alpina”, Journal of Arid Environments, 64(3), 2006, pp. 390–400.

[14] Y. Fan, G. Miguez-Macho, E. G. Jobbågy, R. B. Jackson and C. Otero-Casal, “Hydrologic regulation of plant rooting depth”, PNAS, 2017, 201712381.

[15] S. Rhizopoulou and G. Kaporas, “In situ study of deep roots of Capparis spinosa L. during the dry season: Evidence from a natural “rhizotrun” in the ancient catacombs of Milos Island (Greece)”, Journal of Arid Environments, 119, 2015, pp. 27–30.

[16] A. G. Bengough, “Water dynamics of the root zone: rhizosphere biophysics and its control on soil hydrology”, Vadose Zone Journal, 11(2), 2012, doi: 10.2136/vzj2011.0111.

[17] J. L. Maeght, B. Rewald and A. Pierret, “How to study deep roots and why it matters”, Frontiers in Plant Science, 4, 2013, doi: 10.3389/fpls.2013.00299.

[18] C. Chimona, A. Stamelou, A. Argiropoulos and S. Rhizopoulou, “Study of variegated and white flower petals of Capparis spinosa expanded at dusk in arid landscapes”, Journal of Arid Land, 4(2), 2012, pp. 171–179.

[19] T. Obata and A. R. Ferne, “The use of metabolomics to dissect plant responses to abiotic stresses”, Cellular and Molecular Life Sciences, 69(19), 2012, pp. 3225–3232.

[20] B. H. Yancey, M. E. Clark, S. C. Hand, R. D. Bowlu and G. N. Somero, “Living with water stress: evolution of osmolyte systems”, Science, 217(4566), 1982, pp. 1214–1222.

[21] F. A. Hoekstra, E. A. Golovina and J. Buitink, “Mechanisms of plant desiccation tolerance”, Trends in Plant Science, 6(9), 2001, pp. 431–438.

[22] J. C. Cushman, “Osmoregulation in plants: implications for agriculture”, American Zoologist, 41(4), 2001, pp. 758–769.

[23] L. Szabados and A. Savouré, “Proline: a multifunctional amino acid”, Trends in Plant Science, 15(2), 2009, pp. 89–97.

[24] S. Sivararamakrishnan, V. Z. Patel, D. J. Flower and J. M. Peacock, “Proline accumulation and nitrate reductase activity in contrasting sorghum lines during mid- season drought stress”, Physiologia Plantarum, 74(3), 1988, pp. 418–426.

[25] S. Rhizopoulou, S. T. Diamantoglou and L. Passiakou, “Free proline accumulation in leaves, stems and roots of four Mediterranean native phrygana species”: Acta Oecologica, 11(4), 1990, pp. 585–593.

[26] P. D. Hare, W. A. Cress and J. Van Staden, “Proline synthesis and degradation: a model system for elucidating stress-related signal transduction”, Journal of Experimental Botany, 50(333), 1999, pp. 431–434.

[27] F. Ain-Lhou, M. Ziaunzegui, M. D. Barradas, R. Tirado, A. Clavijo and F. G. Novo, “Comparison of proline accumulation in two Mediterranean shrubs subjected to natural and experimental water deficit”, Plant and Soil, 230(2), 2001, pp. 175–83.

[28] S. Diamantoglou and S. Rhizopoulou, “Free proline accumulation in sapwood, bark and leaves of three evergreen sclerophylls and a comparison with an evergreen conifer”, Journal of Plant Physiology, 140(9), 1992, pp. 361–365.

[29] Z. Zegaoui, S. Planchais, C. Cabassa, R. Djebbar, O. A. Belbachir and P. Caroll, “Variation in relative water content, proline accumulation and stress gene expression in two cowpea landraces under drought”, Journal of Plant Physiology, 218, 2017, pp. 26–34.

[30] H. J. Boernert and R. G. Jensen, “Strategies for engineering water-stress tolerance in plants”, Trends in Biotechnology, 14(3), 1996, pp. 89–97.

[31] M. M. Chaves, J. P. Maroco and J. S. Pereira, “Method for assessing cell membrane stability as a water stress tolerance gene expression in two cowpea landraces under drought stress”, Journal of Plant Physiology, 97, 1996, pp. 264.
S. Rhizopoulou and W. J. Davies, *Oecologia* 627 and shade leaves from four Mediterranean evergreen sclerophylls, *Environmental and Experimental Botany* J. B. Passioura, Management, S. Rhizopoulou, M. S. Meletiou-Christou and S. Diamantoglou, sclerophylls relations for sun and shade leaves of four Mediterranean evergreen proline for water-stress studies L. S. Bates, R. P. Waldren and I. D. Teare, Measurement and characterization by UV-VIS spectroscopy H. K. Lichtenthaler and C. Buschmann, Protocols in Food Analytical Chemistry A. A. Albalasmeh, A. A. Berhe and T. A. Ghezzehei, species of Epirus/Greece and Apulia/Italy landscaping and agricultural cultivations - A Review regarding local plants in drying soil. A. R. Accogli and G. Karras, plants in drying soil. R. E. Sharp and W. J. Davies, doi:10.1104/pp.16.00581. H. K. Lichtenthaler and A. R. Wellburn, doi:10.1007/s11738-016-2330-4. M. S. Meletiou-Christou and S. Rhizopoulou, doi:10.1371/journal.pone.0001622. R. J. Stirzaker and J. B. Passioura, doi:10.1109/TPS.2015.2437161. R. E. Sharp and W. J. Davies, Plant Physiology, chlorophyll fluorescence to predict mortality from drought, Plant Physiology, 2017, 34 pp. 253–261. S. Munné-Bosch and L. Alegre, Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in Rosmarinus officinalis plants*, Planta, 210(6), 2000, pp. 925–931. R. E. Sharp and W. J. Davies, “Root growth and water uptake by maize plants in drying soil.”, *Journal of Experimental Botany*, 36(9), 1985, pp. 1441–1456. R. J. Strzaker and J. B. Passioura, “The water relations of the root–soil interface”, *Plant, Cell & Environment*, 19(2), 1996, pp. 201–208. H. K. Lichtenhaler and A. R. Wellburn, “Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents”, *Biochemical Society Transactions*, 11, 1983, pp. 591–592. H. K. Lichtenhaler and C. Buschmann, “Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy”, *Current Protocols in Food Analytical Chemistry*, vol. 1, 2001, F4.3.1–F4.3.8. L. S. Bates, R. P. Waldren and I. D. Teare, “Rapid determination of free proline for water-stress studies”, *Plant and Soil*, 39(1), 1973, pp. 205–207. A. A. Albalasmeh, A. A. Berhe and T. A. Ghezzehei, “A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry”, *Carbohydrate Polymers*, 97(2), 2013, pp. 253–261. J. J. Irigoyen, D. W. Einerich and M. Sánchez Díaz, “Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (Medicago sativa) plants”, *Physiologia Plantarum*, 84(1), 1992, pp. 55–60. S. Rhizopoulou, M. S. Meletiou-Christou and S. Diamantoglou, “Water relations for sun and shade leaves of four Mediterranean evergreen sclerophylls”, *Journal of Experimental Botany*, 42(5), 1991, pp. 627–635. M. S. Meletiou-Christou, S. Rhizopoulou and S. Diamantoglou, “Seasonal changes of carbohydrates, lipids and nitrogen content in sun and shade leaves from four Mediterranean evergreen sclerophylls”; *Environmental and Experimental Botany*, 34(2), 1994, pp. 129–140. S. Rhizopoulou and W. J. Davies, “Influence of soil drying on root development, water relations and leaf growth of Cercidiphyllum japonicum L.”, *Oecologia*, 88(1), 1991, pp. 41–47. J. B. Passioura, “Roots and drought resistance”, *Agricultural Water Management*, 7, 1983, pp. 265–280. P. Baltzoi, K. Potia, D. Kyrkas, K. Nikolau, A. T. Paraskevopoulou, A. R. Accogli and G. Karras, “Low water-demand plants for landscaping and agricultural cultivations - A Review regarding local species of Euphorbia/Greece and Apulia/Italy”, *Agriculture and Agricultural Science Proceed*, 4, 2015, pp. 250–260.

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