

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 period. Sugar, proline and chlorophyll content were measured in above and below ground tissues of the considered species, during the 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

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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



Fig. 1 Samples of the studied plants. *N. oleander* in plastic tubes (a, b), *R. officinalis* and *C. siliqua* in rhizotrons (c and d, respectively).

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 ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$ 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 grounded 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 $\mu\text{g g}^{-1}$ 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 $\mu\text{mol g}^{-1}$ 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 \pm 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*.

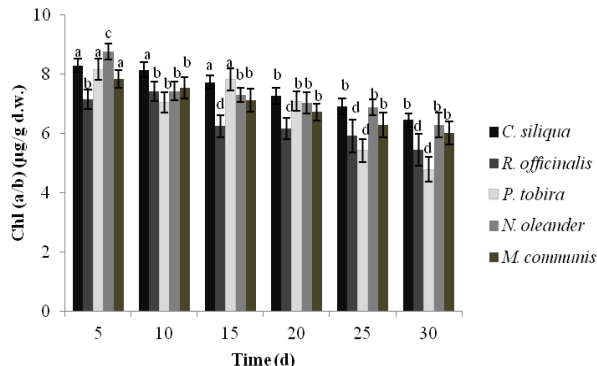


Fig. 2 Chlorophyll content. Values are means \pm SE. Significant differences ($p \leq 0.05$) of mean values are marked using lowercase letters.

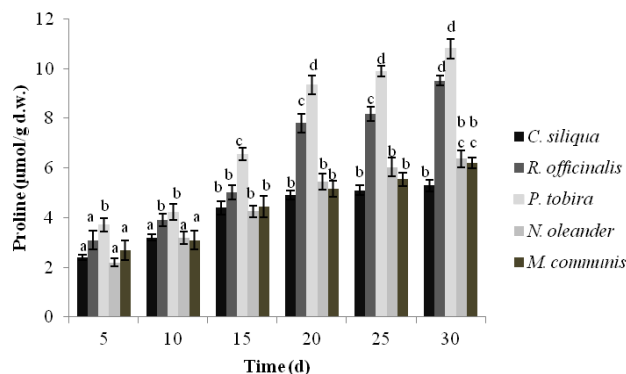


Fig. 3 Shoot total sugar content. Values are means \pm SE. Significant differences ($p \leq 0.05$) of mean values are marked using lowercase letters.

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].

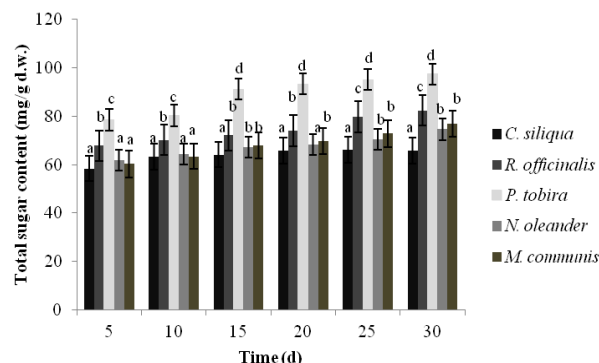


Fig. 4 Root total sugar content. Values are means \pm SE. Significant differences ($p \leq 0.05$) of mean values are marked using lowercase letters.

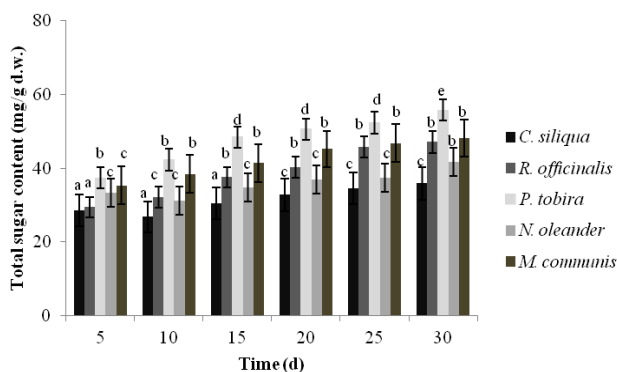


Fig. 5 Root proline content. Values are means \pm SE. Significant differences ($p \leq 0.05$) of mean values are marked using lowercase letters.

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.

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