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Saving Water while Enhancing Transplanting Success of *Pinus halepensis*

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Abstract

Mediterranean ecosystems are of high ecological importance, mainly due to their increased biodiversity levels. Nonetheless, restoring them is a hard task, mainly due to the semi-arid climate that prevails particularly during summer months. The aim of this study was to observe the effect of drought preconditioning of three-year-old *P.halepensis* seedlings in order to produce better equipped seedlings for restoration purposes. The hypothesis was that water deficit conditions induce physiological cues that enhance characteristics such as Root Growth Potential (RGP) that help seedlings for further water and nutrient exploitation. The seedlings were subjected for one month under three irrigation frequency treatments; watering twice per week, once per week and once per two weeks. At the end of a month, they were evaluated for characteristics, such as height and RGP. The results revealed that the seedlings that were watered more frequently had increased growth, while the seedlings that were watered the least had greater RGP. Therefore, the species of *P.halepensis* under water deficit conditions invest in the development of new roots that enable the species to survive and tolerate the semi-arid Mediterranean Greek ecosystems. Consequently, the benefit is dual; saving water while preconditioning seedlings to enhance growth traits like root growth that enhance transplanting success.

Keywords: Biodiversity; Conservation; Ecophysiology; Preconditioning; Restoration; Seedling production

Introduction

Mediterranean ecosystems are of high ecological value mainly due to their increased biodiversity levels. Despite their importance, their restoration is a hard task that relates to the prevailing semi-arid climate [1-3]. The stressed water deficit condition that plants experience, particularly during the hot-dry summers, is the main factor that negatively affects the survival and welfare of the transplanted seedlings [4]. Due to climate change, the intensity and frequency of aridity becomes more profound for the Mediterranean region [4,5]. As a result, restoration and reforestation efforts increasingly fail.

One of the methods used by forest nurseries to enhance transplanting success is preconditioning [6-8]. Specifically, seedlings undergo a period of stressed growth conditions prior to transplanting. This triggers physiological cues that enhance growth traits that help seedlings successfully establish under field conditions [8]. One of the growth traits is the ability to produce new roots. For the semi-arid ecosystems, drought preconditioning works as a signal for the seedlings to produce new roots in order to tolerate post-planting drought conditions [4,9]. "Root Growth Potential, RGP" is a useful tool to assess the quality of the seedlings and their ability to develop new roots [10,11]. Research showed that RGP was positively correlated with seedling viability as well as the above ground seedling biomass development under field conditions [6, 7,10-14]. So, the estimation of RGP provides a good surrogate on the ability of the species to grow new roots for water and nutrient exploitation.

Pinus halepensis Mill. (Aleppo pine) [15], is a thermophilic species that can tolerate high temperatures and drought growth conditions. It covers 2.5 million hectares at the western Mediterranean region in areas such as Albania, Israel and Greece [16]. It usually dominates coniferous fire prone forests ecosystems. It has a wide distribution range at the western Greek areas at an altitudinal variation between 0 to 600m [17]. Due to its wide spread and ability to dominate and grow under semi-arid climatic conditions, *P. halepensis* is highly used for reforestation and restoration purposes [18].

Consequently, the study is aiming to investigate seedling responses of *P. halepensis* that have undergone a preconditioning period of irrigation treatments. It was hypothesized that seedlings that experience reduced water conditions would have increased ability to develop new roots for further water and nutrients exploitation in order to successfully establish on a site. By studying seedling behavior under water stress conditions, we are able to mimic field conditions and determine the best irrigation frequency prior to transplanting to increase the percent of survival. Therefore, the benefit is dual; saving water by reducing the irrigation frequency while increasing the transplanting success.

Material and Methods

Three-year-old seed-derived seedlings were grown under field conditions in 55x60x160cm (QuickPot QP 24T/16) containers filled with standard soil substrate mixture (peat, perlite, fertilizer, 10% soil) at the forest nursery of Chalkidona, Greece. For experimental purposes, seedlings were placed in a controlled growth chamber under light of high-pressure sodium lamps (PPFD 300μmol m^{-2} s^{-1}) for photoperiod of 14 hours and room temperature of 21 ± 2°C. The Root Growth Potential (RGP) was estimated as described by Mattson's methodology [11]. The seedlings were placed in stainless steel trays with soil substrate 1(peat):1(sand) that enabled the development of new roots. The trays were placed in a water bath with controlled temperature of 21 ± 2°C and room humidity of 40 ± 10%.

According to the experimental design, three were the irrigation frequency treatments: watering twice per week, watering once per week and watering once per two weeks. The "control" referred to seedlings that were evaluated prior to the initiation of the experiment. All treatments were selected based reduced watering levels compared to the "daily irrigation frequency" that is used by forest nurseries under field conditions. After the irrigation treatment, the excess water was pumped up from the trays through a pipe, while there was no application of fertilizers. At the end of the experimental month, the seedlings were extracted from the trays and carefully cleaned. For each irrigation treatment five seedlings were used for evaluation.

Specifically, the height (HT) and the root collar width (RCW) were evaluated with a digital caliper and the leaf area (LA) was measured with a special leaf area instrument (Portable Area Meter, LI- 3000C). The dry weights of the seedling's subparts were also estimated by cutting and placing them in an oven for 72 hours at 80°C [19]. Specifically, the leaf dry weight (LDW), the shoot dry weight (SDW), the total root dry weight (TRDW), the total dry weight above ground seedling parts (TDWup) and the total seedling dry weight (TSDW) were measured. The RGP was evaluated based on the new roots dry weight (NRDW) and the maximum new root length (MaxNRL). The Specific Leaf Area (SLA, ratio of leaf area/dry weight of leaves) was also estimated [20], as well as the ratio (RATIO) of the total root dry weight by the total dry weight above ground seedling parts (TRDW / TDWup) [21].

For the statistical analysis one-way ANOVA was used to compare means among irrigation treatments for each studied variable, with data been tested for normality and homogeneity [22]. Mean differences were compared with the Tukey's multiple range tests at the significance levels of $p < 0.05$.

Results and Discussion

Based on the study, the results indicated different growth responses among the three irrigation frequencies treatments. Overall, the seedlings that received more frequent irrigation (twice or once a week) had a greater development than the seedlings that were watered the least (once every two weeks). However, the least watered seedlings had greater development of new roots.

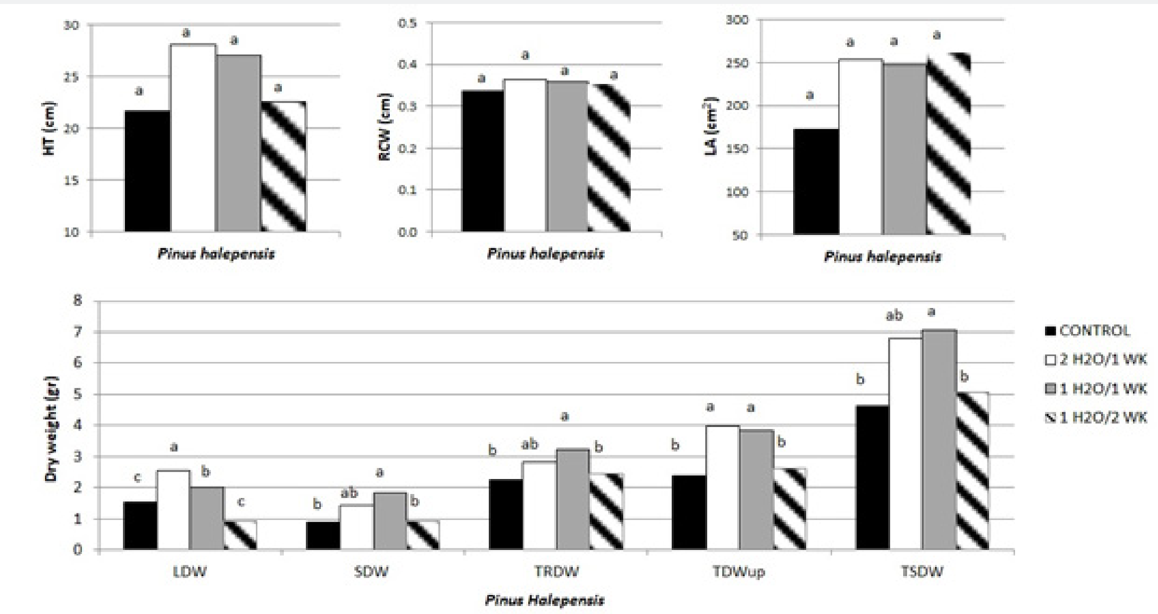

Figure 1: Seedling performance of *P. halepensis* based on irrigation frequency treatments for the variables of HT, RCW, LA and dry weights for LDW, SDW, TRDW, TDWup and TSDW.

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Further, the HT, the RCW and the LA did not indicate differences among the irrigation treatments (Figure 1). Consequently, those variables didn't show any immediate seedling response for the evergreen species of *P. halepensis* based on the irrigation treatments for the experimental period of a month. The effect was more profound by the biomass partitioning of the seedlings' subparts based on their dry weights. Specifically, the seedlings that were watered once or twice per week had the greatest growth above and below ground; specifically the LDW, SDW, TRDW, TDWup and TSDW (Figure 1). Similar results were found for one year *P. halepensis* [4] as well for the species of *Myrtus communis* [23], with reduced irrigation treatments having decreased root biomass. Based on this study, three-year-old seedlings of *P. halepensis* maintain their growth even under the reduced irrigation frequency of one watering per week. Consequently, the amount of water that can be saved is tremendous while also maintaining its physiological welfare.

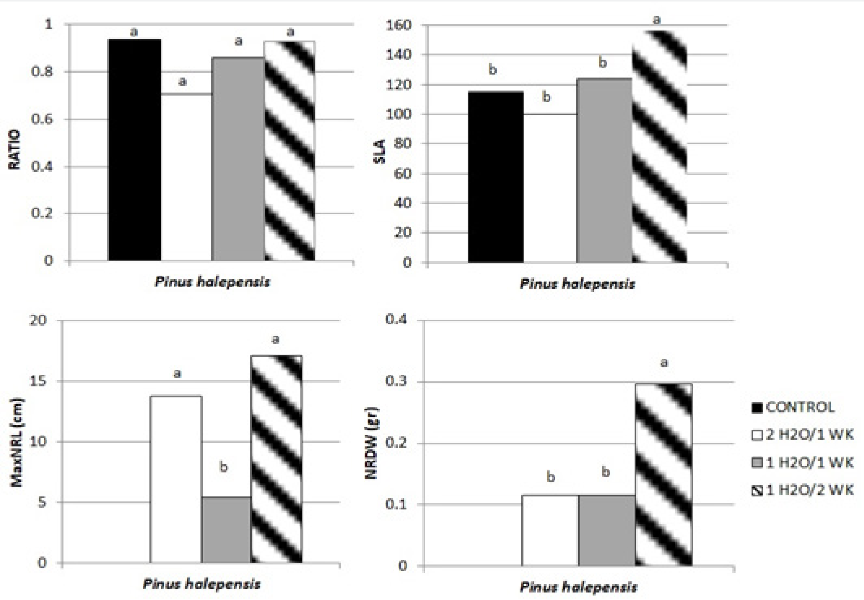

Figure 2: Seedling performance of *P. halepensis* based on irrigation frequency treatments for the variables of RATIO (root to shoot dry weight), SLA (leaf area to leaf dry weight) and RGP as determined by the MaxNRL and the NRDW.

Further, although the RATIO did not indicate differences among treatments, the SLA was greater for seedlings that were watered once every two weeks (Figure 2). These findings differentiate from other studies that showed that species experiencing water stress conditions were associated with lower SLA values; therefore, they had thicker leaves [21,24]. In this study, despite that the LA did not differ among irrigation treatments, the LDW showed the least dry weight for the seedlings that experienced the most water stress (one irrigation per two weeks). That could be the result of reduced leaves due to leaf abscission as a physiological response in order for the species to cope with water deficit conditions (experimental observation).

According to the RGP, both the NRDW and MaxNRL were greater for the seedlings that were subjected at the least irrigation frequency of one watering every two weeks (Figure 2). So, although those seedlings received the least water and had the least above ground biomass accumulation, they had the greatest RGP. Therefore, the experimental preconditioning period of one month of one irrigation every two weeks was adequate to induce a metabolic shift towards the development of new roots at the expense of the rest of the seedling growth traits, such as leaf production. Therefore, this suggests that one of the physiological mechanisms of *P. halepensis* that enhance its survival in semi-arid environments like Greece, is their fast metabolic shift towards the production of a new roots for further water and nutrient exploitation. In terms of conserving water, the benefit is tremendous by watering only twice a month. Consequently, both saving water while preconditioning seedlings to enhance growth traits like root development that enhance transplanting success [25].

Conclusion

According to the results, the seedlings of *P. halepensis* that were exposed under water deficit conditions were able to maintain their growth. The seedlings with the least water frequency had greater SLA and RGP (as indicated by NRDW and MaxNRL). These results show that the seedlings of *P. halepensis* invested in the development of new roots for further exploitation of water and nutrients for their survival. This could be one of the main characteristics that enable the species to dominate the semi-arid areas of Greece; it triggers cues for further root growth. Undoubtably, we benefit by both saving water and preconditioning to produce better equipped seedlings for successful transplant..

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