



Growth Phenology of Pistachio Seedlings under Water Stress and Rehydration Conditions

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Abstract

Pistacia atlantica Desf., a wild pistachio species of major medicinal, ecological interests. It is used as a principal rootstock for *Pistacia vera* L., a domestic pistachio species. Unfortunately, it is endangered of disappearance. To study comparatively the responses of the two species to water stress and rehydration, 6 months aged seedlings of both species were subjected to a water stress period, imposed progressively by withholding irrigation, for a period of 40 days, followed by two weeks under well-watered conditions. The number of green leaves, number of senescent leaves, leaf area, shoot phytomass, stem, root phytomass and root/shoot phytomass ratio were assessed.

Under water deficit treatment, the main effect of water stress was a marked reduction in growth phenology (number of green leaves, leaf area leading to lower phytomass production). Significant differences were found between species. *P. vera* showed a decrease in shoot and root phytomass, attributed to higher leaf senescence. At the contrary, *P. atlantica* had a relatively high growth and phytomass production. After rehydration, growth and phytomass production was partially recovered only in *P. atlantica*, suggesting a good tolerance to water stress. However, the higher effects of water stress in *P. vera* inhibit it to recovery after restoring water.

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Introduction

The scarcity of water in Mediterranean ecosystems and the current water deficit are leading to the urgent need to better manage water use for irrigation in arid and semi-arid areas [1]. Plants grown under such conditions are often exposed to a long period of drought which affect plant growth and production [2-10]. These constraints are expected to be accentuated in later years [11-13]. Especially under global change all that leads to the disappearance of many species and desertification [14].

Thus, one solution to these problems is to select resistant species whose ecophysiological characteristic enables them to survive and produce under such conditions. In addition to forests, woody plants contribute well to biodiversity and area protection [15], especially under arid bioclimate.

Pistachio is considered as drought and saline-resistant species [16-23]. It is mainly grown under low rain conditions in the Mediterranean basin. In Tunisia, pistachio culture is concentrated at 95% in arid and semi-arid regions. Pistachio is becoming

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an interesting alternative to traditional crops. Indeed, its cultivation is highly encouraged in such areas. *P. vera* (*Mateur* variety) is the most important in Tunisia). *P. atlantica*, a spontaneous pistachio species, although it is frequently used as principal rootstock for *P. vera* and of a really important medicinal and ecological interests, it is threatened by the disappearance. For example in Tunisia, only a few aged, dispersal and endangered species exist in the Center and the South (no more than 1500 individual). The responses of pistachio species to water stress has been rarely studied [16,19,22].

Water stress leads to a significant reduction of growth and phytomass production). Recent studies have shown that growth rates of several plants are directly proportional to the soil water availability [24]. Water stress may affect the plant functioning mechanisms through several interrelated changes at the morphological [25], anatomical [18,26,27] physiological [28,29] and biochemical levels [18,30,31].

Therefore, the purpose of this paper was: 1) to study the effect of water stress and re-watering conditions on growth maintenance and phytomass allocation in *Pistacia vera* (cv *Mateur*) and *P. atlantica*, 2) to compare their morphological adaptation to water availability in the soil and 3) to identify the morphological mechanisms of their adaptation to water stress.

Materials and methods

Experimental site and plant material

The experiment was conducted from September 2011 to June 2012 at the survey field in the Faculty of Sciences of Gafsa (Tunisia). For the purposes of the present study, mature seeds of *P. vera* (cv *Mateur*) were sampled from the region of Gafsa (Tunisian South-Est area) while the seeds of *P. atlantica* were gathered from wild trees in Meknassy (Tunisian Centre-West area). The experimental assay was firstly initiated in the laboratory conditions: Temperature 22°C, photoperiod 14h/10h light/obscurity. The seeds were cultivated in small plastic pots (250mL) contained (2/3) clean sand and (1/3) peat. After a growth period of one month, the seedlings with five leaves were transplanted in 11 L pots outside laboratory (one plant per pot) with a total of 120 pots. The soil used was a mix of soil, sand, peat, and gravel in 45:40:10:5% respectively. During establishment, individuals were regularly irrigated to ensure maximum growth. To reduce the lag effect of growth induced by germination, the individuals having the same growth were selected for treatment. Pots were arbitrary moved every week to minimize border position effects. **Irrigated treatments**

The irrigation treatments were full irrigation (Control treatment) and no irrigation (stress). Before starting the water stress treatment, the control and stressed plants were drips irrigated until slight drainage occurred. To determine pot weight at field capacity, all pots were weighted early every day, always at the same hours. The soil used has a water content of 17%.

At the beginning of treatments, the pots were protected from rainfall by a plastic cover. In plants subjected to water stress, irrigation was withdrawn for 40 days. Once the stress period was completed, six stressed pots were re-watered up to field capacity to study re-hydration effect. To examine the effect of water deficit and re-hydration on growth and phytomass production of *P. vera* and *P. atlantica*, different parameters were measured for the control, water deficit and re-hydration treatment: Number of green leaves, senescent leaves, leaf area, shoot, stem, root phytomass and root/shoot phytomass ratio.

The period of measurements (March-June 2012) was characterized by a temperature between 25 and 30°C.

Measurements

Growth parameters

-The number of green leaves was recorded just before application of treatments and weekly after treatment application until the end of the experiment at a number of six repetitions.

- During the period of the plants growth, the visual symptoms in the leaves were assessed: The number of senescent leaves was accounted since the senescence phenomenon appears.

- After water stress and rehydration periods, leaf area was estimated on six seedlings for each treatment: The entire surface of the leaves by the plant was determined by scanning the leaves, then, the software of image processing *Mesurim pro 8* made it possible to determine leaf area, expressed in Cm².

-Each plant was separated into leaves, stems and roots. Each part was weighted and then washed once with tap water and once with distilled water, dried at 85°C during 48 h, and the dry weight (g/plant) of each plant part was determined after the stress and after re-hydration period. The number of repetition was six. This parameter was estimated twice (After water stress and after rehydration period).

-The ratio R/S (%) is the relationship between the dry weight of roots and the dry weight of shoots. It is given as follows: Ratio R/S=(R/S)*100. The number of repetition was six. R/S was estimated after water stress and after rehydration period.

Statistical analyses

Data Were Analyzed Using (ANOVA) test according to a factorial model with fixed factors (day of treatment, species, treatment (water stress or rehydration) with the SPSS (Statistical Package for the Social Sciences, SPSS Institute Inc., Cary, NC, USA) base 11.5 software. The Sigma Plot Version 8.0 software was used to represent the different features. Means are presented with standard errors of the mean and significance is expressed at p<0.05. Duncan test one ANOVA factor was used to compare means in each date.

Results

Effects of water stress on the growth phenology and phytomass production

Growth phenology

Regarding the number of green leaves, we found that values were identical in both irrigated species and no differences were found until day 8 of the experiment (Figure 1). From day 12 onwards, the number of green leaves increased significantly (p<0.001) compared to the stressed plants. In irrigated treatment, *P. vera* had more leaves than *P. atlantica*. Under water stress, *P. atlantica* showed slight but, not a significant reduction in leaf number. Differences between the control and stress treatment were only 37%. In contrast, in *P. vera*, leaf number was the most affected by water stress conditions (81%).

At the end of rehydration period, 13 days after, the responses of both species to re-watering were not significant. Only in *P. atlantica*, few new leaves appeared at the end of experimental period. Thus, both species showed significant differences (p<0.001) between irrigated and rewatered plants at the end of

the experimentation.

Similarly, no differences existed between species until day 20 of the experiment for senescent leaves (Figure 2). However, from day 28 onwards, senescent leaves, recorded only in *P. vera*, increased significantly as water stress increased. It reached 45% at the end of water stress treatment. On the contrary, *P. atlantica* didn't show leaf senescence, only brown color in the inferior face of old leaves were observed.

In the control condition, *P. vera* showed a higher leaf area than *P. atlantica* (approximately 10% higher) (Figure 3). Water stress affected significantly the leaf area development of *P. vera*, with *P. atlantica* once again being the less affected, followed by *P. vera*. The largest decrease of leaf area occurred in stressed seedlings reaching about 56% in *P. vera* nevertheless, it did not exceed 26% in *P. atlantica*.

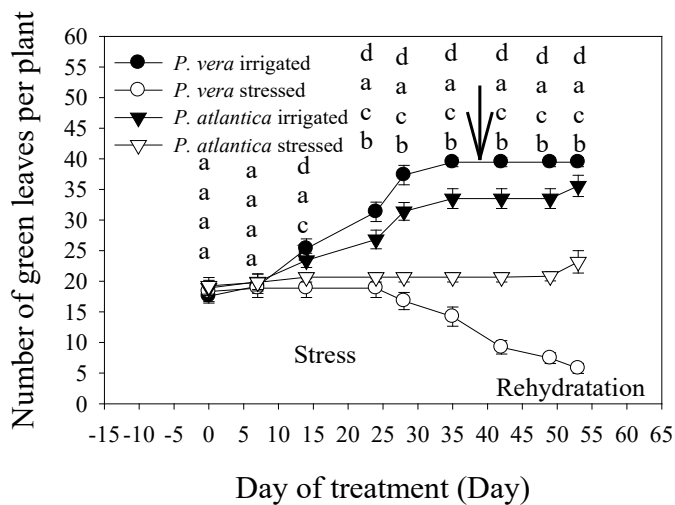


Figure 1: Effect of water stress and re-watering on number of green leaves of the seedlings of *P. vera* and *P. atlantica* (n=6). Bars denote the standard error. Means followed by the same letter are not significantly different according to Duncan test at 5%.

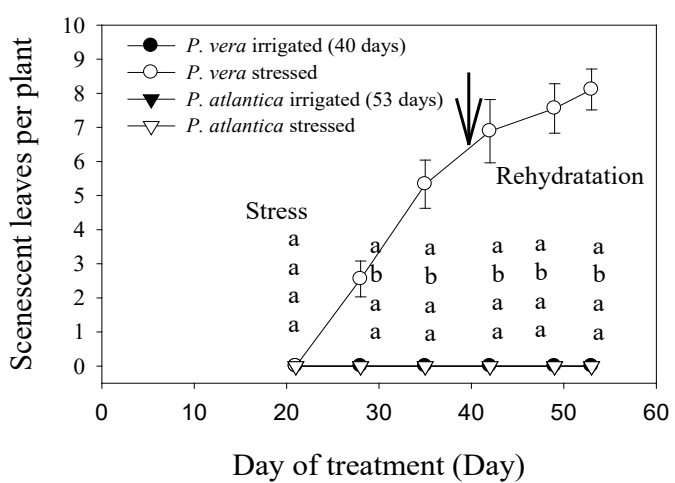


Figure 2: Effect of water stress and re-watering on number of senescent leaves per plant (n=6). Bars denote the standard error. Means followed by the same letter are not significantly different according to Duncan test at 5%.

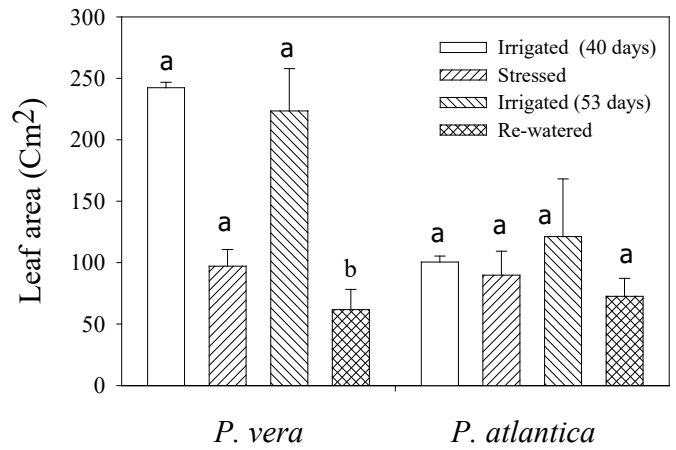


Figure 3: Leaf area of the seedlings of *P. vera* and *P. atlantica* at the end of the experiment (n=6). Bars denote the standard error. Means followed by the same letter are not significantly different according to Duncan test at 5%.

Phytomass production

For both species, water stress led to a reduction of shoot phytomass (Figure 4). Although *P. vera* plants present higher shoot phytomass in the control condition. *P. vera* shoot phytomass decreased significantly under water stress. This decrease was as a result of the reduction of the number of green leaves and leaf area in both species and the increase of the number of senescent leaves in *P. vera*. This reduction was again, very marked in *P. vera*. Nevertheless, *P. atlantica*, maintain a shoot phytomass near those of the control plants with a reduction of only 37%. However, it reached 81% in *P. vera*.

After re-watering, the *P. atlantica* re-watered seedlings remain with similar shoot phytomass as stressed ones. However, *P. vera* rewatered plant showed more reduced shoot phytomass even than stressed plants.

As it was observed in shoot phytomass, in the control treatment, *P. vera* showed significantly higher stem dry weight than *P. atlantica* (Figure 5). Water stress resulted in a significant reduction in stem dry weight in *P. vera*, but not in *P. atlantica* (Figure 5). Stressed plants of *P. atlantica* showed once again higher stable values of stem dry weight than *P. vera*. In this situation, the reduction was about 40%. However, it reached 48% in *P. vera* stressed seedlings. Although rehydration was restored, *P. vera* didn't improve dry stem phytomass and it was reduced again (63%). At the contrary, *P. atlantica* remains with a similar dry stem phytomass as stressed seedlings. Differences between species were highly significant (p<0.001).

Root phytomass in control plants was higher in *P. vera* than *P. atlantica*. Drought stress led to a significant decrease of root phytomass for both species (p<0.001) (Figure 6). These reductions were 36% and 38% in *P. vera* and *P. atlantica* respectively. After 13 days of rehydration, both species improves slowly their root phytomass. This improvement was only 11% and 3% compared to the stressed plants. Thus, they didn't reach values assessed in control plants.

Water stress ratio root/shoot phytomass was improved in both species, with 39% and 6% in *P. vera* and *P. atlantica* respectively (Figure7). After 13 days of rehydration, both species increased slightly their ratio root/shoot phytomass.

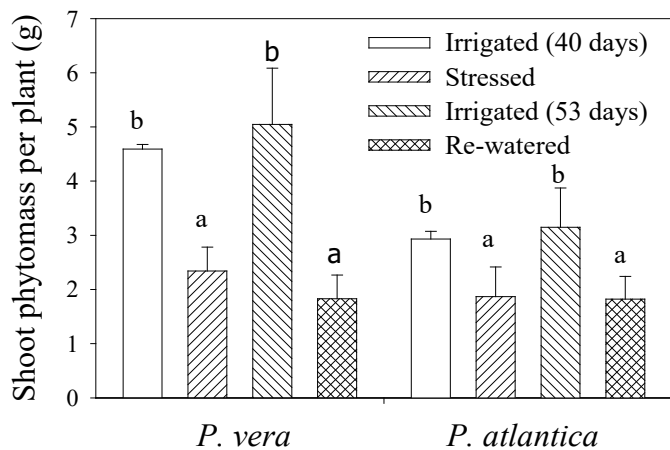


Figure 4: Effect of water stress and re-watering on shoot phytomass of the seedlings of *P. vera* and *P. atlantica* at the end of the experiment (n=6). Bars denote the standard error. Means followed by the same letter are not significantly different according to Duncan test at 5%.

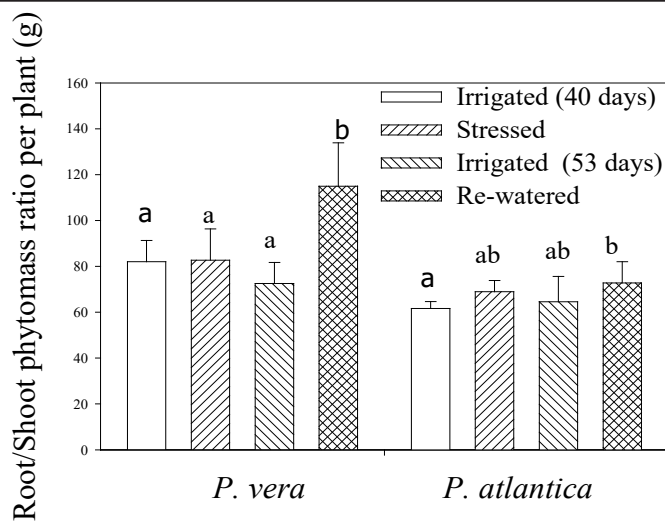


Figure 7: Effect of water stress and re-watering on ratio root/shoot phytomass of the seedlings of *P. vera* and *P. atlantica* at the end of the experiment (n=6). Bars denote the standard error. Means followed by the same letter are not significantly different according to Duncan test at 5%.

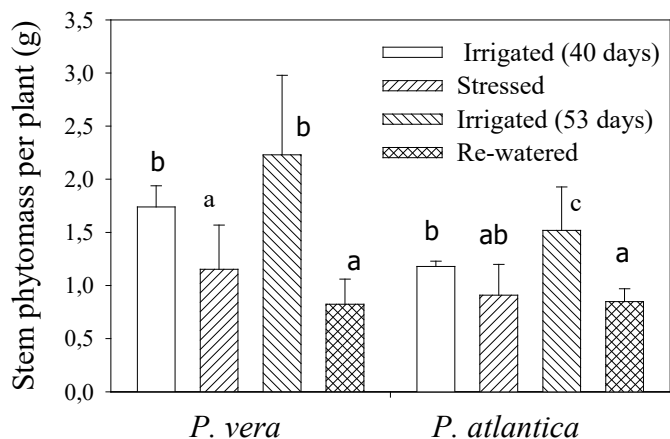


Figure 5: Effect of water stress and re-watering on stem phytomass of the seedlings of *P. vera* and *P. atlantica* at the end of the experiment (n=6). Bars denote the standard error. Means followed by the same letter are not significantly different according to Duncan test at 5%.

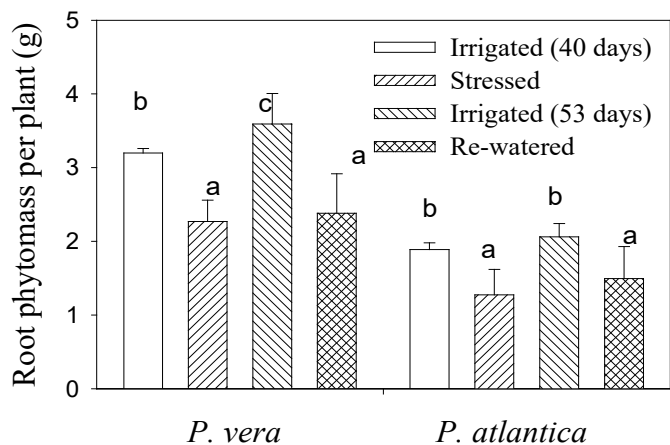


Figure 6: Effect of water stress and re-watering on root phytomass of the seedlings of *P. vera* and *P. atlantica* at the end of the experiment (n=6). Bars denote the standard error. Means followed by the same letter are not significantly different according to Duncan test at 5%.

Discussion

The growth phenology and phytomass productions of plants are ultimately reduced by water stress although plant species differ in their tolerance to water stress [6,9,28]. It appeared that plants perceive and respond rapidly to reduction, even little, in water status through a series of changes ranging from physiological to molecular events developing in parallel [32]. In addition to a plant's ability to avoid and/or tolerate water stress, recovery following rehydration is pivotal to dictate a plant's resistance to drought [33]. The effects of both treatments (water stress and re-hydration) on growth and phytomass production will be discussed.

Effect of water stress and rehydration on growth phenology

Under favourable hydric conditions for growth, a high leaf area ratio provides a greater photosynthesizing area and consequently a higher growth rate but it result in higher water loss [34,35]. Therefore, growth data suggests that *P. vera* is more productive under well-watered conditions, but it looks the more affected by water stress. Growth is extremely sensitive to drought and is strongly influenced by the ability of the roots to grow in drying soil and maintain an optimal water status [36]. Reduction in plant vegetative growth following water stress suggests that available water is one of the major factors that determines growth and development of young pistachio plants. It is a special survival mechanism adapted to prevent damaging survival chances [37]. Similar responses have been reported in several fruit trees, including pistachio [16], olive [38] and other saharan and mediterranean species [28,39,41]. Indeed, the increase in water stress was followed by a reduction in the number of green leaves of the seedlings, a reduction of the new leaves and an acceleration of the senescence of old ones. The fall of the old leaves contributes to saving hydration [42]. This mechanism can be regarded as a program of recycling inside the plant allowing the redistribution of the resources stored in the old leaves to the young ones or stems [43].

It was expected that plants which were re-watered after a brief water stress period would resume normal or greater growth compared with plants watered continuously depend-

ing on species [37]. However, this did not happen in this study. Although the growth of leaves was partially recovered only in *P. atlantica*, renewed growth seems not significant. Our results suggest that the difference in performance observed between the two species could be attributed to anatomical and to physiological characteristics [22].

Effects of water stress and rehydration on phytomass production

To study the response of plant to water stress, it is relevant to approach it at the phytomass production scale as well, since productivity is dependent on the morphological adjustment and the photoassimilates produced at the whole plant level. Reduced phytomass production occurs via the inhibition of new leaf growth, leaf area reduction or the earlier senescence of older leaves, in the case of prolonged stress as shown in *P. vera*. This reduction in foliage dimension leads to decreased transpirational area but also to lower intercepted radiation throughout the growing season and ultimately to decreased biomass production [44]. This phenomenon was observed in both studied species, more pronounced in *P. vera* with enrolled leaves. It has long been reported that plants having small leaves are typical of dry environments. Their growth rate and biomass are relatively low [45]. In many crops, alteration of the leaf angle with dehydration, towards smaller angles, will also decrease total intercepted radiation and therefore carbon assimilation by the plant, but will have an important protective role against excess solar energy. Photosynthetic resilience to drought is known to vary with leaf age [26]. Younger leaves tend to be more resistant to drought than older leaves, and this increased tolerance may be particularly relevant in plants where a severe reduction in the size of the leaf canopy occurs as a result of shedding of older leaves, because it allows a fast recovery following rehydration [44]. It was shown that recovery from a severe stress was a two-stage process: The first stage occurs during the first hours or days upon re-watering, corresponding to the improvement of leaf water status and stomatal re-opening [47-49]. The second stage lasts several days and requires de novo synthesis of photosynthetic proteins [50]. However, the root/shoot ratio increased as water stress increased, indicating that shoot growth is more sensitive than root growth. This result coincides with reports in the literature of increases in root/shoot ratio with increased water stress [51].

The progressive dehydration, induced a significant reduction in leaf area and the phytomass production in *P. vera*, which continues even after rehydration. However, *P. atlantica* showed more stable root growth under both water regimes compared to *P. vera*. Thus, *P. atlantica* presented morphological changes that enable it to better adapt to the different availability of water in the soil. In addition, it is characterized by a faster recovery after rehydration. Its potential can justify its use as rootstock for improving the *P. vera* vigor and production.

Conclusion

Under water stress and rehydration treatments, *P. atlantica* plants had stable and higher growth and phytomass production compared to *Pistacia atlantica*. Thus, *P. atlantica* seems more resistant to water stress than *P. vera*. That resistance makes it an important rootstock for *P. vera* in Tunisian arid zones. The difference in performance observed between the two species could be attributed mainly to anatomical and to physiological characteristics of *P. atlantica* roots. Since roots are the only source to acquire water from the soil, the root structural and

functional traits are the key component of plants responses to water stress.

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