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Effect of hydric and light stress on biomass, nutrient uptake and enzymatic antioxidants of *Argania spinosa* seedlings

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Abstract: Sunlight and water are factors that affect seedling development. However, the effects of acclimatization of seedlings to sunlight and water stress remain poorly understood. This study aimed to examine the interactive effects of acclimatization to light and water stress on argan tree (*Argania spinosa*) seedlings in nurseries. An experiment was conducted with 504 seedlings using two sunlight treatments (L1 and L2, i.e. 100% and 60% of full sunlight, respectively), and three watering treatments (well-watered, moderate stress and severe stress, i.e. 100%, 50% and 25% of field capacity, respectively). According to our results, water stress treatments caused a reduction in total biomass accumulation, nitrogen and phosphorus uptake. Water stress significantly increased other macroelements, H_2O_2 and MDA levels and antioxidant enzyme activities compared to well-watered seedlings. Seedlings grown under moderate shade (L2) showed higher macroelement uptake, which probably contributed to the increase in total biomass in all water treatments. The highest membrane stability index (MSI) values, H_2O_2 and MDA levels and lowest antioxidant enzyme activities were recorded in acclimatized argan seedlings under moderate shading (L2). These results suggest that moderate shade can effectively prevent stress caused by light excess and can also mitigate the harmful effects of water stress on *A. spinosa* seedlings.

Keywords: *Argania spinosa*; water stress; sunlight stress; macroelement uptake; shading

INTRODUCTION

Climate change and anthropogenic activities in recent decades have caused a regression of argan forests [1]. To ensure the survival of argan forests in general and the argan tree in particular, artificial regeneration remains the only possibility [2]. However, several reforestation failures have been observed after transplantation of the argan tree in the field. These failures can be partly attributed to poor acclimatization of seedlings to transplant shock, and they represent the main obstacle to the success of the plantations. Indeed, from the nursery to transplantation to the field, seedlings move from a resource-rich environment to a more challenging one.

At the beginning of field transplantation, water scarcity, extreme temperatures and solar radiation are usually the types of stress that plants are exposed to [3]. In particular, water stress leads to reduced seedling growth [4] and can have a very significant impact on plant relationships and nutrient uptake [5]. It was shown [6] that drought stress decreases the concentration of nitrogen and phosphorus in plant tissues. In addition, the generation of reactive oxygen species (ROS) is one of the first biochemical responses under water stress in the argan tree [7]. Increased production of ROS, such as H_2O_2 by water stress, can lead to damage in cell structures as a result of oxidative stress. To maintain homeostasis and prevent oxidative stress, plants such as the argan tree have developed defense systems involving of certain enzymes such as superoxide dismutase (SOD), peroxidase (POD) and polyphenol oxidase (PPO). When ROS production is in

excess under dry conditions, they can cause significant oxidative damage to membrane lipids [7,8]. Variations in malondialdehyde (MDA) levels, which essentially reflect lipid peroxidation, allows the quantification of this damage [7].

Like water, light is another environmental factor essential to plant performance [9]. It also plays a role in regulating plant growth and development [10], and the light that is optimal for growth is species-specific [11]. However, a severe increase in sunlight intensity can also induce photoinhibition of photosynthesis, which can lead to significant damage in non-acclimatized plants [12,13]. The interactive effects of sunlight and water on plants remain poorly documented, resulting in contrasting hypotheses. Nevertheless, it was pointed out [14] that shade can alleviate the effects of drought on plants. Indeed, moderate shade was shown to buffer the effects of drought on *Torreya grandis* seedlings [9].

For *A. spinosa*, there are several studies on water stress [2,7,15,16], however, none of them have focused on the acclimatization aspect in the nursery. Indeed, in the literature there is a lack of information either on the acclimatization of argan tree seedlings or on the interactive effects of acclimatization of argan tree seedlings to changing environmental stresses (drought and sunlight).

It is conceivable that acclimatization to water and sunlight stress and their interaction have a considerable impact on most biological processes and thus on the improvement of the quality of seedlings (stress resistance capacity) of argan trees. The objective of this study was to examine the interactive effects of acclimatization to sunlight and water stress on *A. spinosa* seedlings in nurseries. We assessed (i) how the intensity of water stress and the level of sunlight affect the variables studied, (ii) whether moderate shading diminishes the adverse effects of water stress on seedlings, and (iii) how the responses of the variables studied are related to each other under interaction between drought and sunlight. The assessment of these parameters will allow us to better understand sunlight and water regime requirements for better acclimatization to extreme conditions (drought and sunlight) in semi-arid environments. Such knowledge is needed to optimize the regeneration of *A. spinosa* and thus contribute to the conservation of biodiversity.

MATERIALS AND METHODS

Plant material and experimental design

The study was conducted at the Regional Forestry Research Center of Marrakesh, Morocco (31°40'04"N 7°58'04"W). Argan seeds were collected in July 2018 from twenty argan trees in Essaouira (31°32'07.8" N 9°28'34.4" W). The seeds were dried in open air and stored at room temperature in a storage room. On March 24, 2019, the seeds were soaked in tap water for 24 h before germinating on a damp cloth on March 30, 2019. Then, 560 healthy and uniform seeds were selected and sown in 20 containers of 500 cm^3 on April 1, 2019. Forest soil (same origin as the seeds) and commercial peat (TS3) were air-dried and mixed (1:3, v/v) as a substrate for the tree seedlings. The mixture had a pH of 7.5 and contained an average of 8.21% total nitrogen, 129.67 mg kg^{-1} assimilable phosphorus and 115.67 mg kg^{-1} potassium. All seedlings remained for four months (April-July 2019) in the growing sector of the nursery. This sector was covered with a 40% shade net (exposure to 60% full sunlight) and watered daily. The height, diameter, aerial fresh mass and root fresh mass of the seedlings averaged 17.65±2.76 cm, 3.17±0.29 mm, 2.95±0.29 g and 2.22±0.11 g, respectively. After the growing period, the argan tree seedlings were kept in their respective containers and then divided into two equal groups to study the interaction of water and light stress. The experimental design was completely randomized for three months (from August 15 to November 13, 2019), with the application of three watering regimes (well-watered (C), medium stress (MS) and severe stress (SS), respectively 100%, 50% and 25% of field capacity), and two levels of sunlight (L1 and L2, respectively 100% and 60% of full sunlight). The argan tree seedlings exposed to a level of 60% of full sunlight were maintained in the nursery growth sector. Soil moisture was measured daily by time reflectometry (FieldScout TDR 200 Soil Moisture Meter, Spectrum Technologies Inc. Plainfield, IL, USA) with 12-cm probes to maintain the moisture content of each water treatment. Each treatment consisted of three blocks and each block had 28 argan tree seedlings (with 504 seedlings in total).

Growth variables and foliar mineral analysis

At the end of the experiment, ten seedlings from each treatment were randomly sampled. Shoots and roots

were separated. The samples were oven-dried (at 105°C for 48 h) to constant weight. This allowed us to calculate the biomass accumulation. The dried leaf samples that were collected allowed us to analyze and determine macroelements (N, P, K, Ca and Mg). Total foliar nitrogen was analyzed by the Kjeldahl method [17]. Briefly, dry leaf (250 mg) powder was wet-digested in 250-mL macro-Kjeldahl tubes using concentrated H_2SO_4 (10 mL) and Kjeldahl tablet at 350-400°C. After digesting the samples with NaOH (40%), NH_4-N was fixed in H_3BO_3 (1%) and titrated with 0.02 N H_2SO_4. The other elements were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) after calcination of the samples as described [17].

Membrane stability index (MSI), H_2O_2 and MDA contents

At the end of the experiment, membrane stability was estimated by measuring electrolyte leakage (conductivity) according to the described method [18]. Briefly, 20 uniformly similar leaf discs (0.2 cm^2 per disc) per replica were sampled from the developed leaf blade and carefully washed with distilled water to remove surface electrolytes. Leaf discs were placed in separate, sealed vials filled with 15 mL of distilled water and shaken at 25°C for 4 h. Then, the initial conductivity (Ci) of the solutions was measured using a precalibrated conductivity meter (Hach, Model. sensION+EC7, Co, USA). The total conductivity (Ct) was determined after the solutions were autoclaved for 20 min at 120°C and then cooled to room temperature. The membrane stability index (MSI) was calculated using the formula:

$$MSI = [1- (Ci/Ct)] \times 100.$$

At the end of the experiment, the concentration of hydrogen peroxide was determined spectrophotometrically [19]. Briefly, fresh leaf (100 mg) powder was homogenized with 5 mL 10% (w/v) trichloroacetic acid (TCA) and centrifuged at 10000 ×g for 10 min at 4°C. The supernatant (0.5 mL) was then recovered, and 1 mL 1 M iodic potassium and 0.5 mL 10 mM potassium phosphate buffer, pH 7, were added. After 1 h of incubation in the dark at room temperature, the absorbance was read at 390 nm and plotted against a standard H_2O_2 curve.

At the end of the experiment, lipid peroxidation was determined by estimating the MDA content as described [20]. Fresh samples (0.1 g) were homogenized in 2 mL of 0.1% (w/v) TCA and centrifuged at 15000 ×g for 10 min at 4°C. The supernatant (0.5 mL) was then recovered and mixed with 1.5 mL of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA. The mixture was incubated in a water bath at 90°C for 20 min. After stopping the reaction in an ice bath, the samples were centrifuged at 10000 ×g for 5 min. Absorbance was recorded at 532 nm subsequent to subtraction of nonspecific absorption at 600 nm. The MDA content was calculated based on its extinction coefficient of 155 mM^{-1} cm^{-1}.

Antioxidant enzymes

At the end of the experiment, fresh leaves were immediately ground in liquid nitrogen to obtain a fine powder and stored at -20°C. Antioxidant enzymes were extracted by homogenizing the powder. Briefly, 100 mg of the finely ground powder were homogenized in 50 mM K_2HPO_4/ KH_2PO_4 buffer, pH 7.8, containing 5 mM 2-mercaptoethanol, 0.1 mM ethylenediaminetetraacetic acid (ETA), 1% (w/v) polyvinyl pyrrolidone (PVP), 0.1 mM phenylmethanesulfonyl fluoride (PMSF) and 0.2% (v/v) Triton X-100 to determine antioxidant enzymes SOD and POD, while K_2HPO_4/KH_2PO_4 buffer, pH 5.5, was used for PPO. To determine the specific activities of the enzymes, the total soluble protein content was determined using bovine serum albumin (BSA) as standard [21].

According to the method described in [22], SOD activity was determined by its ability to inhibit the photochemical reduction of nitrotetrazolium blue chloride (NBT) at 560 nm. A unit of SOD activity was defined as the amount of enzyme that induces 50% inhibition of NBT reduction. Based on the method described in [23], POD activity was determined using the production of tetraguaiacol with the extinction coefficient of 25.5 mM^{-1} cm^{-1} at 436; PPO activity was measured at 410 nm following catechol oxidation [24]; PPO activity was expressed in enzyme units U mg^{-1} protein.

Statistical analysis

To evaluate the effects of watering and sunlight treatments and their interaction on biomass accumulation, macroelement uptake and antioxidant enzyme activities, the data were subjected to several analyses of variance

Table 1. Two-way ANOVA results on the effects of sunlight, watering regime and their interaction on biomass accumulation (SDM, RDM and TDM), macroelement uptake (N, P, K, Ca and Mg), H_2O_2, MDA and MSI levels and antioxidant enzyme activities (SOD, POD and PPO) of *A. spinosa* seedlings under different sunlight and watering treatments.

Variables	Means squares and their significances		
	L	W	L*W
Biomass accumulation			
SDM	7.05**	63.17***	0.73 NS
RDM	1.31*	35.08***	1.67**
TDM	14.45**	192.28***	4.34*
Macroelement contents			
N	2.92**	33.36***	1.25**
P	0.10***	0.38***	3.71 NS
K	0.45***	1.87***	0.005*
Mg	5.22***	7.64***	0.15***
Ca	0.91***	4.41***	0.09***
Biochemical traits			
MSI	857.05***	4919.67***	198.26***
H_2O_2	684.04***	5538.35***	8.307***
MDA	932.37***	5611.68***	181.33***
SOD	267774.74***	5549874.79***	45247.37***
POD	335577.77***	1557411.05***	14815.08***
PPO	159329.71***	3490683.64***	12641.35***

NS – not significant *P<0.05; **P<0.01; ***P<0.001; all treatments were made in triplicate.

(ANOVA) using SPSS software (version 20.0). Separation of the means was performed by Tukey's post-hoc test at P≤0.5. Before ANOVA, the data were checked for normality and variance homogeneity. All treatments were made in triplicate. Principal component analysis (PCA) was performed using R Studio software.

RESULTS

Effect of sunlight intensity and water stress on biomass accumulation and foliar macroelement uptake

Water stress, sunlight and their interaction significantly affected biomass accumulation and foliar nutrient uptake in argan tree seedlings (Table 1). Compared to seedlings in well-watered treatments, water stress treatments (MS and SS) reduced shoot and root dry mass and thus total dry mass under both sunlight regimes (P<0.001) (Table 2). Moreover, parallel with the reduction in total biomass, a decrease in nitrogen and phosphorus contents and an increase in other macroelements (calcium, potassium and magnesium) in leaves were recorded in stressed seedlings under both sunlight regimes. Moderate shading (60% of full sunlight) resulted in higher total biomass, higher foliar nitrogen and phosphorus contents and an accumulation of calcium, potassium and magnesium in water-stressed seedlings (MS and SS) as compared to seedlings grown under full sunlight (100%). The interaction of the water and sunlight regime had a significant impact on total and root biomass but did not affect the aboveground biomass (Table 1).

Effect of sunlight intensity and water stress on H_2O_2 and MDA contents and on membrane stability

Water stress had a significant impact on H_2O_2 and MDA contents and on the membrane stability index (MSI) under both levels of sunlight (Table 3). Compared to seedlings subjected to the treatments (well-watered), the water stress treatments (MS and SS) increased the H_2O_2 and MDA contents and decreased the MSI.

Table 2. Mean values of shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM), N, P, K, Ca and Mg of *A. spinosa* seedlings grown under two sunlight regimes (L1 and L2, 100% and 60% of full sunlight, respectively), exposed to three water regimes (well-watered (WW), medium stress (MS) and severe stress (SS); 100%, 50% and 25% of field capacity, respectively).

		SDM (g)	RDM (g)	TDM (g)	N (mg/g DW)	P (mg/g DW)	K (mg/g DW)	Mg (mg/g DW)	Ca (mg/g DW)
L1	**WW**	4.49 ± 0.99ab	3.82± 0.26a	8.31 ± 1.07ab	6.12± 0.38a	1.29± 0.07b	2.20 ± 0.01e	7.10 ± 0.05e	7.39 ± 0.05e
	MS	2.69 ± 1.03c	2.25 ± 0.90b	4.94 ± 1.69c	4.00 ± 0.30b	0.94 ± 0.03d	2.80 ± 0.02c	8.88 ± 0.04b	8.51 ± 0.02c
	SS	1.04 ± 0.11d	1.03 ± 0.12c	2.07 ± 0.21d	1.64 ± 0.34c	0.80± 0.00e	3.37 ± 0.01a	9.55 ± 0.05a	9.34 ± 0.03a
L2	**WW**	5.05± 1.05a	3.63± 0.69a	8.68 ± 1.54a	6.52± 5.78a	1.44± 0.04a	1.95 ± 0.03f	6.38 ± 0.04f	7,23 ± 0,09f
	MS	3.80± 0.87b	3.19± 0.30a	7.00 ± 1.03b	5,85± 0,24a	1.08± 0.01c	2.45 ± 0.04d	7.53 ± 0.18d	7.95 ± 0.05d
	SS	1,41± 0,54d	1,16± 0,16c	2,58 ± 0,68d	1,80± 0,33c	0.95± 0.01d	3.01 ± 0.08b	8.39 ± 0.10c	8.71 ± 005b

The means for each character followed by the same letter are not significantly different at 5% (Tukey's test); all treatments were made in triplicate.

Table 3. Mean values of the MSI, H_2O_2 and MDA of *A. spinosa* seedlings grown under two sunlight regimes (L1 and L2, 100% and 60% of full sunlight, respectively), exposed to three water regimes (well-watered (WW), medium stress (MS) and severe stress (SS); 100%, 50% and 25% of field capacity, respectively).

		MSI (%)	H_2O_2 (nmol/g FW)	MDA (nmol/g FW)
L1	**WW**	69.08 ± 0.53a	52.13 ± 0.50e	33.20 ± 0.29e
	MS	40.17 ± 1.34b	74.22 ± 0.41c	62.45 ± 0.89c
	SS	8.53 ± 0.06d	118.13 ± 0.77a	99.44 ± 1.29a
L2	**WW**	72.21 ± 0.45a	48.47 ± 0.25f	30.45 ± 0.51e
	MS	66.15 ± 1.04a	58.88 ± 0.58d	37.84 ± 1.95d
	SS	20.82 ± 3.94c	100.13 ± 0.77b	83.61 ± 0.51b

The means for each character followed by the same letter are not significantly different at 5% (Tukey's test); all treatments were made in triplicate.

Table 4. Mean values of superoxide dismutase (SOD), peroxidase (POD) and polyphenol oxidase (PPO) of *A. spinosa* seedlings grown under two sunlight regimes (L1 and L2, 100% and 60% of full sunlight, respectively), exposed to three water regimes (well-watered (WW), medium stress (MS) and severe stress (SS); 100%, 50% and 25% of field capacity, respectively).

		SOD (U mg^{-1} protein)	POD (nmol min^{-1} mg^{-1} protein)	PPO (U mg^{-1} protein)
L1	WW	494.80 ± 37.80e	525.34 ± 100.29de	878.99 ± 2.72de
	MS	1021.59 ± 39.34c	892.38 ± 106.62c	1208.02 ± 108.97c
	SS	2492.77 ± 23.91a	1614.68 ± 75.71a	2406.80 ± 70.77a
L2	WW	444.85 ± 15.47e	362.53 ± 5.19e	788.25 ± 10.54e
	MS	724.71 ± 20.44d	591.68 ± 96.27d	1007.32 ± 41.35d
	SS	2107.78 ± 16.65b	1258.69 ± 19.34b	2133.73 ± 6.93b

The means for each character followed by the same letter are not significantly different at 5% (Tukey's test); all treatments were made in triplicate.

Moderate shading (60% of full sunlight) was accompanied by the lowest H_2O_2 and MDA levels and the highest MSI in all water treatments (Table 3). Thus, the interaction of water regime and sunlight had a significant impact on H_2O_2, MDA and MSI (Table 1).

Effect of sunlight intensity and water stress on antioxidant enzyme systems

The effects of water and sunlight, and their interaction on the activities of antioxidant enzymes (SOD, PPO and POD) are shown in Table 1. Compared to seedlings grown in well-watered conditions, regardless of sunlight level, moderate or severe water stress significantly increased the activities of these enzymes (Table 4) ($P<0.001$). Water stress significantly increased the activities of the antioxidant enzymes under high light (100% full sunlight) in all water treatments (Table 4).

Principal component analysis (PCA)

The application of PCA is based on the use of mean values of data to establish similarities between different treatments and different variables (Fig. 1). Results showed that 97.5% of the total observed variability was explained by the first two dimensions (Dim); most of the variation was captured by Dim1 (89.7%), while Dim2 represented 7.8%. Antioxidant enzymes, H_2O_2, MDA, K, Ca and Mg were positively correlated to the first dimension (Dim1) (Fig. 1A). The parameters N, P, MSA, MSR, MST and MSI correlated with the second dimension (Dim 2) (Fig. 1A). PCA allowed for net discrimination between the different treatments associating with sunlight and water (Fig. 1B). The results revealed that the treatments, well-watered and medium stress under moderate shade, were characterized by higher values of N, P, MSA, MSR, MST and MSI in the left side, whereas severe stress treatments were related to high values of antioxidant enzymes, H_2O_2, MDA, K, Ca and Mg in the right side. However, the medium stress treatment under 100% sunlight where almost centered on Dim1 with intermediate values for all parameters.

DISCUSSION

Sunlight and water are two important factors affecting tree seedling development and seedling survival [9]. Our study evaluated the interactive effects of sunlight acclimatization and water stress on *A. spinosa* seedlings in nurseries. Water stress and high light (100% sunlight) limited total biomass accumulation and macronutrient uptake and increased antioxidant enzyme activities. Moderate light conditions (60% of full sunlight) reduced the detrimental effects of water stress on the growth of seedlings by increasing the total biomass in stressed seedlings, compared to full sunlight (100%), which could be a morphological response to water and light stress. These results are in agreement with several papers that showed that under moderate light levels, seedlings exhibited greater overall biomass accumulation than those exposed to full sunlight [9,25]. However, sunlight, in interaction with water stress, did not have a significant effect on the SDM/RDM ratio when compared to seedlings under well-watered treatments.

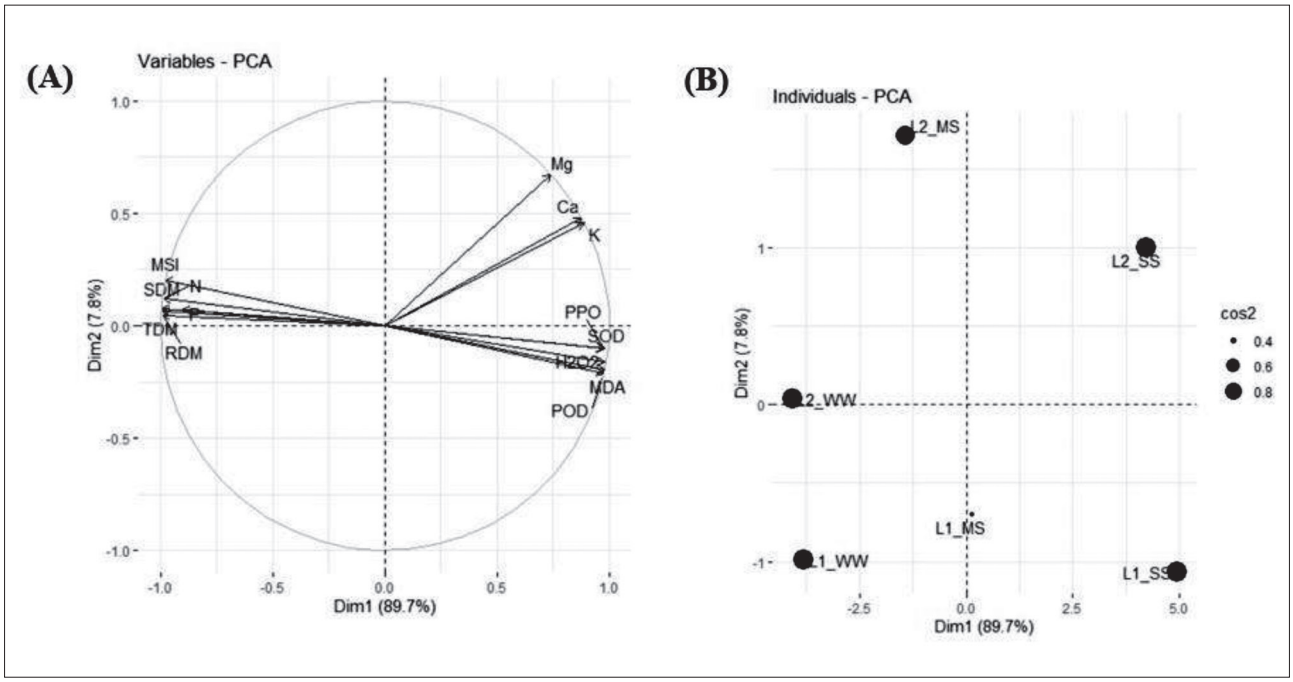

Fig. 1. Principal components analysis of the studied parameters (**A**) and the applied treatments (**B**) on *A. spinosa* seedlings after 3 months of acclimatization to water and light stress (sunlight). N: nitrogen, P: phosphorus, K: potassium, Ca: calcium, Mg: magnesium, SDM: shoot dry mass, RDM: root dry mass, TDS: total dry mass, MSI: membrane stability index, SOD: superoxide dismutase, POD: peroxidase, PPO: polyphenol oxidase, H_2O_2: hydrogen peroxide, MDA: malondialdehyde, L1WW: sunlight (100% of full sunlight) and well-watered, L1MS: sunlight (100% of full sunlight) and medium stress, L1SS: sunlight (100% of full sunlight) and severe stress, L2WW: sunlight (100% of full sunlight) and well-watered, L2MS: sunlight (60% of full sunlight) and medium stress, L2SS: sunlight (60% of full sunlight) and severe stress.

Water stress influences the absorption of macroelements, partly due to the decrease in soil moisture, which leads to a decrease in the rate of diffusion of nutrients from the soil to the roots, resulting in imbalanced plant mineral nutrition [26]. Drought reduces the transport of mineral nutrients from the roots to shoots due to reduced transpiration rates and changes in the functioning of membrane transporters [26]. We observed that as water stress intensifies, the uptake of nitrogen and phosphorus decreases, and that of potassium, calcium and magnesium increases. Nitrogen is an important macroelements needed for plant growth [27]. Under drought conditions, reduced transpiration of soybean and rice crops was reported to decreases nitrogen transport from roots to shoots, thus limiting nitrogen uptake [28]. Indeed, water stress perturbs nitrogen mobility in soil, resulting in a reduction in plant growth [27]. Regarding phosphorus uptake, its decreased concentrations were reported in watermelon and cherry tomato under drought conditions [29,30]. As for nitrogen, water stress also reduces phosphorus mobility because it moves mainly through diffusion [27]. Furthermore, the high leaf concentrations of potassium, calcium and magnesium recorded in stressed argan tree seedlings can be regarded as a physiological tolerance mechanism. Likewise, under drought conditions, argan seedlings were shown to accumulate high concentrations of potassium, calcium and magnesium [16]. Under water stress conditions, increased accumulation of potassium in grapevine plants was recorded, while also showing its contribution to the adjustment of osmotic potential [31]. The uptake of calcium can improve the hydrophobicity of the cell membrane and reduce its permeability, thus ensuring plant resistance to drought [32]. Increased accumulation of magnesium is essential for plant growth, and it was reported that Mg allows the activation of over 300 enzymes and plays a role in the synthesis of organic molecules necessary for plant growth [33]. Reduced mobility as well as the uptake of macroelements under water stress conditions reduces plant growth [27]. However, compared to seedlings in L1 (100% of full sunlight), argan tree

seedlings under moderate shade (60% full sunlight) appeared to be more tolerant considering their high contents of nitrogen and phosphorus and increased accumulation of potassium, calcium and magnesium under all water treatments. These results suggest that moderate shade can help *A. spinosa* seedlings overcome some of the detrimental effects of water stress.

ROS (including H_2O_2) are increased in plants subjected to different stresses [34], including drought stress[35], and they can lead to structural and functional changes in cells [36]. In our study, the increase in hydrogen peroxide (H_2O_2) content with water stress under both levels of sunlight (60 and 100%) indicates that stressed argan tree seedlings are exposed to oxidative stress. To estimate oxidative stress damage to cell membranes, MDA and the MSI served as indicators [37]. Generally, the content of MDA byproducts is a measure of the degree of lipid peroxidation caused by oxidative stress [38,39]. In the current study, the increase in MDA content and the decrease in the MSI in water-stressed seedlings under both sunlight treatments points to the degree of damage to leaf cell membranes by drought [40], and that lipid peroxidation decreased the MSI. Changes in cellular membrane caused by lipid peroxidation increase electrolyte leakage and osmotic imbalance [41]. In addition, increased water stress leads to decreased membrane stability and increased MDA [42]. However, the increase in H_2O_2 and MDA contents and the decrease in the MSI were lower in argan seedlings grown under moderate shade (60% of full sunlight), suggesting that moderate shade reduced the detrimental effects of oxidative stress by increasing the protective mechanism in plant cells.

The excess ROS and increased lipid peroxidation can be countered by various defense mechanisms, including enzymatic antioxidant mechanisms [7,15,39]. Under drought conditions, a significant increase in the activities of these enzymes was demonstrated in the argan tree, confirming their importance as effective defense mechanisms against oxidative damage [7,15]. SOD was considered the most powerful enzymatic antioxidant, providing the first line of defense against ROS [43]. SOD removes superoxide and then reduces the potential risk of hydroxyl radical formation [44]; POD plays a key role in preventing oxidative damage and maintaining cell membrane integrity by removing MDA and decreasing H_2O_2 content [7]; PPO catalyzes o-hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenols to quinones in the presence of oxygen [45]. In our study, the activities of these antioxidant enzymes increased with increasing water stress under both sunlight regimes. However, under the drought conditions, *A. spinosa* seedlings growing in moderate shade (60% of full sunlight) displayed significantly lower activities of the antioxidant enzymes SOD, POD and PPO when compared to those acclimatized under high light (100% of full sunlight). These results indicate that moderate shading can minimize the damage to water-stressed foliage [9].

CONCLUSION

Sunlight and water are important factors in the reforestation in semi-arid and arid regions. In this study, the effects of sunlight and water were evaluated and their effects on variables including biomass accumulation, macroelement (N, P, K, Ca and Mg) uptake, MSI, H_2O_2 and MDA contents and antioxidant enzyme (SOD, POD and PPO) activities in *A. spinosa* seedlings were compared. The growth, macroelement uptake and biochemistry of *A. spinosa* seedlings were influenced by the intensity of sunlight and water regime. Moderate shading (60% of full sunlight) resulted in a significant accumulation of biomass and macroelements, significant membrane stability and a reduction in H_2O_2 and MDA levels and antioxidant enzyme activities as compared to seedlings exposed to full sunlight (100%). These results reinforce the idea that the detrimental effects of water stress are relieved by moderate shade (60% of full sunlight). In addition, PCA showed that moderate shading was more effective in reducing or even eliminating the adverse effects of average water stress in seedlings. These findings suggest that for effective acclimatization to water and light stress, seedlings should be grown under moderate shade (60% of full sunlight) and exposed to medium water stress. However, to assess and confirm these conclusions, further field research will be needed.

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Author contributions: A. Hachemi, O. Said Ali, T. Belghazi and S. El Messoussi conceived and designed the experiments. A. Hachemi,

O. Said Ali, T. Belghazi, A. Lahrouni, S. El Mercht and C. El Hassan performed the experiments and the acquisition, analysis and interpretation of data. A. Hachemi, T. Belghazi and S. El Messoussi wrote the paper. T. Belghazi and S. El Messoussi supervised the work. All authors reviewed and approved the final manuscript.

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