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Effect of soil moisture content and nitrogen fertilizer on survival, growth and some physiological characteristics of *Platycladus orientalis* seedlings

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Abstract: Given the important applications of oriental arborvitae (*Platycladus orientalis* /L./ Franco), we examined the interaction effects of soil moisture content (*SMC*) and urea fertilizer on survival, growth and some physiological characteristics of seedlings of this species. In this study, 270 uniform oriental arborvitae seedlings were transplanted in 2 kg containers filled with the top soil of the natural stand in Soorkesh forest reserve. After 2 months, 6 levels of *SMC* (100%, 75%, 50%, 25%, 15% and 10%) and 3 levels of urea [control: 0, low nitrogen (*LN*): 75 mg·kg^{-1}, and high nitrogen (*HN*): 150 mg·kg^{-1}] were used from June till the end of the first growing season in 3 replications and 5 seedlings per replication as subsamples. The results showed that all seedlings survived under *SMC* above 25% in control and urea fertilizer treatments. However, under 15% and 10% *SMC*, the seedling survival decreased significantly in control treatment, but adding urea fertilizer significantly increased the survival, especially in *HN* treatment. Growth and morphological properties of seedlings, including root and shoot length, number of first-order lateral roots and total biomass, showed a significant decrease at 75%. Seedling quality index gradually decreased with *SMC* reduction, but the addition of urea to the soil significantly increased it under all *SMC* treatments. The results also indicated that a decrease in *SMC* significantly decreased relative water content of leaves and total chlorophyll content, and urea fertilizer increased total chlorophyll under all *SMC* treatments, but it increased relative water content only at 100% to 25%. Moreover, water use efficiency of *P. orientalis* seedlings significantly increased under low soil moisture treatments, and urea significantly increased it, especially under *HN* treatment. In conclusion, urea fertilizer, especially in the higher amount (150 mg·kg^{-1}), improved growth and quality of seedlings under different soil moisture conditions, and increased their survival under severe drought.

Keywords: soil moisture content; nitrogen fertilizer; oriental arborvitae; seedling quality; water use efficiency; urea

Oriental arborvitae (*Platycladus orientalis*) is the only species in its genus within the family *Cupresssaceae* that was previously included in the genus *Thuja* (Bonner, Brand 2008; Li et al. 2016). This is an important medium-sized long-lasting evergreen tree that is widespread naturally in some areas of Asia (northwestern China, Korea and Far East Russia) and naturalized in Europe, North America, eastern Africa and some other Asian countries, including Japan, India and Iran. This species is widely used in designing green space and forest parks, expanding forest habitats (Bansal et al. 2011), pharmaceutical industry (Amit et al. 2011), and phytoremediation practices (Nuhoglu, Oguz 2003; Malkoc 2006). Commonly, oriental arborvitae is used as a forestation species in vulnerable areas due to its resistance to cold, dry, and salt environment, and there are about 50 countries suitable for

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introduction and cultivation of this species with an area of 2.0 × 10^7 km^2, which occupies 13.8% of land area on the Earth (Li et al. 2016). Harsh environmental conditions such as relatively long dry season, mountains and steepness, shallow and poor soil, activities of various socio-economic destroying factors, and increased environmental stresses, reduce the natural regeneration of this species in its natural distribution areas, so that it has recently been categorized as a near-threatened species in the IUCN red list (Farjon 2013). Therefore, study of factors affecting growth and survival of *P. orientalis* seedlings, as well as strategies to increase their resistance to environmental stresses are of high importance in forestry and nursery management. Drought is the most important environmental limiting factor for plant growth with its increase forecasted in most of the climate change global scale scenarios (Handmer et al. 2012; Dai 2013). In this situation, soil moisture decreases to the point when plants are hardly able to absorb water quickly enough to compensate transpiration (Schmidt 1973). Because drought and water stress reduce water content in plant tissues, they can bring about many changes in uptake, translocation and assimilation of nutrients in plants (French, Turner 1991 (cv. Danja); da Silvae et al. 2011). Therefore, they can reduce photosynthesis, growth and survival of conifer seedlings (Brix 1979). Several authors have studied the effect of water stress and irrigation period on conifer seedling growth, physiology and survival (Edwards, Dixon 1995; Villar-Salvador et al. 2005; Yang et al. 2007; Aaltonen et al. 2017). However, other environmental factors also vary within the light gradients in a correlative manner. Specifically, the leaves exposed to higher irradiance suffer from more severe heat, water, and photoinhibition stresses. Research in tree canopies and across gap-understorey gradients demonstrates that plants have a large potential to acclimate to interacting environmental limitations. The optimum temperature for photosynthetic electron transport increases with increasing growth irradiance in the canopy, improving the resistance of photosynthetic apparatus to heat stress. Stomatal constraints on photosynthesis are also larger at higher irradiance because the leaves at greater evaporative demands regulate water use more efficiently. Furthermore, upper canopy leaves are more rigid and have lower leaf osmotic potentials to improve water extraction from drying soil. The current review highlights that such an array of complex interactions significantly modifies the potential and realized whole canopy photosynthetic productivity, but also that the interactive effects cannot be simply predicted as composites of additive partial environmental stresses. We hypothesize that plant photosynthetic capacities deviate from the theoretical optimum values because of the interacting stresses in plant canopies and evolutionary trade-offs between leaf- and canopy-level plastic adjustments in light capture and use. Saplings of one clone of Norway spruce, *Picea abies* (L.) However, few studies investigated the interaction effects of water stress and soil nutrient availability on conifer seedlings (Boyle and Hellenbrand 1991; Cuesta et al. 2010; Dosskey et al. 1993; Villar-Salvador et al. 2013). So, there is no adequate evidence regarding the effect of fertilization on the sensitivity of forest tree seedlings to drought (Binkley, Fisher 2013). The study of Graciano et al. (2005) revealed that soil enrichment with mineral nutrients such as nitrogen (N) can improve seedling water stress resistance. Reinbott and Blevins (1999) showed that soil fertilization can increase the water absorption potential of seedlings. Most of the recent studies have focused on the interaction of drought with nitrogen nutrition, since it is an important nutrient that tree seedlings require in higher amount and changes of its availability make great variations in seedling growth and performance (Villar-Salvador et al. 2013). Additionally, water relations in a plant are greatly influenced by its nitrogen status, so that nitrogen and water often interact (da Silva et al. 2011). Under drought conditions, soil water content decreases extremely, and mobility of nitrogen is restricted severely. Then plant nitrogen uptake decreases and nitrogen deficiency occurs in water stressed plants. Wu et al. (2008) showed that appropriate nitrogen supply, using nitrogen fertilizer, can alleviate undesirable effects of drought on seedling growth, leaf photosynthesis and water use efficiency of plants in *Sophora davidii*, but a high level of nitrogen fertilizer had adverse or low effects (Wu et al. 2008). da Silva et al. (2011) stated that too much nitrogen fertilizer increases a water potential in the soil, and decreases the uptake of essential nutrients, cell and tissue development and growth of plant. In other words, the effect of soil moisture content on survival, growth and physiology of tree seedlings can be changed under different nitrogen fertilizer treatments. Hence, a better understanding of the role of

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nitrogen fertilizer in survival, growth and physiology of tree seedlings under water stress conditions can improve fertilizer management in arid and semi-arid forest nurseries. Urea ($CO(NH_2)_2$) is nowadays the most prevalent nitrogen fertilizer in forest nurseries due to its low price, high (46%) nitrogen content, and quick absorption in plant (Faustino et al. 2015; South 2018). When urea is placed in the soil, it enters the plant directly or in the form of ammonium nitrate after degradation by soil microbes (Witte 2011). Positive effects of urea on growth and physiology of the Cypress family seedlings were reported in some researches, including *Thuja plicata* (Weetman et al. 1988) and *Cupressus macrocarpa* (El-Keltawi et al. 2012). However, some authors stated that applying too much granular urea had depressing effects on young seedlings (South 2018). Therefore, special precautions should be taken in the application of urea in a nursery. Faustino et al. (2015) stated that urea fertilization produces changes similar to those induced with drought, and urea-fertilized plants will better tolerate soil drying conditions (Faustino et al. 2015). In this context, it is important to investigate the response of nursery plants to soil moisture conditions in different doses of urea fertilization. Given the importance of *P. orientalis*, this study aimed to prepare more detailed scientific information on the interaction effects of urea fertilization and soil moisture content on survival, morphological and physiological characteristics of seedlings in the first growing season. We hypothesized that (*i*) seedlings of *P. orientalis* cannot tolerate severe drought for a long time, but using urea fertilizer can improve their survival, growth and physiological characteristics under all soil moisture conditions; (*ii*) the amount of urea fertilizer used can change its effects on *P. orientalis* seedlings under different soil moisture conditions.

MATERIAL AND METHODS

Study area and preparation of materials. This study was conducted in a research nursery of Gorgan University of Agricultural Sciences and Natural Resources (36.8°N latitude, 54.48°E longitude) in the north of Iran. In order to produce required normal seedlings for the study, 4 000 seeds were collected from 10 trees that are located in a *P. orientalis* seed orchard of the Ghorogh forest nursery (elevation 120 m a.s.l.) in November, 2014. Seeds were sown in growing trays (filled with 1 : 1 : 2 mix of sand, compost and soil) and grown at a temperature of 25 °C after two weeks of cold stratification (Dumroese et al. 2008). Newly grown seedlings were transplanted in 2l containers filled with *P. orientalis* forest reserve area (Soorkesh) top soil (upper 25 cm layer composed of O and A horizons) in February 2015. All transplanted seedlings were irrigated regularly till the commencement of water stress study.

Experimental design. Application of water stress and fertilization treatments started on 1st June, 2015. Two hundred and seventy seedlings were grown under six soil moisture content treatments (100, 75, 50, 25, 15 and 10% of available water) and three urea fertilization treatments [150 mg.kg^{-1} (*HN*), 75 mg·kg^{-1} (*LN*), and no fertilizer (Control)], using a randomized complete block design 6 × 3 factorial experiment with 3 replications. In order to estimate survival percentage and reliable average values of growth and physiology of seedlings, 5 seedlings were placed in each of the replications as subsamples. Different characteristics of seedling growing media (typical top soil of the *P. orientalis* forest reserve area in Iran) were determined in the laboratory (Table 1). Based on initial soil information and Equation 1, the relation between the soil matric potential (Ψ_m) and soil volumetric water content (θ_v) was determined, and field capacity and permanent wilting point were estimated at 0.03 and –1.5 MPa, respectively (Kirkham 2005).

$$\Psi_m = A \times \theta_v^B \tag{1}$$

In Equation 1, A and B (θ) were calculated based on Equations 2 and 3, using sandy soil (S) and clay (C) contents. Because the seedlings cannot absorb soil water under permanent wilting point, available water was calculated from the difference between field capacity and permanent wilting point (Kirkham 2005). Then, the weight of absorbable

Table 1. Characteristics of *Platycladus orientalis* forest reserve area (Soorkesh) top soil that was used as growing medium in the study

Soil texture	Clay	Silt	Sand	Bulk density (g·cm^{-3})	Total nitrogen (%)	Available phosphorus (ppm)
	(%)					
Loamy-Silt	21	61	18	1.55	0.09	0.528

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soil water in different soil moisture content (*SMC*) treatments was calculated by multiplying percentage, container soil dry weight, and treatment percentage. Also, the target container weight was calculated in each of *SMC* treatments, using the sum of container weight (102 g), soil dry weight (1 760 g), and weight of absorbable soil water in different *SMC* treatments. Container weight was monitored every three days (40 times) during the *SMC* treatments, and the amount of irrigation for each of the containers was calculated from the difference between current container weight and target container weight. To prevent excessive soil water evaporation, soil surface and container side walls were covered with thin aluminium foils. In order to perform high (*HN*) and low (*LN*) nitrogen fertilizer treatments, 150 and 75 milligrams urea per kilogram of soil were dissolved in irrigation water every 30 days till the end of the experiment, based on Villar-Salvador et al. (2005) proposed nitrogen fertilizer levels for Mediterranean species. No fertilizer was added to the soil in the control treatment.

$$A = \exp\left[-4.396 - 0.0715\,C - 4.88 \times 10^{-4}\,(S)^2 - 4.285 \times 10^{-5}\,(S)^2(C)\right] \quad (2)$$

$$B = -3.14 - 0.00222\,C^2 - 3.484 \times 10^{-5}\,S^2C \quad (3)$$

where:
S – sandy soil;
C – clay contents.

Measurements and calculations. Seedling survival was recorded monthly from the start of the experiment based on visual examination of seedling aboveground parts. All of the survived seedlings were separated from the pot soil, and carefully rinsed in tap water at the end of the first growing season in October 2015. Seedling roots and shoots were separated using small secateurs. Then, shoot length (*H*), shoot diameter (*D*), numbers of first-order lateral roots (*FOLR*) with minimum diameter of 1 mm in each of the seedlings were measured (Davis et al. 1999). The separated parts of seedlings were oven-dried at 80 °C for 48 hours, and weighed using a digital scale (accuracy 0.001 g) to determine seedling root biomass (*RB*), shoot biomass (*SB*) and total biomass (*TB*).

Based on these measurements, seedling quality index (*SQI*) was calculated using Equation 4 (Dickson et al. 1960). In order to determine the relative water content of seedling leaves (*RWC*), three healthy and fully developed leaves bearing branches were collected from the top of seedling crown. Fresh weight (*FW* in Equation 5) of collected leaf bearing branches was determined.

All of the leaf branches were immersed in distilled water in a dark environment and at a temperature of 25 ± 3° C for 24 h. After imbibition period, turgid weight (*TW* in Equation 5) of samples was determined. All of the samples were placed in a preheated oven at 70 °C for 48 h, and weighed using a digital scale with 0.001 g accuracy to determine the dry weight of leaf bearing branches (*DW* in Equation 5). Then, *RWC* was calculated using Equation 5 (Kirkham 2005; Yang et al. 2007).

Total leaf chlorophyll content was estimated four times in each seedling using a SPAD 502Plus Chlorophyll Meter. All seedlings were washed and separated from the soil at the end of the first growing season in December 2015. Seedling roots and aboveground parts were separated using a clipper, and dried at 105 °C for 20 h to determine aboveground, root and total biomass of each seedling. Additionally, seedling water use efficiency (*WUE*) was calculated using Equation 6 (Bacon 2009).

$$SQI = TB/(RB/SB+H/D) \quad (4)$$

where:
TB – total biomass;
RB – root biomass;
SB – shoot biomass;
H – shoot length;
D – shoot diameter.

$$RWC = ((FW-TW)/(TW-DW)) \times 100 \quad (5)$$

where:
FW – fresh weight;
TW – turgid weight;
DW – dry weight.

$$WUE\ (\text{g}\cdot\text{kg}^{-1}) = (TB)/(\text{Water Consumption}) \quad (6)$$

where:
TB – total biomass.

Statistical analysis. Data analysis was performed using the analysis of variance (ANOVA) method based on a randomized complete block factorial design. Normality of model residuals and equality of variances were tested using Kol-

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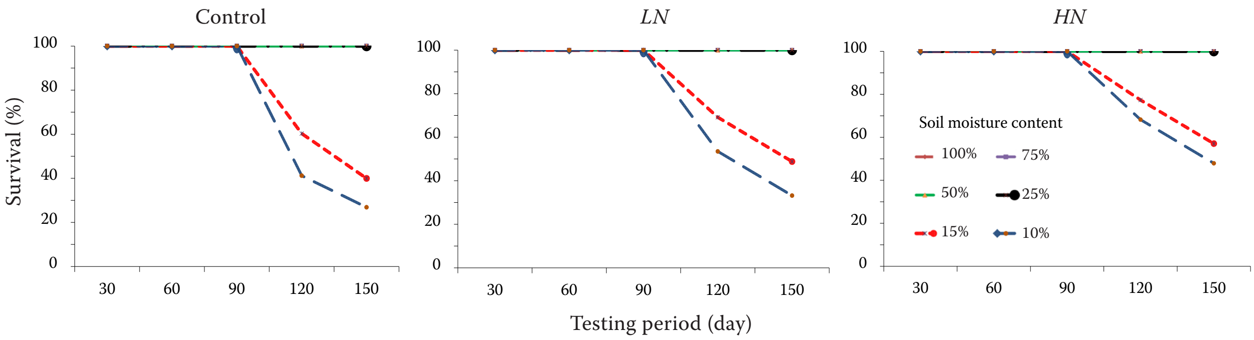

Figure 1. Variation of seedling survival in the testing period under different soil moisture content in control (no fertilizer), low nitrogen (*LN*) and high nitrogen (*HN*) fertilizer treatments; (*LN*, *HN* represent 75 and 150 mg·kg^{-1} urea, respectively)

mogorov-Smirnov and Levene's methods, respectively (Zar 2009). Situations in which ANOVA specified significant differences in main effects or interaction effects are at a 95% confidence level, Duncan's multiple comparisons tests were used for detailed comparisons. In order to summarize common patterns of variation among variables, and for better visualization of the multidimensional relation of *SMC* and seedling properties data under different nitrogen fertilizer treatments, principle component analysis (PCA) was performed using SPSS Statistics (Version 19).

RESULTS

Survival

In treatments with soil moisture content higher than 25%, the survival rate was not reduced from the initial value (100%) until the end of the study, but in 15 and 10% treatments, the survival of seedlings started to decrease to 69% and 54%, respectively, on day 90 (30th August). A decrease in survival rate in these treatments continued till the end of the experiment, so that it reached 49% and 36%, respectively (Figure 1). Though, compared to the control, nitrogen fertilizer (*NF*) treatments had a slight effect on survival rate after day 90.

Analysis of variance showed significant main and interaction effects of *SMC* and *NF* on survival percentage at the end of the first growing season ($F_{(5,34)SMC}$ = 22231.15, P = 0.000; $F_{(2,34)NF}$ = 502.58, P = 0.000; $F_{(10,34)SMC \times NF}$ = 209.78, P = 0.000). So that, in all *NF* levels, survival of seedlings was not reduced from 100% until *SMC* reached 15%, but in 15 and 10% *SMC*, mean seedling survival decreased significantly at all *NF* levels. Moreover, maximum seedling survival was recorded in high nitrogen fertilizer (*HN*) treatments (Figure 2).

Growth and quality index

The results showed that the main effect of *SMC* on all growth characteristics of *P. orientalis* seedlings, including shoot biomass ($F_{(5,34)}$ = 65.84, P = 0.000), root biomass ($F_{(5,34)}$ = 78.05, P = 0.000), total biomass ($F_{(5,34)}$ = 22.70, P = 0.000), shoot to root biomass ratio ($F_{(5,34)}$ = 6.98, P = 0.000), first-order lateral roots ($F_{(5,34)}$ = 14.38, P = 0.000), and seedling quality index ($F_{(5,34)}$ = 24.82, P = 0.000), was significant, but the main effect of *NF* was significant on root biomass only ($F_{(2,34)}$ = 5.41, P = 0.009). Moreover, the interaction effect of *SMC* and *NF* on shoots ($F_{(10,34)}$ = 2.42, P = 0.026), roots ($F_{(10,34)}$ = 2.24, P = 0.038) and shoot to root biomass ratio ($F_{(10,34)}$ = 2.49, P = 0.022) of seedlings was significant. Decreasing *SMC* from 100 to 75% significantly decreased SB in control and nitrogen fertilizer treatments (Figure 2). Gradual decrease in SB continued along the decrease of *SMC* from 75 to 25%, so that the lowest *SB* was recorded in 10% *SMC*. The highest value of *SB* was observed in 100% treatments. *NF* had no significant effect on *SB* at 100% to 15% *SMC*, but compared to control treatment, the use of urea fertilizer under *HN* and *LN* treatments significantly increased *SB* at 10% *SMC*. Moreover, multiple comparisons of means showed that the highest values of *RB*, *FOLR* and *SQI* were estimated at 100% and 75% *SMC* (Figure 2). These average values gradually started to decrease at 50% and 25%, and reached their lowest values at 15% and 10%. *NF* had no significant effect on *TB*, *FOLR* and *SQI*. Also, except for 10%, *RB* was not affected by *NF*. Conversely at 10% *SMC*, *NF* significantly decreased *RB* compared to control treatment.

Water use efficiency and physiological characteristics

The results indicated that main effects and interaction effects of *SMC* and *NF* on water use effi-

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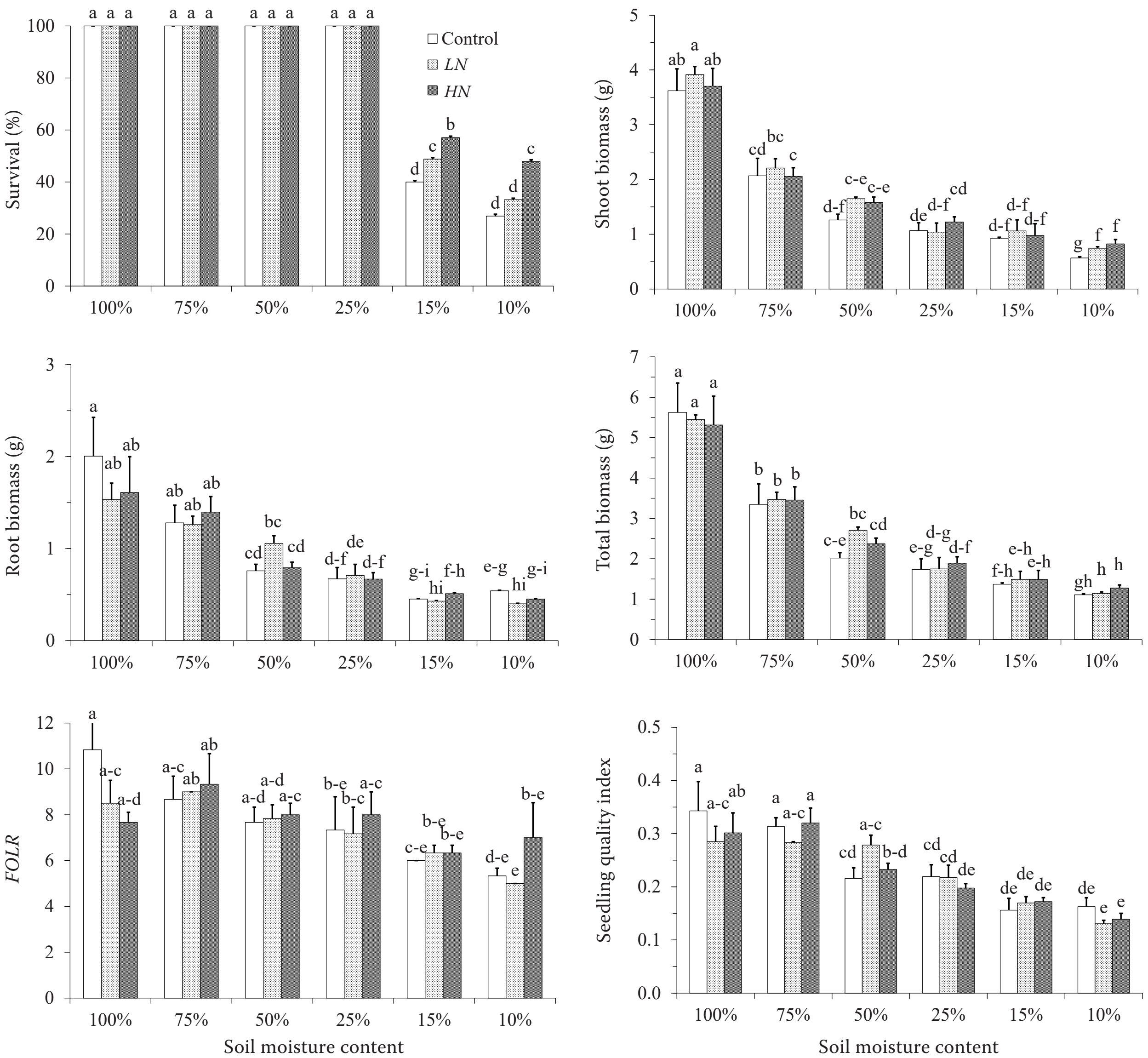

Figure 2. Survival, growth characteristics and quality index (arithmetic mean ± standard error) of *Platycladus orientalis* seedlings for treatment combinations of soil moisture content and nitrogen fertilizer (*LN* and *HN* represent 75 and 150 mg·kg^{-1} urea fertilizer, respectively; means followed by the same letters denote no significant differences at α= 0.05) *FOLR* – first-order lateral roots; *LN* – low nitrogen; *HN* – high nitrogen

ciency ($F_{(5,34)SMC}$ = 30.18, *P* = 0.000; $F_{(2,34)NF}$ = 58.46, *P* = 0.000; $F_{(10,34)SMC×NF}$ = 8.26, *P* = 0.000), relative water content ($F_{(5,34)SMC}$ = 917.62, *P* = 0.000; $F_{(2,34)NF}$ = 98.04, *P* = 0.000; $F_{(10,34)SMC×NF}$ = 17.62, *P* = 0.000), and total chlorophyll content ($F_{(5,34)SMC}$ = 109.58, *P* = 0.000; $F_{(2,34)NF}$ = 13.10, *P* = 0.000; $F_{(10,34)SMC×NF}$ = 4.51, *P* = 0.000) of *P. orientalis* seedlings were statistically significant. Water use efficiency increased in lower moisture conditions, especially at *NF* treatments (Figure 3). In the control treatment, *WUE* did not change at different *SMC* levels until soil moisture was reduced from 100% to 25%, however there was a significant increase in *WUE* at 15% and 10%. Compared to the control, in the *NF* treatments, an increase of *WUE* started from the higher *SMC* levels (25% and 75% at *LN* and *HN*, respectively). In the *HN* treatments, *WUE* increased significantly from 100% to 25% as such, its maximum value (8.33 g·kg^{-1}) was found at 25%, and then the value decreased significantly at 15% and 10%. The highest values of *RWC* and total chlorophyll content (*TCh*) in different *NF* treatments were observed at 100% (Figure 4). More-

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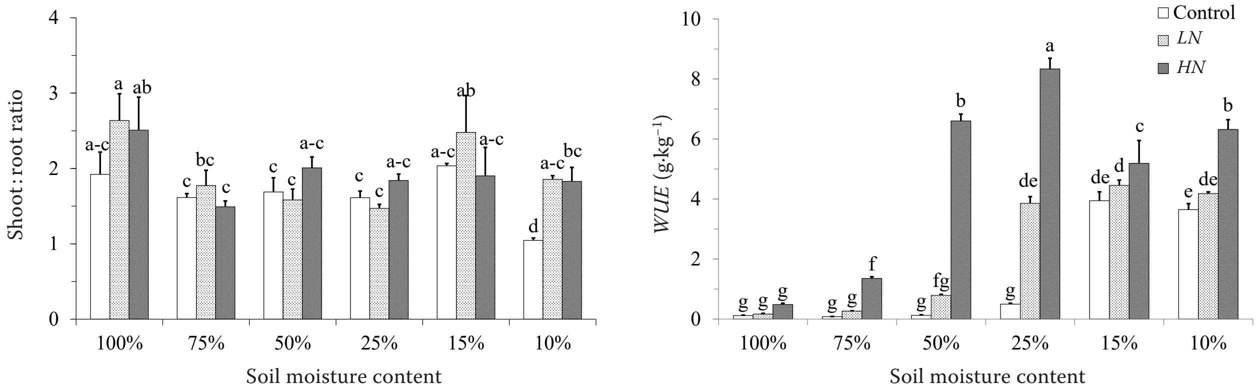

Figure 3. Shoot to root ratio and water use efficiency (*WUE*, arithmetic mean ± standard error) of *Platycladus orientalis* seedlings for treatment combinations of soil moisture content and nitrogen fertilizer (*LN* and *HN* represent 75 and 150 mg·kg^{-1} urea fertilizer, respectively; means followed by the same letters denote no significant differences at α= 0.05) *LN* – low nitrogen; *HN* – high nitrogen

over, the application of *HN* maximized these values. As displayed in Figure 4, *RWC* and *TCh* decreased gradually with a decrease in *SMC*, so that the minimum values of these characteristics were found at the lowest *SMC* level. Application of nitrogen fertilizer, especially in *HN* treatment, significantly increased *TCh* at all *SMC* levels. However, the effects of *NF* on *RWC* were not the same at different *SMC* levels. The results showed that at the higher *SMC* levels (100% to 25%), *HN* treatment increased *RWC* compared to control treatment, but at the lower *SMC* levels (15% and 10%), different results were found (Figure 4). So that, at 15%, *NF* had no significant effect on *RWC*, but at 10% *RWC* decreased significantly by adding nitrogen fertilizer.

Relationships of survival and *WUE* with seedling properties

As shown in Table 2, there was a significant positive correlation between survival rate and morphological and physiological traits of *P. orientalis* seedlings, except for *FOLR* in *HN* treatment. Also, negative correlations of these characteristics and *WUE* were significant. Moreover, the results showed that in many cases, nitrogen fertilizer, especially at *LN* treatment, increased absolute values of these correlation coefficients. Furthermore, the results of principal component analysis (PCA) showed that except for two features (shoot to root ratio and *FOLR*), variation of other traits was almost

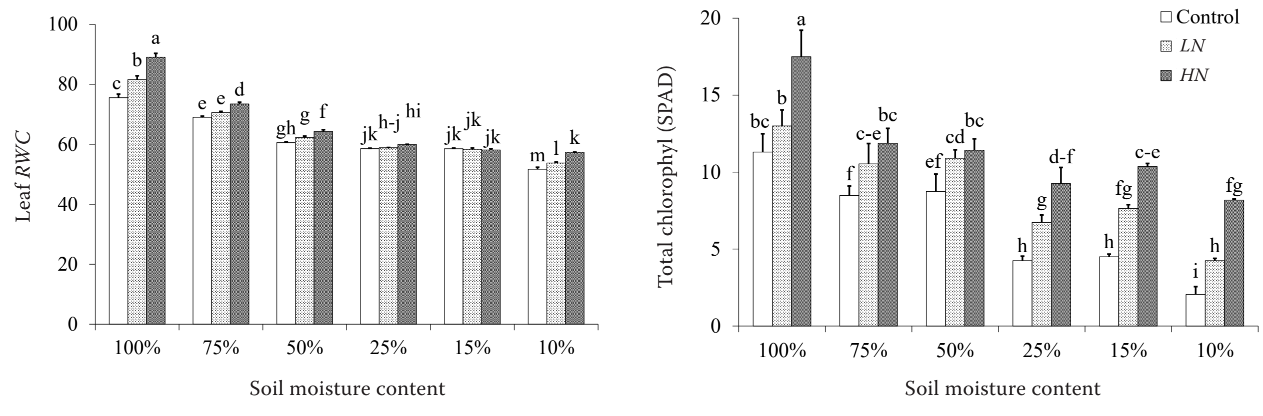

Figure 4. Relative water content (*RWC*) and total chlorophyll content (arithmetic mean ± standard error) of *Platycladus orientalis* seedlings for treatment combinations of soil moisture content and nitrogen fertilizer (*LN* and *HN* represent 75 and 150 mg·kg^{-1} urea fertilizer, respectively; means followed by the same letters denote no significant differences at α= 0.05) *LN* – low nitrogen; *HN* – high nitrogen

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Table 2. Relation of survival and water use efficiency and morphological and physiological traits (correlation coefficients) in *Platycladus orientalis* seedlings in control (no fertilizer), low nitrogen (*LN*) and high nitrogen (*HN*) fertilizer treatments

Item	Treatment	*SB*	*RB*	*TB*	*FOLR*	*SQI*	*RWC*	*TCh*
Survival	Control	0.565*	0.520*	0.558*	0.595*	0.649**	0.675**	0.710**
	LN	0.569*	0.761**	0.645**	0.728**	0.853**	0.635**	0.699**
	HN	0.582*	0.591**	0.600**	0.425^{ns}	0.713**	0.582*	0.484*
WUE	Control	–0.578*	–0.556*	–0.580*	–0.601**	–0.666**	–0.665**	–0.710**
	LN	–0.794**	–0.898**	–0.851**	–0.728**	–0.861**	–0.822**	–0.836**
	HN	–0.774**	–0.750**	–0.786**	0.121^{ns}	–0.705**	–0.845**	–0.726**

*and ** indicate significant correlation at 0.05 and 0.01, respectively; ^{ns} indicates non-significant correlation at 0.05; *FOLR* – first-order lateral roots; *RB* – root biomass; *RWC* – relative water content; *SB* – stem biomass; *SMC* – soil moisture content; *SQI* – seedling quality index; *SR* – shoot to root ratio; *TB* – total biomass; *TCh* – total chlorophyll content; *WUE* – water use efficiency

explained by the first PCA axis, which is closely related to soil moisture content in all fertilizer treatments (Figure 5). Water use efficiency was in the opposite direction to the other attributes. Also, in high nitrogen treatment, the variations of shoot to root ratio and *FOLR* were mostly explained by the second PCA axis, and the location of these features in PCA chart changed to a great extent in the control and low nitrogen fertilizer treatment (Figure 5).

DISCUSSION

Survival

In this study, all *P. orientalis* seedlings remained alive in treatments with soil moisture contents above 25%. This fact shows the high drought tolerance potential of this species in the first growing season. However, the results revealed that a reduction of soil moisture content below 25% field capacity (15 and 10%) can significantly decrease the seedling survival in the first growing season, which is in agreement with the findings of pressure chamber study in this species (Jiyue 1989) and with the studies on some other tree species such as *Sophora davidii* (Wu et al. 2008) and *Quercus macrocarpa* and *Quercus ellipsoidalis* (Davis et al. 1999). Some authors reviewed and described the reasons for plant mortality under drought conditions, including hydraulic failure, carbon starvation and increased attacks by biotic agents (Kin 2015; McDowell et al. 2008). The result of this study revealed that the mortality of *P. orientalis* seedlings did not occur until day 90 even in extreme drought conditions at *SMC* 15% and 10% treatments. This finding can indicate the high drought resistance of this species. Some authors explained the main

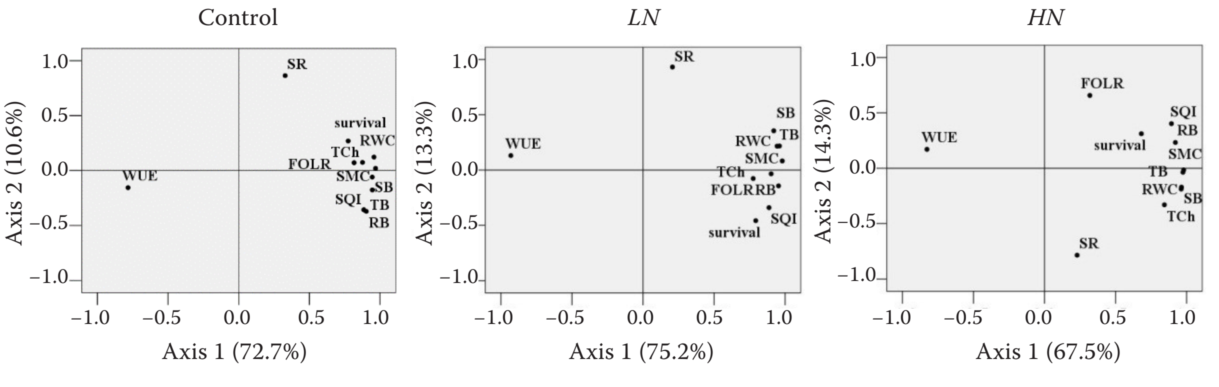

Figure 5. Principal component analysis (PCA) with soil moisture content (*SMC*) and different characteristics of *Platycladus orientalis* seedlings in control, low (*LN*) and high (*HN*) nitrogen fertilizer treatments; *FOLR* – first-order lateral roots; *RB* – root biomass; *RWC* – relative water content; *SB* – stem biomass; *SMC* – soil moisture content; *SQI* – seedling quality index; *SR* – shoot to root ratio; *TB* – total biomass; *TCh* – total chlorophyll content; *WUE* – water use efficiency

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causes of high water stress resistance in some conifers, including *Cupressaceae* species (Pittermann et al. 2010; Kin 2015). The most important factors to withstand water stress conditions in the majority of *Cupressaceae* species, including *Platycladus orientalis*, are specialized xylem tissues with extreme resistance to embolism and cavitation that enable the seedlings to endure extreme water stress for a long period (Brodribb et al. 2014). Moreover, the results showed that the addition of N fertilizer in the soil slightly compensated lethal effects of water stress in *P. orientalis* seedlings. Hence, under 15 and 10% treatments, increasing nitrogen fertilizer from low to high levels in the soil (*HN* to *LN*) increased the seedling survival from 22.3 to 43.1% and 19.7 to 78.1%, respectively. Likewise, some authors indicated that nursery cultural treatments such as nitrogen fertilization can increase the survival of some conifer seedlings (*Pinus contorta*, *Pinus pinea*, *Pinus halepensis* and *Juniperus thurifera*) under xeric and Mediterranean conditions (Villar-Salvador et al. 2013).

Growth and quality index

The results of this study showed that regardless of nitrogen fertilizer treatments, a decrease in *SMC* significantly limited all investigated growth characteristics of *P. orientalis* seedlings, which was in agreement with the findings of studies on some other tree species such as *S. davidii* (Wu et al. 2008), *Pseudotsuga menziesii* (Khan et al. 1996) and four Mediterranean oak species, including *Q. frainetto, Q. conferta, Q. pubescens* and *Q. ilex* (Fotelli et al. 2000). The reasons for the growth reduction of tree seedlings under water stress conditions have been described by some authors so far (Bréda et al. 2006; Shao et al. 2008; Chelli-Chaabouni 2014). Water stress modifies different physiological and biochemical processes involved in plant growth (da Silva et al. 2011).

Root hydraulic conductance, tissue turgor, stomatal conductance, nutrient uptake, leaf growth, photosynthesis rate, and cell division and enlargement are reduced in drought conditions. So that in a long period of water shortage, total biomass of seedlings can be reduced and specific morphological changes in plant can occur (Pugnaire et al. 1999). Application of *NF* treatments did not alter a diminishing trend of growth which was caused by *SMC* reduction. Besides, the effects of nitrogen fertilizer were significant only under severe drought at 10% *SMC*. So *SB* was increased and *RB* was decreased significantly by the use of *NF* treatments. These findings showed a negative effect of urea fertilizer on the shoot to root biomass ratio at extreme drought conditions. So that an increase in the aboveground biomass of seedlings can increase water loss, and a decrease in root biomass can decrease the water absorption capacity of seedlings in extreme drought. Dickson et al. (1960) indicated that the index of seedling quality (*SQI*) combined morphological features of seedlings, and that soil nutrient content is closely related to *SQI*.

Our results confirmed it: under all soil moisture content treatments, nitrogen fertilizer significantly increased *SQI* of *P. orientalis*, which was in agreement with the findings of Quanhong et al. (1989) in *P. orientalis*; Ahmadloo et al. (2012) in two Cypress species (*C. arizonica* and *C. sempervirens*). Ahmadloo et al. (2012) stated that *SQI* is the most appropriate indicator of potential growth and survival of nursery seedlings in the field, because we can predict good field growth and survival for *P. orientalis* seedlings that were grown under soil nitrogen fertilization in the nursery, but showing this requires a field experiment.

This could also be concluded from the strong and significant correlation coefficient of *SQI* with survival and *WUE* of seedlings at the different *SMC* and *NF* treatments (Table 2). The results showed that the effect of nitrogen fertilizer on root biomass of *P. orientalis* seedlings is dependent on the soil moisture content level. As such, under high *SMC* (100 and 75% *FC*), addition of nitrogen fertilizer significantly increased seedling root biomass, but the reduction of soil moisture content in treatments with soil moisture content less than 50% eliminated this effect.

Several studies confirmed the significant effect of N fertilizer on the root growth of *P. orientalis* (Nahed et al. 2010) and some other conifer seedlings, including *Pinus halepensis* (Oliet et al. 2004), *Picea abies* and *Pinus sylvestris* (Gruffman et al. 2012), *Cupressus arizonica* and *Cupressus sempervirens* (Ahmadloo et al. 2012) and *Pinus taeda* (Faustino et al. 2015) in the absence of soil water stress. da Silva et al. (2011) and DaMatta et al. (2002) indicated that under soil water stress conditions and prolonged period of drought, nitrogen mobility and nutrient supply may be severely restricted.

In this situation, the diffusion rate of nutrients may be decreased to the absorbing root surface

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(Hu, Schmidhalter 2005), because application of nitrogen fertilizer did not increase seedling root biomass.

Physiological characteristics and water use efficiency

The results indicated that a decrease in soil moisture content significantly decreased the *RWC* of seedling leaves. This confirms the findings of previous studies on *Pinus ponderosa* (Anderson, Helms 1994) and *Pinus densiflora* (Lee et al. 2004). Arjenaki et al. (2012) stated that a decrease in *RWC* in plants under drought conditions depends on some microscopic damage to the cell membrane and cytoplasm that causes the reducing power of osmotic adjustment and vigour in plants. Moreover, our study indicated that the effect of nitrogen fertilizer on the *RWC* of seedling leaves is dependent on soil moisture content.

Thus, an increase in nitrogen fertilizer in *LN* and *HN* treatments significantly increased *RWC* compared to the control treatment (100%). Similarly, high nitrogen fertilizer (*HN*) significantly increased *RWC* in 75 to 25% treatments. But urea fertilizer had no significant effect on *RWC* under severe drought (15% and 10%) conditions. Wu et al. (2008) reported almost the same response in *S. davidii*. They stated that *RWC* increased with the increase of nitrogen supply under well-watered seedlings. But no such an increase was observed in water stress conditions.

Under drought conditions, seedlings require nitrogen to support changes in their cellular and whole physiological processes and functions. But seedlings are limited in their ability to access soil nitrogen supply and uptake rate (Yuan, Li 2007), because the addition of nitrogen fertilizer in the soil may not improve some physiological properties of seedlings such as leaf *RWC*.

The results revealed that a reduction of soil moisture content significantly decreased *TCh* content, which is in agreement with previous studies on *Thuja plicata* (Fang-yuan and Guy 2004), *Picea asperata* (Duan et al. 2005) and *Cupressus sempervirens* (Naghipoor et al. 2019). Smirnoff (1995) stated that such a decrease in plant chlorophyll under drought stress is mainly a result of the damage to chloroplasts due to active oxygen species (Mafakheri et al. 2010).

Additionally, the results showed that *P. orientalis* leaf *TCh* significantly increased with an increase in nitrogen supply under all soil moisture content treatments, which is in agreement with Wu et al. (2008) in *S. davidii* (Wu et al. 2008). da Silva et al. (2011) stated that if nitrogen supply is insufficient, plant chlorophyll and thus photosynthesis will decrease, but appropriate nitrogen supply would alleviate photosynthetic damage under water stress conditions. The results showed that *WUE* of *P. orientalis* seedlings significantly increased in low soil moisture treatments. This confirms the findings of previous studies on *Pseudotsuga menziessi*, *Pinus contorta* (Smit, Van den Driessche 1992), *Pinus edulis, Juniperus monosperma* (Lajtha, Getz 1993) and *Thuja stuchuensis* (Jin et al. 2015).

According to Equation 6, the increase in *WUE* of seedlings can be the result of the increase of seedling biomass or carbon gain (nominator) or the decrease of water consumption (denominator).

As stated in the previous section of this study, total biomass of seedlings decreases in low moisture treatments, since the increase in *WUE* under low moisture conditions would be the result of the decrease in water consumption per unit biomass production. However, when water is limited, leaf conductance decreases to conserve water, and finite supplied water should be used more efficiently in drought resistant plants (Zhang et al. 1997). Moreover, our study indicated that nitrogen fertilizer, especially *HN* treatment, significantly increased *WUE* of *P. orientalis* seedlings under water stress conditions. This confirms the findings of Wu et al. (2008) on *S. davidii* seedlings.

DaMatta et al. (2002) stated that nitrogen deficiency occurs under water stress conditions, because nitrogen mobility and the ability of crop to convert water to yield decreased under water stress conditions (da Silva et al. 2011). Thus, the addition of proper nitrogen fertilizer to the soil may enhance seedling nitrogen uptake at a given soil moisture level. Furthermore, the results indicated that the positive effect of nitrogen fertilizer treatments (especially *HN*) on *WUE* depends on the soil moisture level. Although the *WUE* of seedlings was better in *HN* treatment under different intensities of soil water stress, the greatest differences between control and *HN* treatments were observed at 25% and 50% *SMC*.

Due to the fact that at these moisture levels, water consumption of seedlings in *HN* treatment does not show any great changes compared to the control treatment (0.089 L at 50% and –0.023 L at 25%). One of the main reasons for this result is a

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further increase in total biomass of *P. orientalis* seedlings under *HN* compared to control treatment (0.353 g at 50% and 0.155 g at 25%). da Silvae et al. (2011) clarified that the appropriate nitrogen supply should alleviate photosynthetic damage of water stress and increase both shoot and root development of seedlings, which is critical to the final yield. Because it can be concluded that the appreciable nitrogen supply in *HN* treatment might enhance the adaptability of *P. orientalis* seedlings to dry conditions by increasing *WUE* and biomass production.

CONCLUSION

Seedlings of *Platycladus orientalis* can tolerate drought conditions until 25% soil available water for a long time, but a reduction of soil moisture decreased the growth and quality of seedlings. Urea fertilizer improved the growth and quality of seedlings under different soil moisture conditions, and increased their survival under severe drought. Thus, nitrogen enrichment of soil can be useful in seedling production and *P. orientalis* stand regeneration practice.

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