Journal of Plant Physiology and Breeding

2020, 10(2): 121-132 ISSN: 2008-5168

Morphological and physiological responses to drought stress in eleven genotypes of *the Juniperus* species

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Received: September 8, 2020 Accepted: December 20, 2020
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Abstract
Drought is one of the most prevalent and critical environmental stresses affecting a variety of plants, particularly ornamental plants. One of the useful methods to alleviate the effect of drought stress is to screen for and develop drought-tolerant varieties. In this study, a factorial experiment based on the completely randomized design was conducted to investigate the responses of 11 genotypes from different *Juniperus* species at two irrigation regimes (normal, drought: not irrigated for a four-week period) in terms of growth and biochemical characters. Drought stress had a significant negative impact on the assessed growth characters. The G3 and G8 genotypes had the highest root fresh weight and root dry weight at both normal and water-deficit stress conditions. G3 showed the highest root volume at normal conditions but at the drought stress, the highest root volume belonged to G1 and G8. At drought stress conditions, the leaf fresh weight and dry weight of G9, G8, G6, G4, G3 and G11 were higher than other genotypes. The stem fresh weight of G3 and G11 and the stem dry weight of G11 and G8 manifested higher values than other genotypes when water deficit stress was imposed. Stem diameter decreased in the seedlings at the drought stress, however, G2, G3, G4, G8, G9 and G11 had higher values than others at stress conditions. The relative water content decreased in the plants under stress, however, the reduction in G3, G5 and G6 were smaller than the rest of the genotypes. Among the genotypes, G5 and G3 showed the highest antioxidant activity under water-deficit stress. The genotypes G1, G6, G7 and G8 had also a notable increase in the antioxidant activity at drought stress conditions. Under drought stress, the highest increase in the proline content belonged to G3 followed by G5, G6 and G7 and the G5, G6, G10 and G8 genotypes had the highest amount of soluble sugars. In conclusion, G3 (*Juniperus chinensis* var. Sargentii) and G8 (*Juniperus chinensis* 'Kallay's Compact') showed mainly better performance under drought stress, which can be suggested as candidate drought-tolerant genotypes to be used in breeding programs for the sustainable development of urban landscape in arid and semi-arid areas. Although G5 (*Juniperus procumbens* 'Nana') had low biomass in this experiment, it showed high antioxidant activity, proline and soluble sugars at the drought stress conditions. Therefore, further investigation is needed, especially at more severe drought stress conditions, to elucidate its outstanding response to drought stress in terms of antioxidant activity and proline and soluble sugars content.

Keywords: Antioxidant activity; Drought stress; *Juniperus*; Morphological and physiological characters

Citation: Keyghobadi S, Fotouhi Ghazvini R, Tajvar Y and Sabouri A, 2020. Morphological and physiological responses to drought stress in eleven genotypes of *Juniperus*. Journal of Plant Physiology and Breeding 10(2): 121-132.

Introduction

Landscape development worldwide, especially in developed countries, is based on local potentials, including water resources, climate and soil properties. Considerable reduction and fluctuation in the annual precipitation and the emergence of water shortage increase maintenance costs, contributing to the growing concerns about establishing landscape projects. Therefore, determining the suitability of plant species to use for landscaping as well as adaptability to a wide range of climatic conditions is a key step in landscape development, particularly in the arid and semi-arid regions (Rabbani Kheirkhah and Kazemi 2015).

Under water-deficit stress conditions, the physiological activities of plants are directly or indirectly impaired. Since the high cellular turgor pressure is essential for important physiological activities such as cell growth and stomatal functions, plants maintain the high cell turgor pressure using various mechanisms. The osmotic adjustment is one of the effective mechanisms to maintain the turgidity pressure under drought stress conditions. Plants increase the concentration of some metabolites in their cells (Mohammadi *et al.* 2016) including soluble sugars, free organic acids and proline (Vendruscolo *et al.* 2007). Accumulation of free proline in many plant species occurs in response to the low water potential as the result of drought and salinity, in which rapid proline aggregation coincides with the onset of decline in the leaf water potential (Kuznestov and Shevyakova 1999; Mohammadi *et al.* 2016). Drought stress also increases the production of reactive oxygen species (ROS) such as radical superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl ($OH^\cdot$), which their cellular accumulation leads to severe oxidative stress (Mittler *et al.* 2004; Zamani *et al.* 2011; Farzaneh *et al.* 2020). In the absence of ROS protection mechanism, ROS can disrupt the normal cell metabolism through oxidative damage to lipids, proteins, nucleic acids and cell membrane, which eventually leads to cell death (Ozkur *et al.* 2009). Plants have an antioxidant system that controls the excess production of ROS under stress conditions and thus, provides protection. On the other hand, the cell maintains an adequate level of ROS for growth and message transmission paths (Di Venere *et al.* 2009).

Some studies have shown that water-deficit stress decreases biomass production through reducing leaf area, height, dry weight, photosynthesis and chlorophyll, and amino acid accumulation (Hung *et al.* 2005; Salehi-Lisar and Bakhshayeshan-Agdam 2016). Research has been conducted on the effects of drought stress on various plants such as rice (Yang *et al.* 2019), sweet corn (Ghassemi *et al.* 2020), wheat (Shayan *et al.* 2019) and tree plants including apple and quince (Bolat *et al*. 2014), sour cherry (Sivritepe *et al*. 2008), pear (Tatari *et al.* 2019), poplar (Arshad *et al*. 2019) and olive (Baceler *et al.* 2009).

The *Juniperus* species are universal plants found almost everywhere and belong to the family Cupressaceae which composes of 60 species. Species of this genus are evergreen, including the tall, short, or shrubby and creeping trees, mainly dioecious and in some cases monoecious. *Juniperus* species have been spread in the northern hemisphere from the cold and arctic regions such as Siberia and Alaska to the high mountains of the tropics (Mao *et al.* 2010). Landscape development using drought-resistant plants such as *Juniperus* is especially important in arid and semi-arid regions. One of the screening methods for achieving resistant genotypes or varieties of *Juniperus* is based on growth and biochemical traits. In this study, some growth and biochemical characters of different genotypes of *Juniperus* were evaluated at drought stress conditions to identify the drought tolerance potential of these genotypes.

Materials and Methods

In this study, the three-year seedlings of eleven different *Juniperus* genotypes (Table 1) were used. The seedlings were planted in the 4.5-liter pots containing a 2:1:1 soil mixture (Field soil: sand: animal manure). Factors included different genotypes of *Juniperus* and two irrigation levels: normal irrigation (control) and drought stress (no irrigation for four weeks). The soil mixture moisture content was calculated according to the soil properties curve. The matrix potential of the medium texture was obtained (0.03 MPa for the control and -1.5 MPa for drought). The pots were divided into two groups: (1) the first group was fully irrigated at the beginning of the experiment, and (2) the second group was exposed to drought stress over four weeks. In both groups of pots, after complete drainage of the surplus water, the lower part of the stem was covered with a plastic black bag to prevent evaporation from the surface of pots. By the appearance of drought symptoms in the second group of plants, samples were collected to evaluate the vegetative and biochemical traits at Ramsar Citrus and Tropical Fruits Research Institute, Iran.

Evaluation of growth characters

To measure the fresh and dry weight of the roots and shoots, after removing the roots from the soil, the roots were separated from the crown junction and washed thoroughly with distilled water, and after removing excess moisture, their fresh weight was measured. They were then placed in an oven at 105 °C for 48 hours and their dry weight was measured. The stem diameter (SD) was measured from the crown with a digital scale and to measure the root diameter, the tallest root near the collar was measured with a digital scale. Archimedes' law was used to measure root volume. For this purpose, by placing the roots in a graduated cylinder and determining the amount of change in water level, the root volume was measured. To measure the relative water content (RWC), the fresh weight (FW) of the detached leaves from the mother plants was recorded. Then, the leaves were soaked in the distilled water for 24 h at 25 ^{o}C. Then, the turgid weight (TW) was measured after removing the surface water using the towel paper. Thereafter, the samples were dried in the oven at 70 ^{o}C for 48 h and the dry weight (DW) was recorded (Smart and Bingham 1974). RWC was calculated according to the following formula:

$$RWC = (FW – DW / TW– DW) \times 100.$$

Table 1. The properties of *Juniperus* species that were used in this experiment.

Code	Scientific name	Variety
G1	*Juniperus horizontalis*	-
G2	*Juniperus sabina* (green scales)	-
G3	*Juniperus chinensis*	Sargentii
G4	*Juniperus squamata*	Blue Carpet
G5	*Juniperus procumbens*	Nana
G6	*Juniperus* × *pfitzeriana*	Arctic
G7	*Juniperus chinensis*	Globosa
G8	*Juniperus chinensis*	Kallay's Compact
G9	*Juniperus chinensis*	Expansa Aureospicata
G10	*Juniperus chinensis*	Shimpaku
G11	*Juniperus sabina* (golden scales)	-

Antioxidant assay

The ability of the plant extract to scavenge free radicals was determined by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method (Miliauskas *et al.* 2004). An amount of 0.1 g sample plant was soaked in five ml of methanol and centrifuged at 10,000 rpm for five minutes; the supernatant was used to estimate the antioxidant activity. Different concentrations of the plant extract were brought to 2 mL with methanol in a test tube and two ml of 0.004% DPPH methanol solution was added and the extract was kept in the dark for 30 minutes at the room temperature. Then, the absorbance of the samples was measured at 517 nm as compared with the control. Finally, the following formula was used to determine the free radical scavenging (% I) of the extracts:

$$\% I = [(Control - Sample) / Control] \times 100$$

Control: Absorbance of the control solution at 517 nm

Sample: Absorbance of the samples at 517 nm

Proline assay

To estimate the proline content, two ml of the root extract (extracted with 10% sulfosalicylic acid solution) was mixed with two ml of ninhydrin reagent and two ml of acetic acid. The resulting solution was stirred in a warm water bath at 100 °C for one h and then, immediately cooled with ice and reached room temperature. Four ml of toluene was added to the above solution and two separate phases were formed after mixing. The proline concentration of the samples was evaluated by reading the supernatant phase absorbance at 520 nm using the standard curve (Bates *et al.* 1973).

Soluble sugars assay

Total soluble sugars content of the root tissue was determined by the phenol sulfuric acid method (Kochert 1978). Five ml of ethanol (70%) was added to 0.05 g of dry root samples and maintained in the refrigerator for one week. Then, the mixture was centrifuged at 10,000 rpm for 15 minutes at room temperature and the supernatant was used for the determination of the content of soluble sugars. A 0.5 mL plant extract was brought to 2 mL with distilled water in a test tube. Then, 1 ml of 5% phenol and 5 mL of concentrated sulfuric acid were added to each tube. After stirring the mixture well, it was kept at room temperature for 30 minutes. The absorbance of these solutions was recorded at 485 nm. Glucose was used to prepare the standard curve and the data were expressed as mg g^{-1} DW.

Statistical analysis

The experiment was conducted as factorial based on the completely randomized design. 0.05 probability level. Cluster analysis of genotypes was carried out by Ward's method using Euclidean distance based on the morphological and biochemical characteristics.

Results and Discussion

Growth characters and relative water content

The results of the analysis of variance (Table 2) showed that growth characters in *Juniperus* were significantly affected by the irrigation levels and genotypes. The irrigation × genotype interaction

was also significant ($p \leq 0.05$) for the recorded traits except for root dry weight (RDW) and stem diameter (SD). As shown in Table 3, in the seedlings under drought stress, all growth characters decreased as compared with the control. The lowest root fresh weight (RFW) was observed in G6, at both control and water-deficit stress conditions (3.77 and 6.66 g, respectively). As expected, G6 had the lowest RDW at the control (5.76 g) and drought stress (2.69 g) conditions, while G3 (10.50 g) and G8 (13.51 g) had the higher RFW in the seedlings under drought stress as compared with other genotypes. Similarly, these two genotypes had the highest quantity of RDW, 7.49 g and 10.20 g, respectively, when water stress was imposed. At drought stress conditions, the leaf fresh weight (LFW) of G9 (11.30 g), G8 (10.80 g), G6 (10.1 g), G4 (9.71 g), G3 (9.58 g) and G11 (8.94 g) was higher than other *Juniperus* genotypes. The same trend was observed for the leaf dry weight (LDW) except that the ranks of G3 and G11 were reversed. The capability of genotypes to produce biomass under drought stress was also monitored by evaluating the stem weight. G3 and G11 (with 8.79 g and 7.26 g, respectively) had the highest stem fresh weight (SFW). However, in terms of stem dry weight (SDW), G11 (6.38 g) had the highest value followed by the G8 genotype (4.63 g).

One of the important root traits that can show the impact of drought stress is the root volume (RV) because roots experience the early effects of water shortage. In this study, although G3 had the highest RV (24.50 ml) at normal conditions, it failed to produce a similar result under drought, and G1 and G8 had the highest RV amongst the genotypes (14.20 and 16.70 ml, respectively). In general, SD decreased in the seedlings under drought stress, however, G2, G3, G4, G9, G8 and G11 with 6.80, 6.76, 7.05, 6.62, 6.29 and 6.62 mm SD, respectively, had higher values than others at the drought stress conditions. Drought stress decreased the RWC of the genotypes. Although there was considerable fluctuation among genotypes, G3, G5 and G6 with the RWC of 61.20, 52.90 and 56.50%, respectively, maintained reasonably higher values under drought stress. The sharp reduction in G11 under stress (34.30%) compared with the control (88.10%) may reflect the effect of genotype by environment interaction on regulating responses to drought stress.

The existence of genetic diversity for tolerance to stress conditions has been frequently reported in other plant species; i.e. alfalfa (Hosseini Boldaji *et al.* 2012) and wheat (Zebarjadi *et al.* 2012). Also, the observed decrease in growth characters may be the result of a decrease in the photosynthesis rate under drought stress, which can be attributed to the closure of stomata or a decrease in the leaf area in response to drought stress. Furthermore, the reduction in growth may be due to the fact that a lot of energy is used to produce enzymes and osmolytes. The decrease in the leaf area under drought conditions can be due to stomatal closure, and reduced water potential, leaf cell turgor pressure, photosynthesis, chlorophyll content, and Rubisco's carboxylase activity. A decrease in the

Table 2. Analysis of variance of the measured traits for the eleven genotypes of *Juniperus*.

		Mean squares											
SV	df	RFW	RDW	LFW	LDW	SFW	SDW	RV	SD	RWC	Antioxidant activity	Proline	Soluble sugars
Irrigation (I)	1	405.2**	139.5**	179.1**	116.7**	205.5**	40.35**	798.1**	69.90**	31264.61**	2212.72**	44.72**	418.12**
Genotype(S)	10	102.6**	50.68**	111.1**	28.04**	41.20**	15.97**	154.6**	9.88**	485.71**	3445.28**	16.11**	58.30**
D×S	10	4.67**	0.97	36.22**	3.60**	3.53**	5.00**	22.93**	0.35	333.82**	972.51**	10.65**	14.14**
Error	66	1.58	0.77	2.66	0.66	0.99	0.36	3.82	0.28	28.81	37.34	6.10	1.161
CV %	-	13.21	12.98	12.41	9.96	15.19	12.99	15.24	7.81	9.29	13.38	17.08	11.76

SV = Source of variation; df = Degrees of freedom; RFW = Root fresh weigh; RDW: Root dry weigh; LFW= Leaf fresh weigh; LDW= Leaf dry weigh; SFW= Stem fresh weigh; SDW= Stem dry weigh; RV= Root volume; SD: Stem diameter; RWC: Relative water content; **Significant at 1% probability level.

Table 3. Mean values of the measured traits for eleven genotypes of *Juniperus*.

				Normal					
Genotype	RFW (g)	RDW (g)	LFW (g)	LDW (g)	SFW (g)	SDW (g)	RV (ml)	SD (mm)	RWC (%)
G1	12.1±1.62^{cd}	9.55±0.61^{b}	11.9±1.61^{e}	7.68±1.06^{ef}	5.91±0.34^{e-g}	3.92±0.29^{f-i}	20.7±1.58^{bc}	7.19±0.58^{de}	71.2±3.68^{de}
G2	7.66±0.23^{gh}	5.76±0.40^{ef}	10.4±1.58^{e-g}	6.07±0.45^{gh}	8.27±0.45^{b-d}	5.23±0.63^{de}	10. 2±1.16^{i-k}	8.67±0.38^{ab}	72.1±3.87^{de}
G3	18.4±1.84^{a}	11.5±0.95^{a}	22.1±1.80^{b}	11.4±0.79^{a}	15.1±2.54^{a}	9.99±0.41^{a}	24.5±2.44^{a}	8.87±0.78^{a}	84.5±5.68^{ab}
G4	10.2±0.84^{d-f}	5.58±0.55^{e-g}	26.2±2.01^{a}	11.3±0.96^{a}	8.29±1.24^{b-d}	4.73±0.47^{ef}	12.5±1.19^{g-i}	8.67±0.70^{ab}	72.1±4.21^{de}
G5	7.00±0.84^{hi}	5.67±0.49^{e-g}	8.47±1.40^{g-i}	5.47±0.94^{h}	3.47±0.12^{i-k}	2.81±0.24^{j-l}	8.75±0.89^{j-l}	5.50±0.48^{h-j}	73.7±5.32^{de}
G6	6.66±0.87^{hi}	4.35±0.98^{gh}	21.1±1.49^{b}	10.3±0.61^{ab}	6.82±0.60 $^{d-f}$	3.65±0.63^{h-j}	9.00±1.69^{j-l}	5.67±0.23^{h-j}	78.2±4.46^{b-d}
G6	9.91±0.85^{ef}	6.72±0.74^{c-e}	21.9±2.07^{b}	11.1±0.47^{a}	6.75±2.10^{d-f}	4.06±0.50^{f-i}	14. 2±2.86^{f-h}	6.91±0.72^{d-f}	75.7±9.19^{cd}
G8	18.2±1.52^{a}	12.4±1.32^{a}	21.8±1.90^{b}	10.3±0.85^{ab}	8.72±1.37^{bc}	6.17±1.12^{bc}	22.5±2.87^{ab}	8.85±0.27 ^{a}	77.4±6.90^{b-d}
G9	11.2±1.65^{d-f}	7.72±0.88^{cd}	16.3±0.90^{d}	9.32±0.55^{bc}	8.65±1.04^{bc}	5.91±0.23^{cd}	18.7±3.05^{cd}	7.99±0.82^{bc}	82.8±1.45^{ac}
G10	15.1±1.62^{b}	9.47±0.90^{b}	18.7±2.06^{c}	8.41±0.93^{c-e}	7.96±0.96^{b-d}	3.69±0.69^{e-h}	17.7±0.89^{de}	7.50±0.26^{cd}	67.1±6.22^{ef}
G11	11.6±0.90^{cd}	9.47±0.33^{b}	15.0±2.97^{d}	10.94±0.20^{a}	8.99±0.61^{b}	6.87±0.72^{b}	15. 2±3.05^{e-g}	8.06±0.42^{a-c}	88.1±6.31^{a}
				Drought					
G1	9.55±0.55^{fg}	7.99±1.24^{c}	6.35±0.75^{jk}	5.03±0.69^{h}	3.93±0.34^{h-j}	3.15±0.174^{i-k}	14.2±3.05^{i-k}	5.84±0.66^{g-i}	21.5±4.67^{k}
G2	4.33±1.04 ^{j}	3.13±0.53^{i}	6.61±0.88 ^{jk}	5.20±0.18^{h}	5.42±0.58^{f-h}	4.56±0.34^{e-h}	6.50±1.19^{e-h}	6.80±0.62^{d-f}	36.3±5.31^{ij}
G3	10.5±1.32^{d-f}	7.49±1.12^{cd}	9.58±0.75 $^{e-h}$	7.39±0.28^{ef}	8.79±0.90^{bc}	4.07±0.43^{f-i}	9.00±0.76^{f-i}	6.76±0.12^{d-f}	61.2±5.67^{fg}
G4	5.53±0.86^{ij}	3.51±0.73^{hi}	9.71±1.25^{e-h}	8.48±1.29^{c-e}	5.00±0.65^{g-i}	4.40±0.64^{e-h}	8.01±0.76^{e-h}	7.05±0.29^{d-f}	28.1±2.69^{jk}
G5	4.10±1.11^{j}	3.55±0.98^{hi}	4.32±0.83^{k}	3.86±0.64^{i}	2.23±0.29^{k}	1.96±0.32^{l}	4.75±0.89^{l}	4.14±0.21^{k}	52.9±5.08^{h}
G6	3.77±0.96 ^{j}	2.69±0.49^{i}	10.1±1.58^{e-h}	7.69±1.16^{ef}	3.26±1.01^{jk}	2.55±0.75^{kl}	4.75±0.89^{l}	4.31±0.36^{k}	56.5±4.14^{gh}
G7	5.29±1.53^{ij}	3.81±0.81^{hi}	9.14±0.81^{f-i}	7.03±0.49^{fg}	4.33±0.66^{g-i}	3.60±0.61^{g-i}	11.1±1.69^{g-j}	4.92±0.64^{jk}	39.8±2.02^{i}
G8	13.5±1.30^{bc}	10.2±0.83^{b}	10.8±1.33^{e-g}	9.14±0.73^{b-d}	5.45±0.69^{f-h}	4.65±0.55^{e-g}	16.7±1.58^{e-g}	6.29±0.25^{f-h}	29.7±5.60^{jk}
G9	6.91±0.90^{hi}	5.22±0.58^{fg}	11.3±0.92^{ef}	9.63±0.80^{bc}	5.10±0.55^{gh}	4.03±0.18^{f-i}	11.5±1.19^{f-i}	6.62±0.33^{e-g}	28.4±1.85^{jk}
G10	9.46±0.50^{fg}	6.49±0.40^{d-f}	7.60±1.06^{h-j}	5.81±0.29^{h}	4.45±0.41^{g-i}	3.69±0.41^{g-j}	12.5±1.77^{g-j}	5.33±0.19^{ij}	39.4±4.70^{i}
G11	7.97±0.51^{gh}	6.50±0.92^{d-f}	8.94±0.74^{f-i}	7.91±0.67^{d-f}	7.26±0.85^{c-e}	6.38±0.74^{bc}	9.01±1.69^{bc}	6.62±0.50^{e-g}	34.3±4.02^{ij}

RFW = Root fresh weigh, RDW: Root dry weigh, LFW= Leaf fresh weigh, LDW= Leaf dry weigh, SFW= Stem fresh weigh, SDW= Stem dry weigh, RV= Root volume, SD= Stem diameter, RWC: Relative water content. Each value in the table is represented as mean ± standard error (n = 3). The mean values for each character followed by different letter(s) in a column are significantly different by Duncan's multiple range test ($p\leq$ 0.05).

growth rate of plant organs and leaf area due to increased drought stress can also be the result of depressed biosynthesis of growth hormones and induction of inhibitors such as abscisic acid (Saruhan *et al.* 2012; Krouma *et al.* 2015). The results of the current study are in agreement with the outcomes of other investigations in several crops (Emam *et al.* 2011; Toupchi Khosrowshahi *et al.* 2018; Pourasadollahi *et al.* 2019).

In this study, the reduction in LFW and SFW at the drought stress can be partly attributed to the decrease in the leaf area and photosynthesis. Silva *et al.* (2007) by examining the effect of drought stress on sugarcane showed that there was a direct relationship between the photosynthesis and growth characteristics (leaf area, biomass, and plant height) since pigment degradation and stomatal closure limit the photosynthetic activity and result in the reduced plant growth under drought stress.

Under normal circumstances, plants have the proper cellular turgor and absorption of nutrient ions, whereas water shortage conditions hamper the absorption of nutrients and consequently

prevent shoot and root development (Silva *et al.* 2007). Under drought stress, the nutritional constraints are created by the reduction in the elemental uptake and consequently reduces the production of aerial organs. Therefore, under stress and low cellular turgor, the allocation ratio of the nutrients to roots increases against aboveground parts and the plant will not be able to continue to its normal growth (Yang and Miao 2010).

Biochemical responses

Some biochemical mechanisms are involved in conferring tolerance to drought stress in plants (Li *et al.* 2015). One of the common mechanisms in plants under stress is an increase in the antioxidant activity to limit the oxidative damage, however, numerous factors affect the potential of antioxidant induction (Salehi-Lisar and Bakhshayeshan-Agdam 2016; Hanafy Ahmed *et al.* 2017). In our study, the antioxidant activity among the *Juniperus* genotypes was significantly different. Additionally, the effect of water regime and interaction of water regime × genotype was found to be significant for the antioxidant activity ($p \leq 0.05$) (Table 2). Drought stress had a positive impact on increasing the antioxidant potential in the studied genotypes. Among the genotypes, the highest antioxidant activity was related to G5 and G3 under water-deficit stress (Figure 1A). Other genotypes with the notable improvement in the antioxidant activity to quench the DDPH radicals under drought stress were G1, G6, G7 and G8.

Analysis of variance indicated the significant effect of the irrigation regime, genotype and their interaction on proline and soluble sugars in the *Juniperus* seedlings (Table 2). The *Juniperus* genotypes experienced an increase in the proline and soluble sugars content under the water shortage stress. G3 showed the highest enhancement in the proline content at the water deficit stress (3.05 mg/g DW) compared with the well-watered conditions (0.54 mg/g DW). A considerable improvement in the proline content of G5, G6 and G7 was also observed under drought stress (Figure 1B). Additionally, the highest amount of soluble sugars was observed in G3 (98.94%) followed by the G5, G6, G10 and G8 genotypes (Figure 1C).

In this study, the increase in the proline and soluble sugars accumulation as a result of drought stress mirrored the positive influence of stress on these compounds in the *Juniperus* genotypes. One of the biochemical changes that occur in plants under drought stress is the accumulation of ROS. Reports have stated that drought stress increases ROS production (Foyer and Noctor 2000). Drought-induced oxidative stress causes lipid peroxidation and membrane damage. The resistance of the plants to various environmental stresses may be related to the level of activity of the enzymes responsible for scavenging ROS (Wang *et al.* 2009). The antioxidant response to water deficit depends on the severity of stress and the type of plant species. In our study, *Juniperus* genotypes increased their antioxidant activity to reduce the effects of oxidative stress. The highest antioxidant activity was observed for G5 as compared with other genotypes. The accumulation of compatible metabolites such as soluble sugars

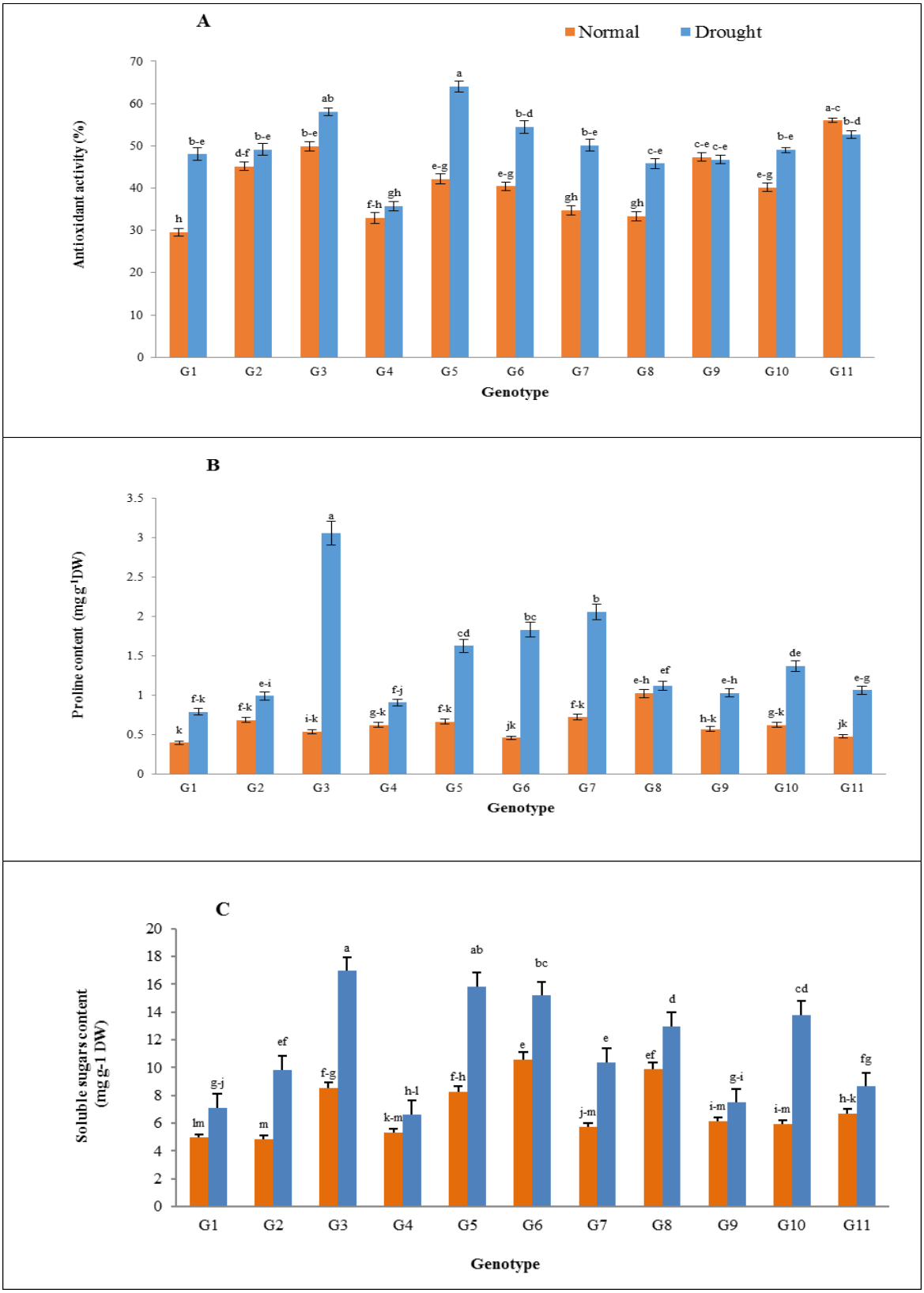

Figure 1. The effect of irrigation regime on antioxidant activity (A), proline (B) and soluble sugars (C) of *Juniperus* genotypes. Bars represent standard errors (n = 3). The values followed by a different letter are significantly different based on Duncan's multiple range test ($p \leq 0.05$).

and proline in plants under drought conditions can help to protect them against stress. Proline protects plants against environmental stresses by several mechanisms, including regulation of osmotic status, scavenging free radicals and stabilizing membranes and proteins. Niknam *et al.* (2006) showed that the proline content in seedlings and calli of *Trigonella foenum-graecum* decreased at 50 mM NaCl but increased at higher salinity levels. However, the proline content in the seedlings and calli of *T. aphanoneura* increased at all salinity levels as compared with the control. The increase in proline accumulation has also been reported in cherry (Nyarukowa *et al.* 2016) and mustard (Mostafaie *et al.* 2018) in response to drought stress.

The increase in the content of soluble sugars under drought stress may be due to a decrease in the need for photosynthetic materials because of reduced growth and increased activity of invertase and amylase enzymes. Therefore, soluble sugars, as an osmotic agent, can allow water absorption and retention, alleviating the adverse effects of the drought stress on plants (Farooq *et al.* 2009). Jimenez *et al.* (2013) have reported the increase of soluble sugars in peach trees under drought conditions.

Conclusions

The results of the current study showed that drought stress adversely affected growth characters in the *Juniperus* genotypes. However, the genotypes studied in this experiment had different responses to drought stress. G3 and G8 were considered drought-tolerant genotypes because they maintained higher biomass production than other genotypes and also had high levels of antioxidant activity, proline and soluble sugars under drought stress. G5 had also high antioxidant activity, proline and soluble sugars at the drought stress conditions; however, it showed low biomass in this experiment when the drought stress occurred. On the other hand, our observations on the G5 plantings show that this genotype has a high survival rate at severe drought stress conditions. Therefore, this genotype should further be evaluated under severe water deficit stress conditions for a possible recommendation for severe drought-prone environments.

Conflict of Interest

The authors declare that they have no conflict of interest with any people or organization concerning the subject of the manuscript.

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بررسی پاسخ‌های مرفولوژیکی و فیزیولوژیکی به تنش خشکی در یازده ژنوتیپ اُرس

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چکیده

خشکی یکی از مهمترین تنش‌های محیطی است که انواع گیاهان از جمله گیاهان زینتی را تحت تاثیر قرار می‌دهد. یکی از روش‌های مفید برای کاهش اثر تنش خشکی، غربالگری و تولید واریته‌های مقاوم به خشکی است. در این مطالعه پژوهشی به صورت آزمایش فاکتوریل در قالب طرح کاملا تصادفی با ۱۱ ژنوتیپ رونده اُرس، در دو سطح آبیاری (آبیاری نرمال و تنش خشکی برای چهار هفته) از نظر صفات رشدی و بیوشیمیایی انجام شد. نتایج نشان داد که تنش خشکی تأثیر منفی قابل توجهی بر ویژگی‌های رشد مورد ارزیابی دارد. ژنوتیپ‌های G3 و G8 دارای بیشترین وزن تر ریشه و وزن خشک ریشه در دو شرایط عادی و تنش کمبود آب بودند. G3 بیشترین حجم ریشه را در شرایط آبیاری نرمال نشان داد ولی در تنش خشکی بیشترین حجم ریشه متعلق به G1 و G8 بود. در شرایط تنش خشکی، وزن تر و خشک برگ G9، G8، G6، G4، G3 و G11 بیشتر از سایر ژنوتیپ‌ها بود. وزن تر ساقه G3 و G11 و وزن خشک ساقه G11 و G8 به هنگام اعمال تنش کمبود آب، دارای مقادیر بالاتری در مقایسه با سایر ژنوتیپ‌ها بود. قطر ساقه تحت تأثیر منفیتنش خشکی قرار گرفت ولی ژنوتیپ‌های G2، G3، G4، G8، G9 G11 به طور نسبی بیشترین مقدار را در بین ژنوتیپ‌ها داشتند. محتوای نسبی آب در گیاهان تحت تنش کم‌آبی کاهش یافت. با وجود این، کاهش این صفت در G3، G5 و G6 کمتر از بقیه ژنوتیپ‌ها بود. در میان ژنوتیپ‌ها، G5 و G3 بیشترین فعالیت آنتی اکسیدانی را تحت تنش کمبود آب نشان دادند. ژنوتیپ‌های G1، G6، G7 و G8 نیز در شرایط تنش خشکی افزایش قابل توجهی در فعالیت آنتی اکسیدانی داشتند. در شرایط تنش خشکی، بیشترین افزایش محتوای پرولین متعلق به G3 و پس از آن G5، G6 و G7 بود و ژنوتیپ‌های G5، G6، G10 و G8 از بیشترین مقدار قندهای محلول برخوردار شدند. به طور کلی، G3 (*Juniperus chinensis* var. Sargentii) و G8 (*Juniperus chinensis* 'Kallay's Compact') عمدتا عملکرد بهتری را تحت تنش خشکی نشان دادند که می توان آن‌ها را به عنوان ژنوتیپ‌های مقاوم به خشکی برای استفاده در برنامه‌های اصلاح نبات برای توسعه پایدار فضای سبز شهری در مناطق خشک و نیمه خشک در نظر گرفت. اگرچه G5 (*Juniperus procumbens* 'Nana') در این آزمایش در شرایط تنش خشکی زیست توده پایینی داشت، ولی فعالیت آنتی اکسیدانی و محتوای پرولین و قندهای محلول بالایی را نشان داد. بنابراین، تحقیقات بیشتری، به ویژه در شرایط تنش خشکی بیشتر، مورد نیاز است تا علت پاسخ برجسته آن به تنش خشکی از نظر فعالیت آنتی اکسیدانی و محتوای پرولین و قندهای محلول روشن شود.

واژه‌های کلیدی: اُرس؛ تنش خشکی؛ صفات مورفولوژیکی و فیزیولوژیکی؛ فعالیت آنتی اکسیدانی

Journal of Plant Physiology and Breeding

2020, 10(2): 121-132 ISSN: 2008-5168