



Drought effects on growth, water status, proline content and antioxidant system in three *Salvia nemorosa* L. cultivars

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Abstract

Plant growth and yield are influenced by various environmental stresses, especially drought. An experiment was done to study the comparative effects of water stress on growth, physiology and antioxidant systems in three *Salvia nemorosa* L. cultivars ('Isfahan', 'Violet Queen' and 'Rose Queen'). The cultivars were treated as control or water stress by stopping irrigation for 10 days. The results showed that the highest number of leaves per plant, leaf area, dry weight of root and shoot and total biomass were obtained from native cultivar 'Isfahan' under water shortage. Relative water content, chlorophyll *a*, *b* and total chlorophyll reduced in all studied cultivars under drought; but the rate of reduction was the lowest in 'Isfahan'. Drought stress increased total soluble sugar in the root and leaf tissues in all cultivars and the highest values were obtained from native cultivar 'Isfahan'. Drought stress also increased proline content, total phenols and flavonoids in all tested cultivars; but the rate of increase in 'Isfahan' was higher than the other cultivars. The activities of catalase and peroxidase enzymes enhanced in all cultivars under drought stress conditions; however, their activities were higher in 'Isfahan' than the other cultivars. Among the cultivars studied, it was found that 'Isfahan' was more tolerant which was revealed by physiological and biochemical characteristics.

Keywords Catalase · Environmental stresses · Relative water content · Total flavonoids and phenols · Total soluble sugar

Introduction

The genus *Salvia* belongs to the Lamiaceae family which has more than 1000 species spread in tropical and temperate regions. This genus has 58 species in Iran, 17 of which are endemic in Iran (Farimani et al. 2015). Different species of *Salvia* genus have antibacterial, antifungal, anti-tumour, antioxidant and anti-inflammatory properties. In traditional medicine, this genus is used to treat eczema, colds, bronchitis, digestive disorders, sore throats and tuberculosis. *Salvia* is economically used in the pharmaceutical, perfumery, sanitary, cosmetic and food industries (Bahadori et al. 2015). The secondary metabolites of this genus are terpenoids and phenolic compounds (Lu and Foo 2002). *Salvia nemorosa* L. has been distributed in Central Europe and West Asia,

especially in Iran. *S. nemorosa* is a resistant herbaceous perennial species that used for medicinal and ornamental purposes (Skala and Wysokinska 2004).

Drought is one of the main factors limiting the growth of crop in arid and semi-arid regions (Madani 2014). This stress affects the various aspects of plant growth and development through the anatomical, morphological, physiological and biochemical changes that the severity of drought damage varies depending on the duration of the stress and plant growth stage (Farooq et al. 2009; Zhang and Huang 2013; Khan et al. 2017). Water stress reduces relative water content (RWC), photosynthetic pigments, stomatal conductance, biomass, growth, and ultimately plant performance (Farooq et al. 2009; Bayat et al. 2016; Caser et al. 2018).

Plants have been developed with different adaptive mechanisms to cope with drought stress by inducing physiological and biochemical responses. Osmoregulation is one of the important processes in adapting plant to drought stress tolerance. It preserves a high water pressure within cells and helps the plant to continue the metabolism and survival. Osmoregulation can be provided by many compounds, including soluble sugars and proline (Zhang et al. 2010;

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Chakhchar et al. 2015). These compounds protect cell membranes, proteins, cytoplasmic enzymes by scavenging reactive oxygen species (ROS) under drought stress conditions (Krasensky and Jonak 2012). On the other hand, plants to cope with oxidative stress, increase their antioxidant activity through enzymatic and non-enzymatic defence systems (Rezayian et al. 2018).

A large part of Iran is located in arid and semi-arid areas facing a severe shortage of water resources (Bayat et al. 2016). Therefore, identification and development of species and cultivars with low water requirement are one of the suitable strategies for reducing irrigation demands (Guerfel et al. 2009; Rezayian et al. 2018). Hence, understanding the physiological and biochemical mechanisms of resistant varieties in response to drought stress is important. Genetic variation for water stress tolerance has been observed in many plant species (Bayat et al. 2016; Khan et al. 2019), but until now the response of *S. nemorosa* cultivars to water stress has not been evaluated. Therefore, the present study was done to evaluate the growth, physiology and antioxidant systems of different cultivars of *S. nemorosa* to drought stress.

Materials and methods

Plant materials, growth conditions and treatments

This experiment was done in the research greenhouse of the Faculty of Agriculture, University of Birjand, Birjand, Iran, in May 2017. The seeds of one native Iranian *S. nemorosa* cultivar namely 'Isfahan' (supplied from Pakan seed Company, Iran) and two foreign cultivars namely 'Violet Queen' and 'Rose Queen' (supplied from Jelitto seed Company, Germany) were sown in 128-cell trays containing peat moss. The dimensions of the cell were 3.2 × 3.2 × 5.1 cm, with a volume of 27.50 cm³. The seeds were irrigated daily (about 15 ml of water per cell) during germination and seedling growth. Thirty days after germination, when the seedlings were at 6–8 leaf stage, they were transplanted into plastic pots with 21 cm in depth and 18 cm in diameter containing sandy loam soil (22.8% clay, 32% silt, and 45.2% sand) with pH 7.4, EC = 1.16 dS m⁻¹ and FC = 22%. Before applying drought stress treatments, plants were kept at the field capacity by irrigation three times a week. 120 days after sowing, the plants were treated with two irrigation regimes: (1) drought stress—irrigation was stopped completely for 10 days and (2) control—well-watered plants maintained at the field capacity. A completely randomized design with six replications was used for conducting the experiment. The mean air temperatures in the greenhouse were 24–18 °C (day to night), 16-h photoperiod, 900 μmol m⁻² s⁻¹ of photosynthetic active radiation and air humidity range between 50 and 60%.

Growth parameter measurements

Measurements were performed at the end of the experiment. The number of leaves per plant was counted. The total leaf area was estimated by a Laser Leaf Area Meter (LI-3100, LI-COR Company, USA). The plants were removed from the pot and the maximum length of the roots was measured. Then, the roots and shoots were placed in an oven at 78 °C for 48 h in order to determine their dry weights (Khan et al. 2016).

Determination of RWC and electrolyte leakage (EL)

The fully developed leaves of the plant were removed and their fresh weight (FW) immediately weighed. The leaves were then immersed in distilled water for 6 h at 24 °C to measure turgid weight (TW). Finally, after drying of the samples at a temperature of 78 °C for 48 h, their dry weights (DW) were recorded (Gonzalez and Gonzalez-Vilar 2003). Calculation of the RWC value was done from the following formula:

$$[(FW - DW) / (TW - DW)] \times 100.$$

To determine electrolyte leakage (EL), fresh leaves (0.5 g) were incubated in distilled water (10 ml) and then shaken at 24 °C for 24 h. At this step, the primary electrical conductivity (EC1) was measured by the electrical conductivity meter (Model Jenway, Germany). The samples were then transferred to an autoclave (121 °C) for 15 min and secondary electrical conductivity (EC2) was determined (Barranco et al. 2005). Finally, the EL values were calculated using the following equation:

$$EL = (EC1/EC2) \times 100.$$

Determination of chlorophyll content

The fresh leaves were extracted in acetone. The absorption of pigment was determined spectrophotometrically (Model Unico 2100, China) at 663 and 645 nm (Arnon 1949).

Determination of total flavonoids and phenols

To prepare extract, 1 g of fresh leaf was homogenized with methanol (10 ml) for 24 h, and then filtered and centrifuged (6000 rpm) for 15 min. To determine the total flavonoids, the extract (1 ml) was mixed with distilled water (4 ml) and 0.3 ml of 5% sodium nitrite. After 5 min, AlCl₃ 10% (0.6 ml) and at 6 min NaOH 1 M (2 ml) and distilled water (2.1 ml) were added to the mixture. The absorbance was determined at 510 nm (Yoo et al. 2008). To determine the total phenols, the extract (0.5 ml) was mixed with Folin–Ciocalteu reagent (0.5 ml) (Sigma-Aldrich, Germany) and distilled water (6 ml). After 5 min, sodium carbonate 20% (2 ml) was added

and the mixture was shaken strongly. After 2 h, the absorption was recorded spectrophotometrically at 760 nm (Singleton and Rossi 1965).

Assay of total soluble sugar

0.5 g of leaf samples was extracted with 5 ml of methanol and then centrifuged (6000 rpm) for 10 min. The extract (0.5 ml) was mixed with anthrone reagent (5 ml). The test tubes were boiled in a hot bath (100 °C) for 30 min and transferred immediately to the refrigerator. After cooling, the absorbance was read at 620 nm (Irigoyen et al. 1992).

Determination of proline

Fresh leaves (0.5 g) were extracted with sulfosalicylic acid (5 ml) and centrifuged at 3000 rpm. The supernatant was mixed with glacial acetic acid and ninhydrin and then shaken strongly. The mixture incubated (100 °C) for 1 h and then transferred to a mixture of water and ice to cool. At this stage, 4 ml toluene was added and shaken for 15–20 s. Finally, the absorbance at 520 nm was read by the spectrophotometer (Bates et al. 1973).

Determination of catalase (CAT) and peroxidase (POX) enzyme activities

Fresh leaf (0.2 g) was extracted in 50 mM phosphate buffer (2 ml, pH 7.5) and centrifuged (13,000 rpm) for 15 min at 4 °C. To determine CAT (EC 1.11.1.6) activity, the reaction mixture comprised 50 mM phosphate buffer (2.8 ml,

pH 7.0), H₂O₂ (30 µl) and enzyme extract (0.1 ml). The activity of CAT was recorded with a decrease in absorbance at 240 nm (Zhang and Kirkham 1994). To assay POX (EC 1.11.1.7) activity, the reaction mixture comprised 50 mM phosphate buffer (2.8 ml, pH 7.0), guaiacol (350 µl), H₂O₂ (350 µl) and enzyme extract (0.1 ml). The activity of POX was recorded with an increase in absorbance at 470 nm (Zhang et al. 2012).

Statistical analysis

The analysis of data was performed with the JMP 8 software. The mean comparison was based on the least significant difference (LSD) test at the 5% probability level.

Results and discussion

Growth attributes

Water stress reduced the number of leaves per plant and total leaf area in all studied cultivars in comparison to the control plants (Table 1). Under water stress, the highest leaf number and leaf area were obtained from ‘Violet Queen’ and ‘Isfahan’, respectively. *S. nemorosa* cultivars differed significantly in terms of the root, shoot and total dry weights under both control and water stress conditions and the highest values were obtained in ‘Isfahan’ cultivar (Table 2). The results of this study were in accordance with previous reports in *Salvia miltiorrhiza* Bunge. (Liu et al. 2011), in *Salvia officinalis* L. (Mansori et al. 2016) and in

Table 1 Number of leaves per plant, total leaf area and maximum root length of three *S. nemorosa* cultivars under control and drought stress conditions

Cultivar	Number of leaves per plant		Total leaf area (cm ²)		Maximum root length (cm)	
	Control	Drought	Control	Drought	Control	Drought
Isfahan	66.00 ± 1.30 b	52.82 ± 1.37 de	1826.98 ± 61.59 a	850.21 ± 65.10 c	37.80 ± 0.66 b	44.00 ± 0.91 a
Violet Queen	73.04 ± 0.77 a	57.42 ± 1.52 cd	1760.62 ± 56.35 a	774.98 ± 31.13 cd	35.33 ± 0.80 b	43.16 ± 1.35 a
Rose Queen	59.20 ± 0.73 c	51.83 ± 1.53 e	1274.30 ± 56.54 b	619.26 ± 23.33 d	36.80 ± 0.73 b	41.00 ± 1.15 a

Means in the columns followed by the same letter are not significantly different according to LSD test at $P < 0.05$. The numbers following ± sign are the standard errors

Table 2 Root, shoot and total dry weight of three *S. nemorosa* cultivars under control and drought stress conditions

Cultivar	Root dry weight (g/pot)		Shoot dry weight (g/pot)		Total dry weight (g/pot)	
	Control	Drought	Control	Drought	Control	Drought
Isfahan	4.60 ± 0.10 a	2.81 ± 0.10 b	5.37 ± 0.06 a	3.40 ± 0.10 d	9.97 ± 0.09 a	6.21 ± 0.12 d
Violet Queen	4.56 ± 0.11 a	2.75 ± 0.11 b	4.98 ± 0.12 b	3.36 ± 0.07 d	9.53 ± 0.20 b	6.11 ± 0.15 d
Rose Queen	2.94 ± 0.03 b	1.43 ± 0.05 c	4.37 ± 0.14 c	2.48 ± 0.08 e	7.31 ± 0.12 c	3.91 ± 0.09 e

Means in the columns followed by the same letter are not significantly different according to LSD test at $P < 0.05$. The numbers following ± sign are the standard errors

Salvia sinaloensis Fern. (Caser et al. 2018). The first sign of water scarcity is the reduction of turgor pressure, which reduces the growth and development of the plant by reducing the division and elongation of the cells (Farooq et al. 2009). In this study, a significant reduction was obtained in dry weights of all *S. nemorosa* cultivars under drought stress, however, dry weight loss in ‘Isfahan’ cultivar was less than two other cultivars. Khan et al. (2019) reported that water stress reduced dry matter production in chick-pea cultivars due to the decrease in chlorophyll content and photosynthesis. In this study, our results also showed that the decreased total biomass was correlated with the decrease in chlorophyll content under drought stress. The results showed that drought stress increased root length of all cultivars compared to the control, but no significant differences were observed among cultivars under water stress (Table 1). The increase in the root length caused by drought stress has been reported by other authors (Farooq et al. 2009; Sadok and Sinclair 2011). Longer root system facilitates water and nutrient uptake from the lower depths of the soil under drought stress conditions.

RWC and EL

The mean RWC values showed a significant decrease between the well-watered (83.68%) and drought stress treatments (34.09%) for all evaluated cultivars. In contrast, the mean EL values increased from 20.08% in the control plants up to 66.06% in drought-stressed plants. Among all cultivars, ‘Isfahan’ exhibited the greatest (40.91%) RWC and the lowest (56.06%) EL under drought stress, which suggests ‘Isfahan’ was the most drought tolerant cultivar (Fig. 1). Reduction of RWC and enhancement of EL caused by drought stress have been reported by other researchers (Mardani et al. 2012; Bayat et al. 2016; Tavallali et al. 2017). Relative water content is one of the common parameters used to determine the water status inside the plant, so that tolerant cultivars have more RWC than sensitive cultivars under water stress (Karimi et al. 2015). In our study, the leaf RWC of tolerant cultivar (Isfahan) was significantly higher than in the sensitive cultivar (Rose Queen). In addition, a significant correlation was observed between RWC and photosynthetic pigments, proline content, total phenols and flavonoids, and antioxidant enzymes. Electrolyte leakage is another indicator of plant stress tolerance that increases under drought stress conditions. The ROS damage the cell membrane through peroxidation of the lipids (Tavallali et al. 2017). In our study, the EL of tolerant cultivar (Isfahan) was significantly lower than the other cultivars which is in accordance with the results of Khan et al. (2019). Tolerant cultivars are able to maintain the EL at a lower level by protecting the structure of the cell membrane (Jinrong et al. 2008).

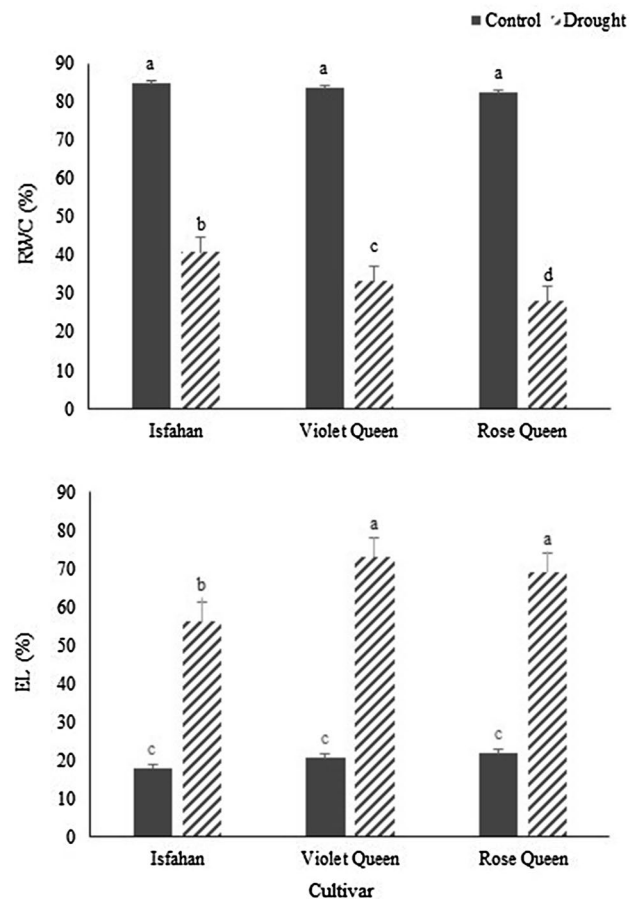


Fig. 1 Relative water content (RWC) and electrolyte leakage (EL) of three *S. nemorosa* cultivars under control and drought stress conditions. Values followed by the same letter are not significantly different according to LSD test at $P < 0.05$. Vertical bars indicate \pm SE

Chlorophyll content

Significant differences were observed among the studied cultivars in chlorophyll content under both control and water stress conditions. The mean of chlorophyll *a*, *b* and total chlorophyll reduced by 22.97%, 26.82% and 24.78% under water stress, respectively. The native cultivar ‘Isfahan’ had the highest chlorophyll content under drought stress condition (Table 3). These results are consistent with the findings of some researchers who reported that a reduction in chlorophyll content under drought conditions (Guerfel et al. 2009; Khakdan et al. 2016; Edziri et al. 2017; Caser et al. 2018). In our study, the percent decrease in chlorophyll content appeared more considerable in sensitive cultivar (Rose Queen) than in tolerant cultivar (Isfahan) which is in agreement with the result of Sadiq et al. (2018) in mungbean (*Vigna radiata*). The leaf chlorophyll content is considered as an appropriate criterion for evaluating the physiological status of the plant. The ROS compounds are produced in the

Table 3 Chlorophyll *a* and *b*, total chlorophyll, total phenolic and flavonoid content of three *S. nemorosa* cultivars under control and drought stress conditions

Cultivar	Chlorophyll <i>a</i> (mg g FW ⁻¹)		Chlorophyll <i>b</i> (mg g FW ⁻¹)		Total chlorophyll (mg g FW ⁻¹)		Total phenolic content (mg g FW ⁻¹)		Total flavonoid content (mg g FW ⁻¹)	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Isfahan	0.84 ± 0.10 a	0.74 ± 0.09 ab	0.53 ± 0.07 a	0.39 ± 0.05 bc	1.38 ± 0.18 a	1.13 ± 0.14 ab	3.50 ± 0.16 b	5.88 ± 0.18 a	0.51 ± 0.06 b	0.76 ± 0.15 a
Violet Queen	0.86 ± 0.04 a	0.60 ± 0.05 b	0.42 ± 0.02 ab	0.31 ± 0.04 cd	1.29 ± 0.08 a	0.91 ± 0.09 b	3.62 ± 0.20 b	5.73 ± 0.25 a	0.45 ± 0.04 b	0.71 ± 0.15 a
Rose Queen	0.54 ± 0.09 bc	0.39 ± 0.06 c	0.30 ± 0.02 cd	0.20 ± 0.03 d	0.84 ± 0.12 bc	0.60 ± 0.09 c	3.57 ± 0.14 b	3.82 ± 0.13 b	0.43 ± 0.04 b	0.64 ± 0.05 a

Means in the columns followed by the same letter are not significantly different according to LSD test at $P < 0.05$. The numbers following ± sign are the standard errors

cell during the drought stress, which causes the destruction of the photosynthetic system and ultimately the decomposition of chlorophyll (Farooq et al. 2009).

Total flavonoids and phenols

Soil drying increased total flavonoids and phenols in all tested cultivars when compared to the control. ‘Violet Queen’ and ‘Isfahan’ had significantly greater total phenolic content than ‘Rose Queen’ cultivar under drought stress, however, there was no significant difference between the studied cultivars in flavonoid content (Table 3). It has been reported that total phenols increased in plants of *S. officinalis* and *S. sinaloensis* (Bettaieb et al. 2011; Caser et al. 2018) and similarly, total flavonoids in *S. sinaloensis* (Caser et al. 2018) under water stress conditions. Phenolic and flavonoid compounds protect crops against environmental stress through non-antioxidant systems. These compounds act as detergents for the ROS and thus stabilize the membrane of the cell and prevent the peroxidation of lipids (di Ferdinando et al. 2014). In the present study, the increment in total phenols of ‘Isfahan’ and ‘Violet Queen’ cultivars were higher than ‘Rose Queen’, demonstrating better drought stress tolerance.

Total soluble sugar and proline content

The values of total soluble sugar in the root and leaf in all studied cultivars increased under water stress. Although the amount of leaf soluble sugar in different cultivars did not differ significantly under drought, it was significant in the root. The native cultivar ‘Isfahan’ had the highest total soluble sugars in the leaf and root under water stress (Fig. 2). Enhancement of total soluble sugar due to water stress has been reported by other researchers (Zhang et al. 2010; Chakhchar et al. 2015). Osmoregulation is one of the important mechanisms of drought stress tolerance in which plants accumulate low molecular weight metabolites, such as sugars, organic acids and amino acids (Chakhchar et al. 2015). The accumulation of soluble sugars could be effective in maintaining the RWC and membrane stability of plant cells (Gil et al. 2011). Hence, selection of varieties that accumulate osmolytes is a reliable strategy for improving drought stress tolerance. Leaf proline content increased in all studied cultivars under water stress and the highest proline content was obtained from native cultivar ‘Isfahan’ (Fig. 3). Under abiotic stresses, proline accumulates in plant as the main osmoprotectant (Chakhchar et al. 2015; Azarmi et al. 2015). The concentration of proline in the cells increases in response to water stress and results in the transfer of water to the plant (Sofo et al. 2004). Proline can contribute to scavenging of the ROS, thereby protecting the cell membrane from oxidative damages of drought stress. Proline

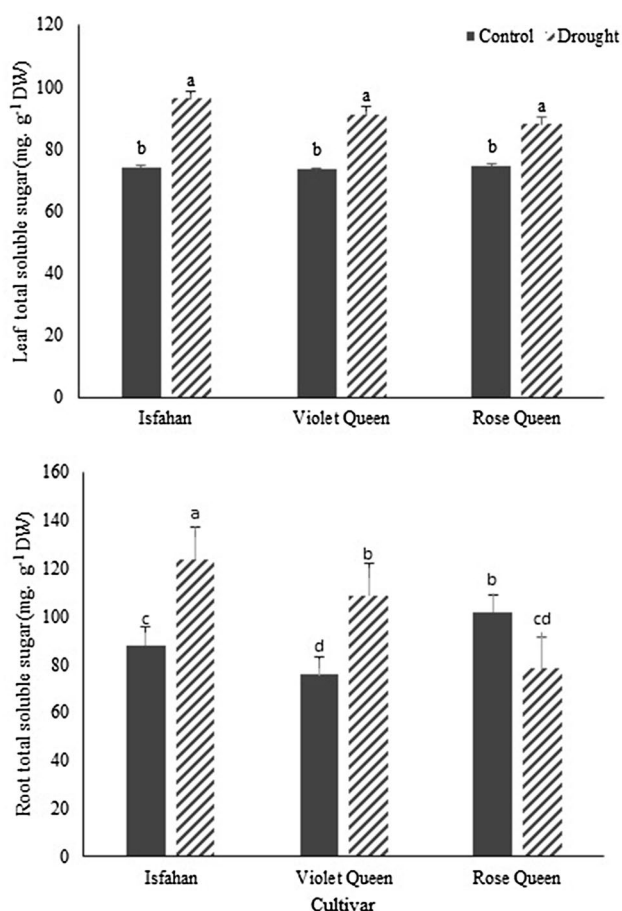


Fig. 2 Total soluble sugar in the leaf and root of three *S. nemorosa* cultivars under control and drought stress conditions. Values followed by the same letter are not significantly different according to LSD test at $P < 0.05$. Vertical bars indicate \pm SE

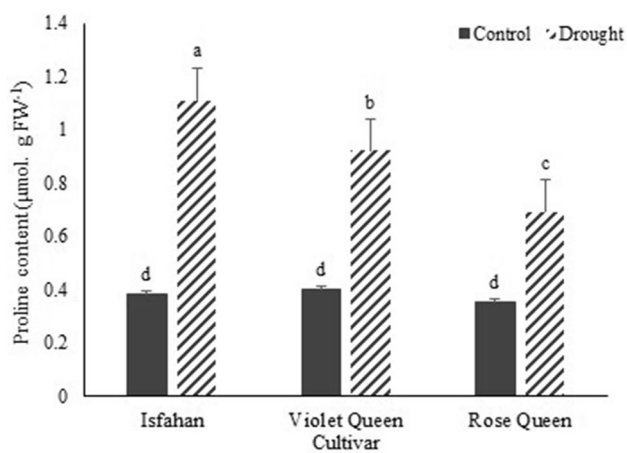


Fig. 3 Leaf proline content of three *S. nemorosa* cultivars under control and drought stress conditions. Values followed by the same letter are not significantly different according to LSD test at $P < 0.05$. Vertical bars indicate \pm SE

accumulation under drought stress is due to the activation of proline biosynthesis enzymes and decreased use of proline in protein synthesis (Tsugane et al. 1999).

Antioxidant enzymes activity

By decreasing soil moisture content, the activity of CAT and POX enzymes increased in all studied cultivars. ‘Isfahan’ cultivar had the highest CAT and POD activities under water stress (Fig. 4). The results are similar to the findings of Rezayian et al. (2018) in canola and Khan et al. (2019) in chickpea where they found water stress increased antioxidant enzyme activities. The enhancement of antioxidant enzyme activities is one of the defence mechanisms in the plant against various abiotic and biotic stresses. When plants are exposed to drought stress, the levels of ROS increase that can damage proteins, lipids, carbohydrates, and nucleic acids. Plants for the purification and detoxification of the ROS in the cells have an enzymatic defence system, which

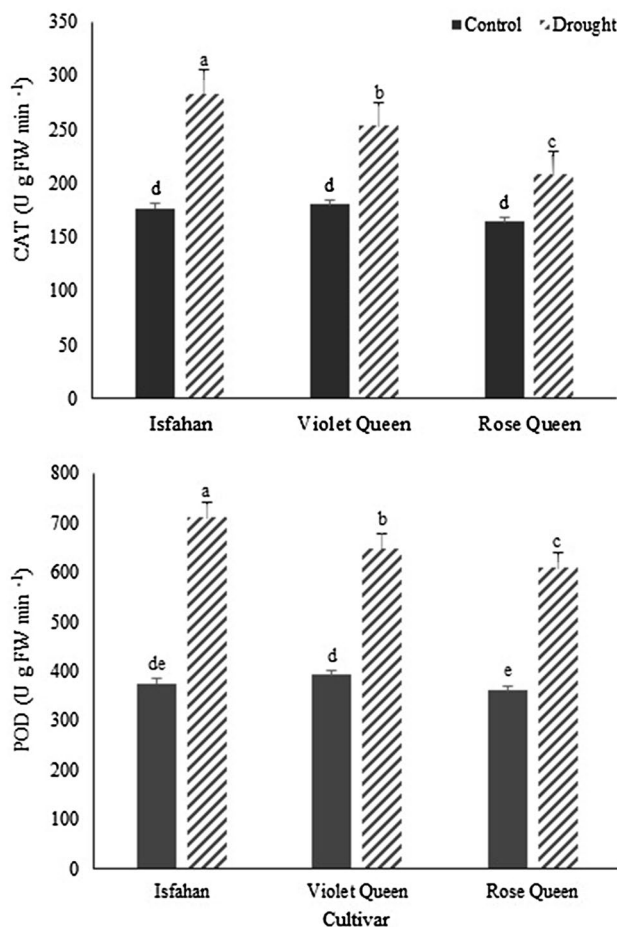


Fig. 4 Catalase (CAT) and peroxidase (POD) enzyme activities of three *S. nemorosa* cultivars under control and drought stress conditions. Values followed by the same letter are not significantly different according to LSD test at $P < 0.05$. Vertical bars indicate \pm SE

increases the tolerance to drought stress (Farooq et al. 2009). Moreover, the comparison between tolerant and sensitive cultivars of different plants showed that the antioxidant enzyme activities in tolerant cultivars of wheat (Huseynova 2012) and chickpea (Khan et al. 2019) were more than sensitive cultivars of these species. Accordingly, we observed higher CAT and POD activities in 'Isfahan' compared to other cultivars under water stress.

Conclusions

In general, the results of this study confirmed that *S. nemorosa* cultivars differed in terms of drought stress tolerance. Among the considered cultivars, native cultivar 'Isfahan' appeared as the most tolerant to water stress. This cultivar because of mechanisms such as higher levels of total soluble sugar, RWC, total phenols, proline, antioxidant enzyme activities and lower EL could survive against water stress. Physiological and biochemical adaptations made the 'Isfahan' cultivar able to maintain its growth and productivity under conditions of water shortage.

Author contribution statement Design and implementation of the experiment: HB, ANM. Analysis and interpretation of data: HB. Paper preparation: HB, ANM. Final revision: HB.

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