

Original Article

Effect of Drought Stress and its Interaction with Salicylic Acid on Fennel (*Foeniculum vulgare* Mill.) Germination and Early Seedling Growth

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Abstract

Environmental stress, particularly drought stress can play an important role in reducing plant growth especially during the germination stage in arid and semi-arid regions in Iran. For cultivation of medicinal plants in arid and semi-arid areas, the assessment of their tolerance is very important. In this research, in order to evaluate the effects of salicylic acid pretreatment on enhancement of seed germination and early seedling growth of *Foeniculum vulgare* Mill. under drought stress, a factorial experiment based on completely randomized design was employed. The factors were the combination of five levels of drought stress induced by poly ethylene glycol (0, -0.1, -0.2, -0.3 and -0.4 MPa) and five concentrations of salicylic acid (0, 0.25, 0.5, 0.75 and 1 mM) with three replicates. The results indicated that an increase in drought stress reduced germination components such as germination percentage and rate, total biomass, seed vigor index, root length, root fresh and dry weight, shoot length, shoot fresh and dry weight and relative water content and increased electrolyte leakage and proline content. Salicylic acid improved germination; therefore, the average time necessary for germination decreased under drought conditions. The seeds treated by salicylic acid, produced a higher root and shoot length, root and shoot fresh and dry weight, total biomass and seed vigor index. Salicylic acid ameliorated the negative effects of drought stress on fennel germination and seedling's growth. Higher concentrations of salicylic acid were more effective than the lower ones. It seems that salicylic acid can enhance the tolerant ability of the seeds to germination under drought stress.

Key words: Electrolyte leakage, Drought stress, Germination, Proline, Salicylic acid

Abbreviations

ABA: abscisic acid, GP: germination percentage, GR: germination rate, IAA: indole-3-acetic acid, PEG: polyethylene glycol, RDW: root dry weight, RFW: root fresh weight, RL: root length, ROS: reactive oxygen species, RWC: relative water content, SA: salicylic acid, SDW: shoot dry weight, SFW: shoot fresh weight, SL: shoot length, SVI: seed vigor index, TB: total biomass

Introduction

Medicinal and aromatic plants are important economic products which represent significant source of economic revenue and foreign exchange and are among the most important agricultural export products. Fennel is one of the most important plants in this regard. Fennel (*Foeniculum vulgare* Mill.) belongs to the family of *Umbellifera* (*Apiaceae*). The generic name derives from the Latin *Foenum* which means hay, referring to the foliar structure. Besides the use as a vegetable, the pharmacopoeia use concentrates on the fruits and the important ingredient is the oil.

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Fennel is used in folk medicine as a stimulant, diuretic, carminative, sedative, galactagogue, emmenagogic, expectorant and antispasmodic. Fennel is also considered as a spice due to terpenic compounds isolated from its fruits volatile oil and used in preparation of various dishes [1]. In aromatic plants, growth is influenced by various environmental factors such as water stress. Drought is one of the most serious world-wide problems for agriculture, which determines the success or failure of plant's establishment. The effects of water deficiency depend on several factors such as its intensity, duration, phenological phase of growth and genetic resistance capacity of plants. Water stress affects different aspects of plant growth (morphology, physiology and anatomy) and causes many changes such as decrease or delay in germination, aerial organ growth reduction, decrease in dry biomass and in rate of growth [2]. The determined metabolically and physiological responses of agricultural plants to the drought stress condition were studied, but for the pharmaceutical and aromatic plants under the lack of humidity are not known. Seed germination is mostly an issue in medicinal plant seed's emergence. Among the plant growth stages, seed germination, seedling emergence and establishment are key processes in the survival and growth of plants [3]. Evaluation of seed germination may be useful to identify resistant seeds and genotypes that are capable of generating sufficiently under low soil moisture conditions. Taylor *et al.* [4] demonstrated that the seed germination and emergence are reduced at a low negative osmotic potential. At the germination stage, drought could decrease shoot and root length [5]; at the vegetative period, it would decrease shoot growth. Furthermore, other studies indicated that stem length is more sensitive to drought stress than root length [6]. The rate of seed germination and the final germination percentages as well as the amount of water absorbed by the seeds were considerably lowered with the rise of osmotic stress levels. Seed priming treatments such as osmopriming, hydro priming, and hormonal priming have been employed to accelerate the germination, seedling growth and yield in most of the crops under normal and stress conditions. Although, the mechanism of seed priming treatments is not fully understood, it has been observed that physiological and biochemical changes take place during the seed treatments [7]. The benefits of seed priming to have been reported, including improving stand establishment at semi-

arid condition and at drought stress [8], enhancing seed with low vigor, improving dormancy breakdown, or increasing productivity. Seed priming accelerates seed germination and seedling establishment under both normal and stressful environments [9].

The results obtained in the last few years strongly prove that salicylic acid could be a very promising and protective compound for the reduction of biotic and abiotic stresses in sensitive crops, because under certain conditions, it has been found to mitigate the damaging effects of various stress factors in plants. Salicylic acid (SA) is an endogenous growth regulator from group phenol compounds that influence many physiological processes such as germination, seedling's growth [10,11], stomatal closure, membrane permeability, the control over ion intake to roots and stomatal conductivity [12], the content of photosynthetic pigments and the rate of photosynthesis [13]. Salicylic acid is a conservative compound of some biological stresses, and it is an important molecular signal for adjustment plant's reaction to environmental stresses [12]. The inclusion of SA at 0.5 mM in the germination medium was associated with increase germination percentage of tomato [4]. Polyethylene Glycol (PEG) compound has been used to simulate osmotic stress effects in Petri dish (in vitro) for plants to maintain uniform water potential throughout the experimental period [14]. Low seed germination and seedling emergence is one of the main problems in arid and semi-arid areas. As regards fennel is known to have low seed germination especially under drought stress. So the present investigation was done in order to understand whether salicylic acid pretreatment could alleviate the harmful effects of water stress and improved plant resistance. Based on obtained results, the application of exogenous salicylic acid can be a method to decrease water stress damages to plants. Also, a very few studies on the interaction of drought stress and salicylic acid was performed on fennel so we conclude that the mentioned study could be a start for further investigation according to different medicinal plant species and different growth stages.

Material and Methods

This study was carried out at the Faculty of Agriculture, Shahid Bahonar university of Kerman, Iran, in 2013. Germination and seedling growth was

studied under control and drought stress conditions. For implementation of drought stress, four different concentrations of PEG (-0.1, -0.2, -0.3 and -0.4 MPa) were used. Distilled water was used as control. Homogenous seeds of *F. vulgare* were surface sterilized using 5% sodium hypochlorite solution for 5 min to eliminate possible Seed-borne microorganisms, and then rinsed three times with sterile distilled water. The seeds of fennel were subjected to seed priming; the first group was hydroprimed [soaked in distilled water], and while the second group was osmoprimed [soaked in 0.25, 0.5, 0.75 and 1 mM SA] for 24 hour, (these concentrations was suggested after some preliminary experiment). After soaking period the seeds were air dried and then subjected to the different concentrations of PEG solution.

Germination test was conducted by three replications of 30 seeds from every treatment in 9 centimeters Petri dishes. Top of Whatman paper No.2 was moistened with 10 ml different concentrations of PEG solution or distilled water (as a control). In order to avoid water losses, Petri dishes were tightly sealed with impermeable colorless paraffin, and then placed in a germination chamber at 21 ± 1 °C with 70% relative humidity. All Petri dishes and filter papers were disinfected in 120 °C for 2 hours. Radicle length of 2 mm was scored as germination [15]. Length of radical and shoot measured by millimetric ruler and for measuring of fresh and dry weight of radical and shoot a balance were used to milligram (Sartorius model: LIBROR AEL- 40 SM). Germination percentage was recorded every day during the study period.

Rate of germination (seeds day⁻¹) was estimated using Maguire's equation [16].

$$GR = S_i/D_i$$

Where GR is germination rate (the number of germinated seeds per day), S_i is the number of germinated seeds at each counting, and D_i is number of days until the nth count. Dry weights of root and shoot (mg plant⁻¹) were measured after drying samples at 70 °C for 24 hours in an oven [5]. Germination was expressed as the Final Percentage of Germination was calculated according to the method of Maguire's equation [16]:

$$GP = (n/N) \times 100$$

GP= percent of Germination.

N=number of total seeds.

n= number of germinated seeds.

Total Biomass = Root Dry Weight + Shoot Dry Weight

The seed vigor index (SVI) of the seed was estimated as suggested by Abdul-Baki and Anderson [17] as follows:

$$SVI = [\text{germination percentage} \times \text{mean (radicle length + plumule length)}] / 100$$

Leaf relative water content (RWC): RWC was calculated as follow:

$$RWC = [(\text{fresh weight} - \text{dry weight}) / (\text{saturated weight} - \text{dry weight})] \times 100$$
 [18].

Electrolyte Leakage: The electrolyte leakage was determined as described by Ben Hamed *et al.* [19]. Shoot samples (0.2 g) were placed in test tubes containing 10 mL of double distilled water. The tubes were incubated in a water bath at 32 °C for 2 hours and the initial electrical conductivity of the medium (EC_1) was measured by an EC meter (Metrohm Filderstadt, Germany). The samples were autoclaved at 121 °C for 20 min to release all the electrolytes, cooled at 25 °C and then the final electrical conductivity (EC_2) of each was measured. The electrolyte leakage (EL) was calculated by using the following formula:

$$EL = (EC_1/EC_2) \times 100$$

Proline determination: Determination of free proline content performed according to Bates *et al.* [20].

Statistical analysis: The experiment was conducted in a factorial arrangement based on completely randomized design with 25 treatments and three replications. Static assays were carried out by one-way ANOVA using LSD test to evaluate whether the means were significantly different, taking $p < 0.05$ as significant. Computations and statistical analysis were done using SAS and MSTATC.

Results

Drought stress induced by Polyethylene glycol (PEG₆₀₀₀) caused a significant reduction in all the measured traits, including germination percentage and rate, total biomass, seed vigor index, root length, root fresh and dry weight, shoot length, shoot fresh and dry weight (Table 1). Pretreatment with salicylic acid (SA) markedly alleviated the effects of water stress and also ameliorated all the measured parameters significantly. Salicylic acid applied through seed soaking was more effective within the range of 0.5–1 mM in protecting fennel seedlings against drought stress.

The highest and lowest germination percentage was recorded for control and -0.4 MPa treatments respectively (Table 2). This treatment reduced the

germination percentage by 91.8% compared to control. Increasing the drought stress from 0 to -0.4 MPa led to a reduction in germination rate. Highest level of osmotic potential caused a reduction of 96.8% in germination rate compared to control (Table 2).

SA affected germination percentage and rate (Table 3). The highest and lowest value of these traits belonged to 0.5 mM SA and control respectively. The concentration of 0.5 mM SA caused an increasing of 21.7% and 26.9% in germination percentage and rate compared to control respectively.

The response of germination percentage to the interaction of SA concentrations and drought levels were different, so that no germination observed at the 0.25 and 0.5 mM SA at -0.4 MPa treatments while 0.75 and 1 mM SA led to germination at the mentioned level of drought (Table 4). Based on these results, it seems that higher concentrations of SA ameliorate germination. Drought stress adversely affected GP in non-primed seeds. The highest reduction of GP was recorded for -0.3 MPa treatments, meantime no germination occurred at the -0.4 MPa treatment. The difference in GP was not statistically significant between -0.1 MPa and control treatments, while both were significantly higher than the other two treatments. Applying SA caused an increase in GP at all levels of drought compared to 0 mM SA. The highest and lowest germination rate belonged to -0.1 and -0.3 MPa treatments under stress conditions at 0.25 and 0.5 mM levels of SA. No Germination rate was recorded for hydropriming and primed treatments with 0.25 and 0.5 mM at -0.4 MPa treatments, while its value for 0.75 and 1 mM were 0.32 and 0.28 respectively (Table 4).

The effect of osmotic potential on total biomass and seed vigor index of fennel were shown on table 2. On the basis of these results, increasing drought levels caused remarkably decrease in total biomass and seed vigor index (Table 2). All the levels of drought stress were significantly different with each other. So that, the highest of total biomass (1.84) and seed vigor index (75.6) were recorded for control treatment, they decreased by increasing osmotic potential and reached to 0.68 and 11.9 respectively.

SA had a significant effect on total biomass and seed vigor index of fennel (Table 3). The difference in these traits was statistically significant between control and all concentrations of SA. The highest concentration of SA caused an increment of 39.7%

and 39.5% in a total biomass and seed vigor index compared to control respectively.

The interaction of drought and SA showed that treatment of *F. vulgare* with SA through seed soaking could prevent the decrease in total biomass and seed vigor index caused by drought stress. Moreover, seedling pretreatment with 0.5 and 1 mM SA had the highest of these traits under normal and stress condition (Table 4).

Root length was also reduced by increasing drought stress, and all the treatments were significantly different from control (Table 2). Root length reduced from 62.1mm at normal condition to 20.9 mm at the highest osmotic potential.

As shown in table 2, the effect of drought levels on root fresh and dry weight was similar and with the increasing drought levels, both traits reduced significantly. The highest value of both traits belonged to control, but the lowest of these traits belonged to -0.3 MPa treatment (Table 2).

The effect of SA pretreatment on root length, root fresh and dry weight was significant. Increasing concentration of SA caused remarkably increase in the mentioned traits (Table 3). The greatest increase in root length, root fresh and dry weight was observed in the concentration of 1 mM SA as 37%, 38.9% and 37.5% compared to non-primed seedlings respectively.

Interaction between drought stress and SA pretreatment indicated that priming with 0.5 mM SA statistically showed the highest root length as compared to control and other concentrations of SA under non stress condition. Root fresh and dry weight also was affected the interaction of drought and SA, So that pretreatment with 1 mM SA caused an increasing of these traits at all levels of drought stress (Table 4).

Various shoot lengths were obtained at the different osmotic potentials (Table 2). The decreasing of osmotic potential reduced shoot length compared to control. The highest reduction in shoot length (53.9%) belonged to the -0.3 MPa treatment compared to control. The shoot fresh and dry weight was negatively affected by drought stress. Drought caused a greater reduction in fresh and dry weight of shoot at higher concentrations compared to control condition (Table 2).

All the concentrations of SA were different from control significantly (Table 3). At the concentration of 1 mM SA, the shoot length, shoot fresh and dry weight were increased by approximately 37.2%, 34.9% and 40% when they were compared with their control treatments respectively.

Maximum shoot length and shoot fresh weight was achieved in seeds primed with 0.5 mM SA, which was statistically similar to all the remaining treatments under normal condition; while this concentration of SA was significantly different from the other levels of SA in shoot dry weight under control condition. But, considering the latter trait, under stress conditions, the level of 1 mM SA was much better than the others (Table 4).

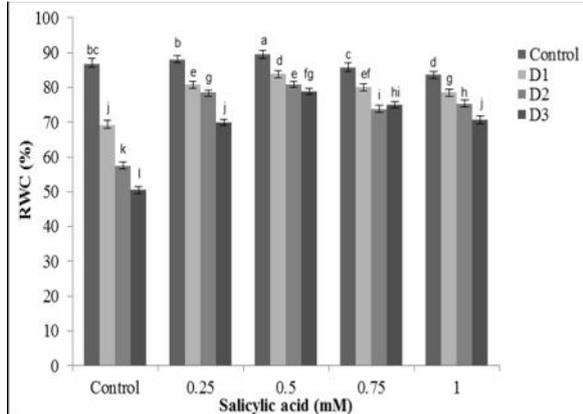


Fig. 1 Effect of salicylic acid pretreatment on relative water content in fennel plant leaves under drought stress conditions. The mean comparisons of treatments were done using LSD method at p 0.05 significant level. (D1: -0.1 Mpa, D2: -0.2 Mpa, D3: -0.3 Mpa levels of drought stress).

The data indicated that polyethylene glycol caused a significant reduction in RWC, but SA pretreatment markedly alleviated the effect of water stress. 0.5 mM SA was more effective than other concentrations (Fig. 1).

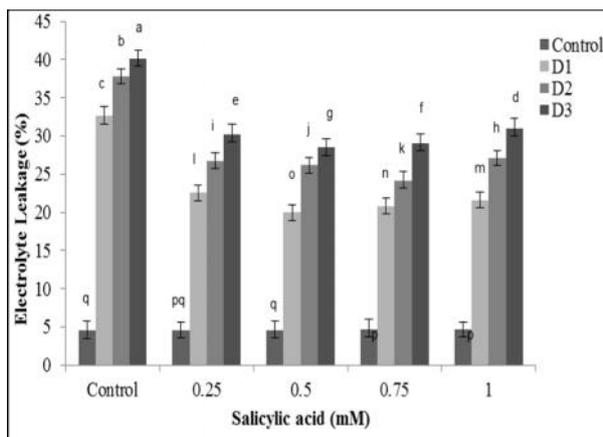


Fig. 2 Effect of salicylic acid pretreatment on electrolyte leakage in fennel plant leaves under drought stress conditions. The mean comparisons of treatments were done using LSD method at p 0.05 significant level. (D1: -0.1 Mpa, D2: -0.2 Mpa, D3: -0.3 Mpa levels of drought stress).

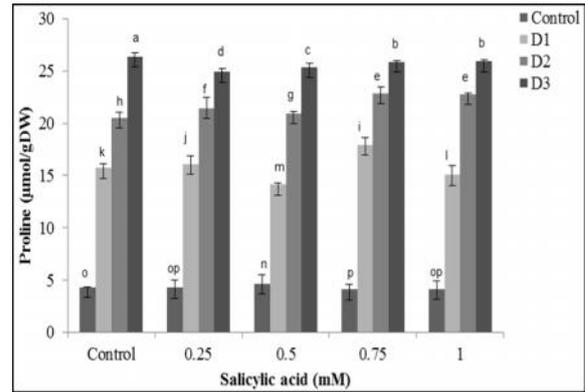


Fig. 3 Effect of salicylic acid pretreatment on proline content in fennel plant leaves under drought stress conditions. The mean comparisons of treatments were done using LSD method at p 0.05 significant level. (D1: -0.1 Mpa, D2: -0.2 Mpa, D3: -0.3 Mpa levels of drought stress).

In order to investigation, the effect of drought stress on membrane permeability, electrolyte leakage was measured. Base on obtained results, drought stress caused an increment of electrolyte leakage to intercellular space and SA pretreatment reduced this leakage at all the levels of drought stress (Fig. 2).

The amounts of proline increased significantly under stress condition. Pretreatment of plants with SA significantly increased the proline content under drought stress (Fig. 3).

Discussion

Drought is an important factor influencing the growth and physiological characteristics of plants. The responses of plants to drought stress depend on the species and genotype, the length and severity of water deficit and the age and stage of development. Severe stresses reduced germination percentage and rate, seedling emersion and vigor [21]. Drought stress induced by PEG decreased germination percentage and also delayed germination time at the highest concentrations due to lower water uptake by seed resulting in decreases of germination.

Table 1 Mean squares for germination percentage (GP) and rate (GR), total biomass (TB), seed vigor index (SVI), root length (RL), root fresh (RFW) and dry weight (RDW), shoot length (SL), shoot fresh (SFW) and dry weight (SDW) of *Foeniculum vulgare* Mill.

SOV	DF	MS									
		GP	GR	TB	SVI	RL	RFW	RDW	SL	SFW	SDW
SA	4	375.5*	0.43 ^{ns}	0.54**	596.84**	503.21**	16.22**	0.05**	176.9**	21.54**	0.3**
Drought	4	13815.5**	30.41**	7.62**	13859.73**	8313.3**	251.95**	0.77**	2448.1**	370.95**	3.56**
SA ×Drought	16	106.3 ^{ns}	0.11 ^{ns}	0.06**	82.31 ^{ns}	138.5**	2.18*	0.008*	17.4 ^{ns}	1.83 ^{ns}	0.04**
Error	50	112.3	0.27	0.02	65.96	49.9	1.03	0.004	19.6	1.5	0.01

** , * and ns denote significant differences at 0.01, 0.05 % levels, and not significant respectively.

Table 2 The effect of drought stress on germination percentage (GP) and rate (GR), total biomass (TB), seed vigor index (SVI), root length (RL), root fresh (RFW) and dry weight (RDW), shoot length (SL), shoot fresh (SFW) and dry weight (SDW) of *Foeniculum vulgare* Mill.

Drought levels (MPa)	GP	GR (Seed/day)	TB (mg/plant)	SVI	RL (mm)	RFW (mg/plant)	RDW (mg/plant)	SL (mm)	SFW (mg/plant)	SDW (mg/plant)
0	82.6 a	3.5 a	1.8 a	75.6 a	62.1 a	10.6 a	0.6 a	32.3 a	12.9 a	1.2 a
-0.1	69 b	2 b	1.4 b	50.5 b	44.6 b	8.3 b	0.44 b	28 b	10.5 b	1.03 b
-0.2	46 c	0.9 c	1.1 c	26.7 c	33.9 c	6.1 c	0.33 c	23.4 c	8.6 c	0.7 c
-0.3	29.6 d	0.5 d	0.6 d	11.9 d	20.9 d	3.8 d	0.21 d	14.9 d	5.9 d	0.4 d
-0.4	6.7 e	0.12 d	0 e	0 e	0 e	0 e	0 e	0 e	0 e	0 e

Means followed by the same letter(s) in each column are not significantly different at the 5% level.

Table 3 The effect of SA on germination percentage (GP) and rate (GR), total biomass (TB), seed vigor index (SVI), root length (RL), root fresh (RFW) and dry weight (RDW), shoot length (SL), shoot fresh (SFW) and dry weight (SDW) of *Foeniculum vulgare* Mill.

SA levels (mM)	GP	GR (Seed/day)	TB (mg/plant)	SVI	RL (mm)	RFW (mg/plant)	RDW (mg/plant)	SL (mm)	SFW (mg/plant)	SDW (mg/plant)
0	39.3 b	1.18 b	0.73 c	23.6 c	25.3 b	4.4 d	0.25 d	14.2 c	5.6 c	0.48 c
0.25	44 ab	1.35 ab	0.99 b	31.1 b	29.7 b	5.6 bc	0.31b c	19.2 b	7.5 b	0.68 b
0.5	50.3 a	1.59 a	1.17 a	38.6 a	36 a	6.2 b	0.34 b	22.5 a	8.4 ab	0.84 a
0.75	51 a	1.55 ab	0.98 b	32.5 b	30.4 b	5.3 c	0.29 cd	20.1 ab	7.9 ab	0.7 b
1	49.3 a	1.5 ab	1.2 a	39 a	40.1 a	7.2 a	0.4 a	22.6 a	8.6 a	0.81 a

Means followed by the same letter(s) in each column are not significantly different at the 5% level.

Table 4 The effect of SA pretreatment on germination percentage (GP) and rate (GR), total biomass (TB), seed vigor index (SVI), root length (RL), root fresh (RFW) and dry weight (RDW), shoot length (SL), shoot fresh (SFW) and dry weight (SDW) of fennel under different levels of drought stress.

	SA × Drought	GP	GR (seed/day)	TB (mg/plant)	SVI
0 mM SA	Control	78.3 ab	3.3 ab	1.4 fg	60.9 bc
	-0.1 MP a	58.3 cde	1.4 def	1.09 h	34.2 ef
	-0.2 MP a	41.6 efgh	0.8 fgh	0.7 jk	17.3 gh
	-0.3 MP a	18.3 ij	0.4 gh	0.4 l	5.5 hi
	-0.4 MP a	0 k	0 h	0 m	0 i
0.25 mM SA	Control	85 a	3.5 a	1.83 bc	77.8 a
	-0.1 MP a	61.6 bcd	1.8 cde	1.5 def	44.5 de
	-0.2 MP a	48.3 defg	1.01 efg	1.1 h	25.6 fg
	-0.3 MP a	25 hij	0.42 gh	0.5 kl	7.6 hi
	-0.4 MP a	0 k	0 h	0 m	0 i
0.5 mM SA	Control	86.7 a	4.05 a	2.27 a	86.3 a
	-0.1 MP a	80 a	2.4b c	1.58 cdef	60.9 bc
	-0.2 MP a	53.3 def	1.02 efg	1.22 gh	33.28 ef
	-0.3 MP a	31.7 ghij	0.45 gh	0.78 lij	12.5 ghi
	-0.4 MP a	0 k	0 h	0 m	0 i
0.75 mM SA	Control	81.7 a	3.69 a	1.73 bcd	73.31 ab
	-0.1 MP a	73.3 abc	2.26 c	1.45 efg	50.3 cd
	-0.2 MP a	41.6 efgh	0.813 fgh	1.09 h	22.8 fg
	-0.3 MP a	40f gh	0.65 fgh	0.64 jkl	15.7 gh
	-0.4 MP a	18.3 ij	0.32 lgh	0 m	0 i
1 mM SA	Control	81.6 a	3.68 a	1.9 b	79.5 a
	-0.1 MP a	71.7 abc	2.12 cd	1.6 cde	62.6 bc
	-0.2 MP a	45d efg	0.84 fgh	1.37 fg	34.5 ef
	-0.3 MP a	33.3 ghi	0.56 fgh	1.03 hi	17.9 gh
	-0.4 MP a	15 jk	0.28 gh	0 m	0 i

	SA × Drought	RL (mm)	RFW (mg/plant)	RDW (mg/plant)	SL (mm)	SFW (mg/plant)	SDW (mg/plant)
0 mM SA	Control	49.3 cdef	9.1 cdef	0.46 cde	28.33 abc	10.22 cd	0.94 def
	-0.1 MP a	37.4 ghij	6.7 gh	0.38 efghi	20.89 defg	7.77 efg	0.71 gh
	-0.2 MP a	26.2 jkl	4.3 ij	0.26 jkl	14.99 fg	6.78 gh	0.44 ij
	-0.3 MP a	13.6 n	1.9 k	0.13 m	6.77 hi	3.22 i	0.28 j
	-0.4 MP a	0 o	0 l	0 n	0 i	0 j	0 k
0.25 mM SA	Control	60.22 bc	10.54 bc	0.65 ab	31.33 ab	13.11 a	1.18 bc
	-0.1 MP a	42.88 fgh	8.44 ef	0.42 defg	29.11 ab	10.74 bc	1.10 bcd
	-0.2 MP a	31.44 hijk	5.67 hi	0.31 hijk	21.44 cdef	8.33 defg	0.80 fg
	-0.3 MP a	14 mn	3.47 jk	0.18 lm	13.88 gh	5.33 h	0.33 j
	-0.4 MP a	0 o	0 l	0 n	0 i	0 j	0 k
0.5 mM SA	Control	82.33 a	12.55 a	0.71 a	34.4 a	14.21 a	1.56 a
	-0.1 MP a	45.67 efg	8.58 def	0.43 defg	30.6 ab	10.88 bc	1.15 bc
	-0.2 MP a	34 hijk	6.22 gh	0.34 fghij	27.7 abcd	9.44 cdef	0.87 efg
	-0.3 MP a	18.22 lmn	3.66 j	0.19 lm	19.6 efg	7.44 fg	0.59 hi
	-0.4 MP a	0 o	0 l	0 n	0 i	0 j	0 k
0.75 mM SA	Control	56.89 bcde	9.73 bcde	0.5 cd	33.22 a	13.33 a	1.23 bc
	-0.1 MP a	39.56 fghi	7.55 fg	0.40 defgh	29.05 ab	10.55 bc	1.04 cde
	-0.2 MP a	30 ijk	5.33 hi	0.28 ijkl	24.44 bcde	9.11 cdef	0.80 fg
	-0.3 MP a	25.56 klm	4.11 ij	0.23 klm	14 gh	6.55 gh	0.41ij
	-0.4 MP a	0 o	0 l	0 n	0 i	0 j	0 k
1 mM SA	Control	61.88 b	11.22 ab	0.67 ab	34.3 a	14 a	1.28 b
	-0.1 MP a	57.55 bcd	10.11 bcd	0.56 bc	30.3 ab	12.4 ab	1.12 bcd
	-0.2 MP a	47.78 defg	8.75 def	0.45 def	28.1 abcd	9.5 cde	0.92 def
	-0.3 MP a	33.33 hijk	6 gh	0.34 ghijk	20.3 efg	7 gh	0.69 gh
	-0.4 MP a	0 o	0 l	0 n	0 i	0 j	0 k

Means followed by the same letter(s) in each column are not significantly different at the 5% level.

The decrease in water potential gradient between seeds and their surrounding media by the effects of PEG₆₀₀₀ adversely affects seed germination. Lower germination due to limited water uptake by the seeds was also reported by Dodd and Donovan [22].

Drought stress reduced germination percentage and rate (Table 2). These results were in agreement with the findings of Gholami *et al* [23] and Basu *et al.* [24]. Germination rate was more sensitive than germination percentage; this is correlated well with the results of AL-Taisan [25] who reported that osmotic potential decreased germination rate more than germination percentage. Root length is one of the most important characters for drought stress due to it contacts with soil and absorbing water. For this reason, root length plays an important role in the response of plants to drought stress [26]. The reduction of enzyme's activity and hormones sprinkle and disorder in photosynthesis and growth in seedlings, which were subjected to drought stress, was probably the reasons of decreasing in shoot length [27]. Gamze *et al.* [2] and Baalbaki *et al.* [28] showed that drought stress caused a reduction in seedling growth, root and shoot length, fresh and dry weight of root and shoot.

In this study, the interactive effect of salicylic acid and drought on *F. vulgare* was investigated. Drought stress induced by PEG reduced germination percentage and rate, total biomass, seed vigor index, root length, root fresh and dry weight, shoot length and shoot fresh and dry weight of fennel, while SA alleviated drought stress damages and increased all the mentioned traits under normal and stress conditions. SA is a compound that able to decrease harmful effects of drought stress on germination, root and shoot length, root fresh and dry weight and shoot fresh and dry weight [29]. Applying of SA stimulated germination due to scavenging of ROS. Baalbaki *et al.* [28] reported that seed priming with SA caused the reduction of oxidative damages and increased antioxidant enzymes activity during germination. The obtained results on sunflower [15] and rice [30] showed that SA is a moderate stimulant for germination. Singh and Usha [31] and Hayat and Ahmad [29] suggested that increase in germination and dry mass of water stressed plants in response to SA may be related to the induction antioxidant responses that protect the plants from damage. Senaratna *et al.* [32] have suggested a similar

mechanism to be responsible for SA induced multiple stress tolerance in bean and tomato plants. Similar results were also reported by Sakhabutdinova *et al.* [10] who found that SA changed the plant hormone's balance and seeds which were treated with SA caused a sharp accumulation of ABA and prevented the decrease in IAA and cytokinin content, which reduced inhibitory effects of water and salinity stresses on plant growth. Singh and Usha [31] reported that SA has an inhibitor role in the ethylene biosynthesis. Furthermore, it was reported that SA regulate cell extension, division and death and in fact, it created a balance between growth and senescence. AL-Hakimi and Hamada [33] have shown that the treatment of wheat plants with SA through seed soaking could ameliorate the inhibitory effect of drought and stimulate grown by enhancing photosynthetic rate and reducing dark respiration. Several reports proved that seed priming with SA caused an increasing of seedling length, seedling fresh and dry weight, total biomass and seed vigor index [10,34].

Drought stress induced by PEG decreased RWC (Fig. 1). Similar results obtained in barely [35] and wheat [36] under drought stress. Lower water uptake by roots resulted in decrease of RWC.

The decrease in water potential gradient between roots and their surrounding media by the effects of PEG₆₀₀₀ adversely affects RWC. SA pretreatment caused an increasing of RWC under drought stress (Fig. 1). These results were in agreement with the findings of Singh and Usha [31]. Increasing of RWC may be related to the role of SA in accumulation of compatible osmolytes in plants, which were subjected to drought stress.

In this investigation SA pretreatment decreased the electrolyte leakage percentage (Fig. 2). Several studies showed that electrolyte leakage in susceptible plants were more than resistant plants [37]. In previous studies, pretreatment with SA evidenced by a reduction in the level of lipid peroxidation and leakage of electrolytes from plant tissues as well as by more intensive growth processes as compared to control plants [29].

Under normal conditions, the total amount of ROS formed in the plants is determined by the balance between the multiple ROS producing pathways and the ability of the enzymatic and non-enzymatic (such as proline) mechanism to deal with them. Under

stress conditions, ROS formation is higher than ability of plants to remove it, and this could result in oxidative damages [12]. In fennel plants under -0.3 MPa of PEG solutions, proline content was elevated over the control (Fig. 3). When SA was applied under drought stress, the proline content increased. The SA greatly improves the dehydration tolerance through the increment of proline content.

In conclusion, PEG-induced drought stress could cause oxidative damage in fennel plant through excessive generation of ROS, and exogenous SA greatly improved the dehydration tolerance through elevated activities of antioxidant systems. Based on our results, it may show that application of SA can be a method to decrease water stress damages to plants

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