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Effect of drought stress and salicylic acid on yield and mucilage content of the medicinal herb *Plantago ovata* Forssk

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Abstract

Since there is limited information on the simultaneous effect of drought stress and salicylic acid on yield and mucilage content of the medicinal herb, *Plantago ovata* Forssk is available, a pot experiment was conducted in factorial form based on a randomized complete block design with three replications in Jiroft, Kerman Province, south of Iran. As the first factor, drought stress included four levels of irrigation: 100% field capacity (FC) (no stress), 75% FC (low stress), 50% FC (medium stress) and 25% FC (high stress). In the second factor, salicylic acid had four levels: 0, 0.01, 0.5 and 1 mM. The results showed that maximum yield and yield components of *P. ovata* Forssk were obtained with 100% FC and simultaneous application of 1 mM salicylic acid. Minimum amounts of yield and yield components resulted from irrigation based on 25% FC with no application of salicylic acid. Such an outcome revealed the significant role of salicylic acid in increasing the tolerance of *Plantago ovata* Forssk to drought stress.

***Keywords**:* Field capacity; Medicinal herb; Pot experiment; Jiroft; Drought stress

1. Introduction

Plantago ovata Forssk is an annual herbal plant belonging to the Plantaginaceae family. It is a precious plant with a good content of mucilage in its dry and mature seeds (Ghahreman, 1981) which is about 25% of seed weight (Hansol et al., 1992). Its seeds are effective in the treatment of diseases such as dysentery and swellings caused by gout and rheumatism (Carruba *et al*., 2002).

Various types of stress like high temperature and extreme light, water logging, nutrient deficiency and disruption in soil texture can affect plant growth and production of a large number of medicinal metabolites; among these, drought stress is the most important factor which limits plant growth and production worldwide (Reddy *et al*., 2004).

Drought stress reduces leaf area index and dry matter in plants and also decreases their yield up to 90% during flowering (Verslues *et al*., 2006). Iran is largely composed of arid and semi-arid regions and has constantly been exposed to drought stress, ruining its field crops and garden productions (Khazaei *et al*., 2007). Evaluating the effect of different irrigation regimes and the amount of cultivated seed on the quantity and quality of *P. ovata*, researchers found out that irrigation regimes significantly affected bush dry weight, bush height, number of spikes per bush and seed weight in spike. Also, reduction in the irrigation intervals led to an increase in the quantity of the aforesaid properties. The impact of this treatment on spike length and seed yield was however, not significant.

In a study by Dehahmadi *et al.* (2012), the effect of drought stress on the morphological

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properties and yield of three medicinal herbs- dill (*Nnethum graveolens*), coriander (*Carianrdum satiivum*) and fennel (*Foeniculum vulgar*) was investigated. The result shows that when the amount of water in soil was lower than FC limit, properties like number of leaf per bush, number of lateral offshoot per bush, number of umbel per bush, number of umbellate per umbel, seed weight per bush, number of seed per bush, thousand seed weight and harvest index were significantly influenced. Another study by Afsharmanesh *et al*. (2008) on the effect of water deficit stress and manure on the quantitative and qualitative yield and morphological properties of *P. ovata* Forssk, showed that such an effect together with the interactive effect of the two factors on seed weight was significant. Therefore, in order to trigger an increase in the yield of medicinal herbs, it is essential to apply novel practical and principled methods with regard to social, economical and environmental issues. Salicylic acid is a phenolic herbal compound and as a herbal hormone and growth regulator is well known for its role in defense mechanisms against biotic and abiotic agents of stress stimulation (Hayat and Ahmad, 2007). Salicylic acid plays a central role in the regulation of various physiological processes like growth, plant development, ionic absorption, photosynthesis, germination (which is dependent on the applied concentration), plant type, species, growth period and environmental conditions. Salicylic acid is also an important molecular signal of fluctuations in plants when they respond to environmental stresses (Senaranta *et al*., 2002). The functional mechanism of salicylic acid against stresses refers to its role in regulating antioxidant enzymes and active oxygen-contained compounds in plants (Khan *et al*., 2003; Shi and Zhu, 2008). Salicylic acid protects plants from the side effects of anti-oxidative reactions by increasing antioxidant enzyme activities. In addition, it increases amounts of polyamine putrescine, spermidine and spemine in plants which contribute to membrane conservation and uniformity under drought stress conditions (Nemeth *et al*., 2002). Treating plants with salicylic acid and use of derivatives in agriculture, horticulture and forestry can increase plant tolerance to different stresses (Senaranta *et al*., 2002). Salicylic acid can stimulate flowering, accelerate the flowering of tobacco tissue and intensify the opening of orchid flower. The mechanism effect of salicylic acid on induction of flowering is not yet known but a hypothesis shows that salicylic acid can contribute to the formation of chelate of metals and consequently induce flowering due to the presence of free hydroxyl groups on the benzoic acid cycle (Raskin, 1992). According to a report (Abdi and Belali, 2010), salicylic acid has a significant role in reducing diseases in potato: the number of tubers increased in plants which received 2 mM salicylic acid under drought stress as compared to those merely under stress, indicating a decline in their oxidative damage. Another study (Metwally *et al*., 2003) showed that a concentration of 0.5 mM salicylic acid increased barley tolerance to cadmium by increasing its antioxidant activity. Also, under salinity conditions, use of 0.1 mM salicylic acid in corn led to an increase in growth parameters like dry and wet weight of root and aerial organs and leaf area as well (Khodraay, 2004). Since there are few studies on the simultaneous effect of drought stress and salicylic acid on yield and mucilage content of the medicinal herb, *P. ovata* Forssk, the present study addressed this issue.

2. Materials and Methods

2.1. Time, location and climatic conditions of the experiment

The research was conducted in the city of Jiroft, Kerman Province, south of Iran in the agronomical year, 2012. Jiroft has a longitude of 57° 45', latitude of 28° 32' northwards and altitude of 575 m from sea level. The region has an arid and semi-arid climate with an average annual precipitation of 175 mm, relative humidity of 55-60% and maximum and minimum temperatures of 49 and 1 or -2°C in some years.

2.2. Design characteristics of the experiment

A pot experiment was conducted in factorial form based on a randomized complete block design with three replications. As factor A, drought stress included four levels of irrigation: 100% field capacity (FC) (no stress), 75% FC (low stress), 50% FC (medium stress) and 25% FC (high stress) and salicylic acid as factor B included four levels: 0, 0.01, 0.5 and 1 mM.

2.3. Planting procedure

Planting was carried out in November. Pots were 20 cm in diameter at the top edge and 18 cm in height. Field capacity and constant wilting point were determined by pressure plate aperture. When the pots were prepared, the seeds were first disinfected in 10% bleach solution for 1 min and then washed with distilled water; afterwards, they were separately (for separate pots) soaked in salicylic acid for 12 h. The seeds were planted manually in small furrows of 0.5 cm depth made in the pots. When seeds turned green after a month, the bushes density was reduced so that each pot contained four bushes left. Weeds were regularly under control by hand. When its seedling settled down, the plant received low irrigation treatments at the 3-4 leaf stage which continued up to its physiological maturation. The seeds of each pot were separately removed and dried after full maturation of seeds and yellowing of husk, offshoots and leaves of the plant. After sifting of the seeds, the seed yield of each pot was measured.

2.4. Mucilage

To determine the amount of mucilage in the seeds, Kalian Sundram's method was applied (Ebrahimzadeh *et al*., 1996).

2.5. Thousand seed weight

To obtain the thousand seed weight, a number of 100 harvested seeds were weighed and the resulted number was multiplied by 10. After data collection, analysis was carried out by MSTATC statistical software. The diagrams were drawn by Excel software. Mean comparisons were also done on the basis of Duncan's multiple range test at the probability level of 5%.

3. Results and Discussion

According to the data variance analysis of the present study (Table 1), drought stress and salicylic acid had a significant effect on bush height, plant dry weight, numbers of spikes, thousand seed weight and mucilage. Furthermore, the interactive effect of drought stress and salicylic acid on these properties, except for mucilage, was significant. The results showed that when different amounts of salicylic acid were used, levels of drought stress were not the same and took different trends.

Table 1. Variance analysis of the studied properties

Mean square					Freedom degree	Source of variation
Mucilage (%)	Thousand Seed Weight (g)	Numbers of spikes	Dry Weight (g)	Height (cm)		
1.73^{**}	0.68^{**}	74.04^{**}	2.72^{**}	3.47^{**}	3	A(drought stress)
0.03^{**}	0.003^{**}	0.23^{**}	0.07^{**}	0.10^{**}	3	B(salicylic acid)
0.0003^{ns}	0.0003^{**}	0.03^{**}	0.009^{**}	0.31^{**}	9	A.B
0.0002	0.000039	0.001	0.0007	0.001	32	Error
					47	Sum
0.03	0.38	0.28	11.80	0.22		C.V (%)

*, ** and ns represent the probability levels of 1%, 5 % and non-significance, respectively

3.1. Bush height

According to data variance analysis (Table 1), drought stress had a remarkable influence on bush height. Maximum (17.78 cm) and minimum (16.49 cm) height of bush were obtained from irrigation based on 100 and 25% FC, in the same order (Table 2). In line with increased irrigation, bush height increased to a noticeable point. Under drought stress, leaf water potential and cell turgor pressure, necessary for growth of plant cells and tissue, reduced. Extreme drought stress inhibits photosynthesis, disrupts metabolism and transfer of materials and finally causes death to the plant (Reddy *et al*., 2004). The impact of salicylic acid on bush height was significant as well (Table 1). Maximum bush height (17.25 cm) stemmed from an application of 0.5 mM salicylic acid, and minimum (17.04 and 17.02) from non-application and 1 mM of salicylic acid, respectively (Table 3). With increase in the use of salicylic acid (from no use to 0.5 mM), the bush height took a rising trend. However, with higher concentrations of salicylic acid from 0.5 to 1 mM, the bush height reduced from 17.25 to 17.02 cm (Table 3). Also, the interactive effect of salicylic acid and drought stress on bush height was significant; Maximum bush height (18.19 cm) was obtained with irrigation based on 100% FC and use of 0.5 mM salicylic acid, whereas the minimum (16.4 cm) was achieved through irrigation based on 25% FC and non-use of this acid (Fig. 1). While increase in irrigation water and salicylic acid led to a higher bush height, a decrease in the water and non-use of salicylic acid resulted in a lower height for the bush.

Mucilage (%)	Thousand Seed Weight(g)	Numbers of spikes	Dry Weight(g)	Height(cm)	Drought stress(A)
40.76[d]	1.75[a]	15.67[a]	2.79[a]	17.78[a]	100 FC
40.81[c]	1.70[b]	13.46[b]	2.45[b]	17.27[b]	75 FC
41.26[b]	1.22[d]	10.68[c]	2.17[c]	16.86[c]	50 FC
41.56[a]	1.61[c]	10.44[d]	1.66[d]	16.49[d]	25 FC

Numbers with similar letters in each column have no significant difference statistically

Table 3. Mean comparison of the impact of salicylic acid on the studied properties

Mucilage (%)	Thousand Seed Weight(g)	Numbers of spikes	Dry Weight(g)	Height(cm)	Salicylic acid (mM)
41.03[d]	1.55[d]	12.42[d]	2.19[d]	17.04[c]	0
41.08[c]	1.56[c]	12.49[c]	2.22[c]	17.18[b]	0.01
41.12[b]	1.57[b]	12.59[b]	2.29[b]	17.25[a]	0.5
41.16[a]	1.59[a]	12.74[a]	2.37[a]	17.02[c]	1

Numbers with similar letters in each column have no significant difference statistically

The plant root system grows better and absorbs more water and nutrients and finally develops more efficiently when it consumes salicylic acid (Gutierrez-Coronado *et al*., 1998). Inanaga *et al*. (2002) reported that shortage of salicylic acid before clustering reduced the height of rice. Salicylic acid is bound to carbohydrate-lignin compounds, the formation of which declines if salicylic is not sufficiently available (Inanaga *et al*., 1995).

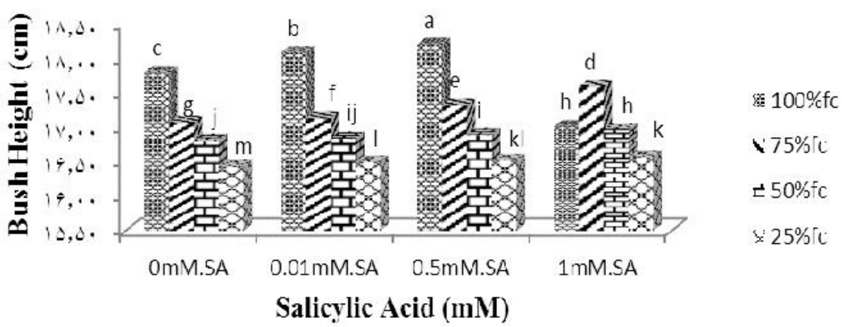

Fig. 1. The interactive effect of drought stress and salicylic acid on bush height

3.2. Plant dry weight

According to data variance analysis (Table 1), drought stress had a strong effect on plant dry weight. The highest (2.79 g) and the lowest (1.66 g) dry weight stemmed from irrigation based on 100 and 25% FC, respectively. Regarding such results, increase in the irrigation water led to a significant rise in plant dry weight. Plant respiration and perspiration are influenced by water deficit, which leads to cellular swelling, and as a result, negatively affects opening and closing of stoma in photosynthesis. Furthermore, water deficit leaves a negative effect on plant growth because it affects enzyme processes which are directly under control by water potential (Zou *et al*., 2007). The dry weights resulted from consumption of various concentrations of salicylic acid, which were significantly different from one another (Table 1). Consumption of 1 mM salicylic acid produced the heaviest dry weight (2.37 g). The lowest dry weight (2.19 g) was obtained when no acid was used. As more salicylic acid was consumed, plant dry weight increased to a remarkable point (Table 3). When salicylic acid was consumed, the leaves stood more upright and their shades were less of an obstacle to one another; thus, there was an increase in the rate of photosynthesis and substance making, particularly with high plant density (Yoshida *et al*., 1969). Moreover, the interactive effect of drought stress and salicylic acid was significant in this case (Table 1). The highest dry weight for the entire plant (2.88 g) was obtained from irrigation based on 100% FC using 1 mM salicylic acid and the lowest dry weights (1.65, 1.64 and 1.67 g) were obtained from irrigation based on 25% FC with non-use of salicylic acid, 0.01, 0.5 and 1 mM of the acid, respectively (Fig. 2).

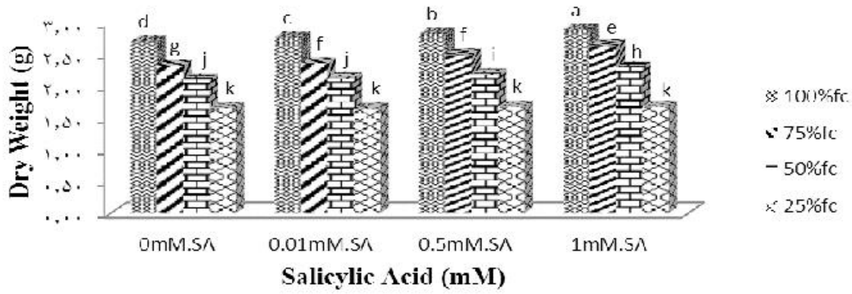

Fig. 2. The interactive effect of drought stress and salicylic acid on dry weight

Therefore, with an increase in the irrigation water and consumption of salicylic acid, plant dry weight also increased. In contrast, low irrigation water, non-use of salicylic acid, or its low concentrations led to a marked decline in the plant dry weight. In fact, salicylic acid plays a vital role in various physiological processes, e.g. growth, plant development, ionic absorption, photosynthesis, concentration-dependent germination, plant type, species, growth period and environmental conditions. This substance is also known as an important molecular signal of fluctuations in plants when they respond to environmental stresses (Senaranta et al., 2002).

3.3. Number of spikes

Irrigation had a significant impact on the number of spikes so as to produce the highest number (15.67) from irrigation based on 100% FC while the lowest (10.44) was from 25% FC. More absorption of water triggered an increase in the number of spikes and vice versa (Table 2). Intervals or extreme stresses of water deficit could disrupt physiological processes and make changes in metabolites of carbohydrates and nitrogen, protein structure and enzymes activities (Zou *et al*., 2007). Different concentrations of salicylic acid resulted in a remarkable difference in the number of spikes. Events such as reduction in the number of spikes, delay in its growth and increase in unproductivity of spike under deficiency of salicylic can occur due to low carbohydrates, decrease in cellular division and weakened formation of cell walls before the clustering stage (Inanaga *et al*., 2002). The interactive effect of drought stress and salicylic acid on the number of spikes was significant (Table 1); the highest number (16.04) stemmed from irrigation based on 100% FC and consumption of 1 mM salicylic acid and the lowest (10.39, 10.42 and 10.44) from 25% FC and non-use of salicylic acid, and its lower concentrations (0.01 and 0.5 mM) (Fig. 3). Overall, the number of spikes increased to a significant point when irrigation was increased and salicylic acid was used at the same time.

Plant germination is indirectly limited when salicylic acid affects growth regulators such as ethylene (Raskin, 1992), abscisic acid and gibberellin (Shakirova *et al*., 2003).

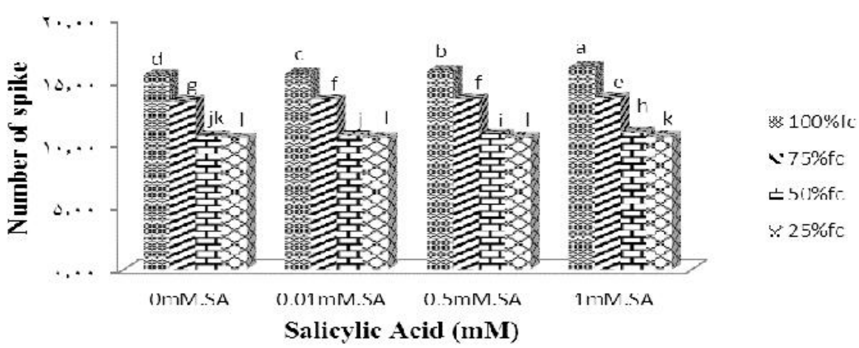

Fig. 3. The interactive effect of drought stress and salicylic acid on numbers of spikes

3.4. Thousand seed weight

As shown in Table 1, thousand seed weight was markedly affected by drought stress. Maximum (1.75 g) and minimum weight (1.22 g) were achieved after irrigation based on 100and 50% FC, respectively. With increased times of irrigation, thousand seed weight increased significantly (Table 2). Each level of salicylic acid concentration led to a different thousand seed weight. The heaviest weight (1.59 g) was obtained with an application of 1 mM salicylic acid and the lightest weight (1.55 g) was obtained without salicylic acid. Therefore, the more the amount of salicylic acid fed to the plant, the higher the thousand seed weight harvested (Table 3). As the amount of salicylic acid increases, more of its sediment remains in the inner and outer coverings of the seed. This stands for increase in seed weight (Balastra *et al*., 1989). The rate of perspiration in plant organs is influential in the transmission and sedimentation of salicylic acid. At seed filling stage, an increase in the rate of respiration in the clusters occurs which gives rise to sedimentation of salicylic acid (Yoshida *et al*., 1962). The interactive effect of drought stress and salicylic acid on the thousand seed weight was significant (Table 1), and from irrigation based on 100% FC and 1 mM salicylic acid, the heaviest weight (1.79 g) was achieved. However, the lightest weight (1.21 g) was produced from irrigation based on 50% FC and no salicylic acid (Fig. 4). Overall, with increased irrigation water and simultaneous use of salicylic acid, the thousand seed weight recorded a significant rise. In contrast, this weight declined when lower amounts of water and salicylic acid were consumed.

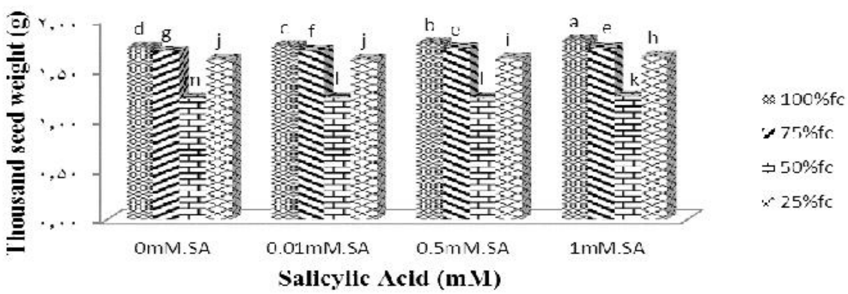

Fig. 4. The interactive effect of drought stress and salicylic acid on thousand seed weight

3.5. Mucilage

As shown by data variance analysis (Table 1), mucilage is heavily affected by drought stress which has a maximum percentage of 41.56 and lowest percentage of 40.76 stemming from irrigation based on 25 and 100% FC, in the same order. There is an inverse relationship between mucilage and irrigation: declined irrigation leads to higher percentage of mucilage and vice versa. After consumption of different concentrations of salicylic acid, there was a statistically significant difference in the final product of mucilage (Table 1). With an application of 1 mM salicylic acid and without it, the highest and lowest percentages of mucilage were 41.16 and 41.03, respectively. In a study, drought stress had no effect on the mucilage content of *P. ovata* Forssk. Based on this result, mucilage content was more influenced by heredity than environmental conditions (Afsharmanesh *et al*., 2008). The highest percentage for mucilage was reported to be obtained from intervals of irrigation within 30 days (Tbrizi, 2004). Furthermore, there was no significance regarding the interactive effect of drought stress and salicylic acid on mucilage. As a result, the simultaneous application of different levels of irrigation and salicylic acid concentrations, resulted in production of the same amount of mucilage. In other words, there was no statistically significant difference in the content of mucilage at each different level.

4. Conclusion

This paper derived the following results: maximum yield and yield components of *P. ovata* Forssk were obtained from irrigation based on 100% FC and simultaneous consumption of 1 mM salicylic acid. This indicated the important role of salicylic acid in alleviating plant response to drought stress and contributing to its production of maximum yield and yield components. Minimum yield and yield components stemmed from irrigation based on 50% FC without any salicylic acid, again an indication of the role of salicylic acid in increasing plant tolerance of drought stress. The highest percentage of mucilage resulted from the lowest level of irrigation (based on 25% FC) and the lowest percentage of mucilage from the highest level of irrigation (based on 100% FC), displaying an inverse relationship between irrigation and mucilage. In addition, minimum and maximum percentages of mucilage were attained via non-application of salicylic acid and using its highest concentration (1 mM), respectively. This showed the direct and positive relationship between salicylic acid and mucilage: higher amount of salicylic acid led to a rise in mucilage content.

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