

## **Effects of salicylic acid and spermine foliar application on some morphological and physiological characteristics of isabgol (*Plantago ovata* Forsk) under water stress**

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**Abstract.** Yield as well as concentration of relevant component in many medical plants are influenced by growing condition water stress. Field experiment was done based on a randomized complete block design with 18 treatments and three replications, at Gonbad Kavous University, Golestan, Iran in winter 2016. Irrigation treatment with three levels (normal irrigation, water stress imposed at flowering stage or at seed filling stage) was placed in main plot. Salicylic acid (SA) with three levels (control, 0.4 and 0.8 mM) and spermine (Spm) with two levels (control and 0.02 mM) were assigned in sub-plots. Result showed the foliar application SA and Spm treatments under normal irrigation and cutoff irrigation at seed filling stage have significantly affected relative water content, electrolyte leakage and membrane stability index, but under cutoff irrigation at flowering stage did not significantly affected measured traits. Exogenous applications of SA and Spm applied increased the plant height, 1,000 grain weight and biological yield in compared to the non-use product at normal irrigation regime. Foliar spraying SA and Spm under cutoff irrigation at flowering stage did not significantly affected measured traits. Application product especially SA<sub>0.8</sub> mM and Spm<sub>0.02</sub> mM under cutoff irrigation at seed filling stage increased the most characteristics in compared to control. Foliar application of phenolic compounds such as salicylic acid and polyamines like spermine can be considered as an effective measure to reduce the adverse effects of water stress and ultimately increase the yield and yield components of isabgol.

**Key words:** isabgol, membrane stability index, relative water content, phenolic compounds, water deficit.

### **INTRODUCTION**

Climate change is a matter of concern in the twenty-first century that warns of rising temperature, unprecedented drought, flood, desertification, radiation, cyclones, forest fires, extreme low temperature that can adversely affect agriculture and human life (Reynolds & Ortiz, 2010). The responses of crops to these abiotic stresses have a number of similarities, although the genetic basis is not necessarily the same (Reynolds & Ortiz, 2010). Plants are challenged by a variety of biotic or/and abiotic stresses, which can affect their growth and development, productivity and geographic distribution (Liu et al., 2015). Abiotic stresses due to physical factors can occur in all stages of a plants lifespan (Venkateswarlu et al., 2012). Growth rate is accelerated due to increased

temperature, which reduces photosynthesis since the life cycle is shortened, while both heat and drought stress may also inhibit growth directly at the metabolic level. Furthermore, harvest index may be reduced if reproductive processes are impaired by stress that occurs at critical developmental stages (Reynolds & Ortiz, 2010). In order to survive adverse environmental conditions, plants have evolved various adaptive strategies, among which is the accumulation of secondary metabolites that play protective roles (Liu et al., 2015).

Increasing the crop production under irrigation has several limitations such as irregular rainfall distribution and non-availability of permanent irrigation. An alternative approach is to apply exogenous phyto-hormones (polyamines, salicylic acid and gibberellic acid), plant growth promoting rhizobacteria or other effective components application that can plant protection and maintain under limited moisture (Hara et al., 2012).

Salicylic acid (SA) is an important phytohormone that plays a role in response to biotic stresses. Apart from this role, recent studies have demonstrated that SA also participates in the signaling of abiotic stress responses, such as drought, high and low temperature, salinity, ozone, UV radiation, and heavy metals (Hara et al., 2012). In addition, abiotic stresses also induce endogenous SA accumulation. The appropriate application of SA could provide protection against several types of environmental stresses (Hara et al., 2012). According to several reports salicylic acid has a significant role in reducing injuries abiotic stresses in plants. The use of salicylic acid under stress condition decreased the lipid peroxidation in basil plant (Delavari et al., 2010). Bayat et al. (2012) reported that application of salicylic acid under salt stress conditions greatly reduced the amount of electrolyte leakage in calendula. In addition, salicylic acid plays a vital role in various physiological processes, e.g. growth, plant development, ion absorption, photosynthesis and germination (Shekofteh et al., 2015).

Polyamines (PA) are a group of phytohormone-like aliphatic amine natural compounds with aliphatic nitrogen structure and present in almost all living organisms including plants. They are involved in many physiological processes, such as cell growth and development and respond to stress tolerance to various environmental factors (Gill & Tuteja, 2010). Recently several reports showed that application polyamines (putrescine (Put), spermidine (Spd) and spermine (Spm)) also improved tolerance of plants against several abiotic stresses including german chamomile (Sharafzadeh et al., 2012), wheat (Gupta et al., 2012), citrus (Shi et al., 2010), pistachio (Kamiab et al., 2013), clerodendrum (Mukherjee & Bandyopadhyay, 2014), jojoba (Lobna et al., 2015), cotton (Loka et al., 2015), valerian (Mustafavi et al., 2015), tomato (Nowruzi Givi et al., 2015) and sweet basil (Pazoki, 2017). Darvizheh et al. (2018a) stated that foliar application of SA (75, 150 mg L<sup>-1</sup>) and Spm (70 mg L<sup>-1</sup>) on purple coneflower, increased secondary metabolites and physiological characteristics in plant under drought stress condition.

Isabgol (*Plantago ovata* Forsk) is an annual herbaceous plant belonging to the Plantaginaceae group. The origin of this plant is the Mediterranean region, North Africa, Southwest Asia, including Iran (Omidbeygi, 2005). The seeds of *Plantago ovata* are commercially known as white or blonde psyllium, Indian plantago or isabgol. India dominates the world market in the production and export of psyllium (Bokaeian et al., 2014). Mucilages are generally polysaccharides, which are polymeric in nature and are obtained from seed, husk and leaves isabgol. Decomposition of isabgol seeds has shown

some sugars and polysaccharide compounds in grain mucilage, including galactose, glucose, xylose, arabinose, rhamnose and galacturonic acid. (Omidbeygi, 2005). Koochaki et al. (2007) reported that water stress had a negligible negative effect on most parameters evaluated except length of spike and seed yield of isabgol were affected negatively by increasing the length of irrigation intervals. In addition, they stated the highest yield was obtained from 10 and 20 day intervals irrigations. Rahimi et al. (2010) also found the significant decrease in leaf chlorophyll *plantago ovata* under drought stress conditions. The aim in the study was to determine first if these products have an ameliorative effects and then to determine the best dose.

## MATERIALS AND METHODS

### Experimental design

The experiment was conducted at the Gonbad Kavous University, located in Golestan province, Iran in 55°21'E, 37°26'N, 45 m above sea level with 450 mm mean 10 years precipitation. The precipitation, average temperature and relative humidity during the experiment were 119.1 mm, 61.7 °C and 300% respectively. The soil had a silt-loam texture. Chemical characteristics of the upper soil layer (30 cm) at the start experimental were: pH (7.92), electrical conductance (1.2 dS m<sup>-1</sup>), bulk density (1.5 g cm<sup>-3</sup>), field slope (≤ 0.2), organic carbon (1.11%), total N (0.11%), available P (21.2 mg kg<sup>-1</sup>) and K (504 mg kg<sup>-1</sup>).

The experiment was arranged as a split plot factorial base on randomized complete block design with 18 treatments and three replications with the following treatments: three irrigation levels (control (non-stress), irrigation cutoff at flowering stage and irrigation cutoff at seed filling stage), three salicylic acid level (SA0= No salicylic acid (water spray), SA2= spraying 0.4 mM of salicylic acid, SA3= spraying 0.8 mM of salicylic acid) and two spermine levels (Spm1= not using spermine (water spray), Spm2= spraying 0.02 mM of spermine). Irrigation was used as main-plot, salicylic acid application and spermine spraying were as sub -plot. The unit plot size was 5 m×1 m; with 5 rows (row spacing and plant distance were 20 and 10 cm, respectively). Blocks distances were considered 3 meters and the distance of main plots and sub plots inside the blocks was 3 and 0.5 meter, respectively. Isabgol was hand sown at a 0.5–1 cm depth soil on March 2016. Isabgol seeds (98% viability and seed purity) were obtained from Sabz Rooyeshe Mahallat Company, Iran.

In this experiment nitrogen and phosphorus fertilizers were added respectively with a dose of 100 kg ha<sup>-1</sup> urea (46% N) and 150 kg ha<sup>-1</sup> triple super phosphate (19/8% P), based on soil test and fertilizer recommendations for isabgol.

Soil moisture content at field capacity and permanent wilting point were 0.9 and 0.7% (equivalent to a weigh moisture of 16.8 to 21.6), respectively (Walter & Gardener, 1986). The depth of irrigation was determined based on the average soil water content that calculated by following equation (Allen et al., 1998):

$$dw = \frac{(\theta_{m1} - \theta_{m2})}{100} \cdot \rho_b \cdot ds \quad (1)$$

In this equation; dw (cm): depth of irrigation;  $\theta_{m1}$ : initial weight moisture (FC) (%);  $\theta_{m2}$ : secondary weighs moisture (WP) (%);  $\rho_b$ : bulk density (g cm<sup>-3</sup>) and ds: depth of soil (cm).

Irrigation (Furrow irrigation system) was carried out in all plots until the complete plant establishment (four-leaf stage) as needed. Then, soil moisture content was maintained before the application of stress treatments in all experimental plots in the same range as mentioned above.

The exogenous salicylic acid (molecular weight 138.1, Sigma) and spermine (molecular weight 202.3, Sigma) were applied on the budding process (flowering stem production), flowering and seed filling in the required treatments.

#### **Measurement of morphological and physiological characters**

After biological maturity 10 plants were randomly sampled from each plot to measure plant height, spikes per plant, spike length, seed per spike, membrane stability, electrolyte leakage and relative water content. We harvested two square meters of three central rows were from each plot to determine the seed yield, biological yield, harvest index, 1,000 seed weight, seed mucilage percent and yield mucilage yield.

Harvest index (HI) is calculated using the formula (2):

$$HI = \text{Seed yield} / \text{Biological yield} \cdot 100 \quad (2)$$

Mucilage contents in the seed were determined according to Kalyanasundaram et al. (1984). Grain mucilage yield (GMY) was calculated by the following equation:

$$GMY = \text{Seed yield} \cdot \text{Content of grain mucilage} \quad (3)$$

Sairam et al. (1994) method was followed for analysis of membrane stability index (MSI).

$$MSI \text{ was calculated as } MSI = [1 - (EC1/EC2)] \cdot 100. \quad (4)$$

Electrolyte leakage (EL) percentage was calculated by the following equation proposed by Tas & Basar (2009).

$$EL = EC1/EC2 \cdot 100 \quad (5)$$

Leaf relative water content (RWC) was estimated gravimetrically according to the method of Andersen et al. (2004). In this method; leaf relative water content was calculated from the following equation.

$$RWC = \frac{fw - dw}{ftw - dw} \cdot 100 \quad (6)$$

In this equation; dw: drought weight, fw: fresh weight and ftw: fully turgid weight.

#### **Statistical analysis**

The data were subjected to analysis of variance (ANOVA) using the SAS package (version 9.1). The LSD test was applied to test significance of treatment means at 0.05 and 0.01 levels of probability. The regression was estimated amount the parameters of the SPSS package (version 22.0).

## **RESULTS AND DISCUSSION**

Analysis of variance (ANOVA) revealed that the 2way interactive effects of irrigation and salicylic acid were statistically significant ( $P \leq 0.01$  and  $P \leq 0.05$ ). The 2way interactive irrigation and spermine had significant effect on measure treat except

for plant height, number of spike per plant, number of seed per spike, 1,000-seed weight, seed mucilage percent, membrane stability index and relative water content. Salicylic acid and spermine had significant effect on plant height, spike length, biological yield, seed yield and seed mucilage yield, but no had significant effect on number of spike per plant, number of seed per spike, 1,000-seed weight, harvest index, seed mucilage percent, membrane stability index, electrolyte leakage and relative water content) on other traits were significant ( $P \leq 0.01$  and  $P \leq 0.05$ ). Analysis of variance (ANOVA) revealed that the 3way interactive effects of irrigation and salicylic acid and spermine was statistically significant on all characteristics ( $P \leq 0.01$  and  $P \leq 0.05$ ) (Table 1).

### **Plant height**

Comparison of means showed that the plant height increased on foliar application of SA<sub>0.8</sub> mM and Spm<sub>0.02</sub> mM under normal irrigation (with 4.42% in compared to control). The plant height was not significantly affected by spray treatments under cutoff irrigation condition at flowering stage (Table 2). In this regard, Shekofteh et al. (2015) reported that maximum plant height in isabgol was obtained with irrigation based on 100% FC (field capacity) and use of 0.5 mM salicylic acid, whereas the minimum was achieved through irrigation based on 25% FC and non-use of this acid. Also foliar application of SA and Spm increased the plant height (except SA<sub>0</sub> mM and Spm<sub>0.02</sub> mM treatment) under cutoff irrigation condition at seed filling stage, although they did not significant different with each other (Table 2). Reducing the plant height in response to drought stress can be due to the blockage of xylem and phloem vessels, after which the transfer of materials and assimilate from the plant is reduced (Khalil et al., 2010). In a study to investigate the use of putrescine and spermidine on vegetative traits of basil herb under drought stress conditions, the application of 0.8 mM putrescine and 0.8 mM spermidine had the highest plant height (Pazoki, 2017). Darvizheh et al. (2018b) evaluated the effects of SA and Spm application at different concentrations on purple coneflower and reported that the growth indices increased in treated plants with SA150 + Spm 70 mg L<sup>-1</sup>.

### **Spikes number per plant**

Comparison of mean of treatments showed that highest spikes number per plant observed in the normal irrigation and non-use of SA and Spm treatment ( $18.60 \pm 0.60$ ). Exogenous application SA<sub>0.8</sub> mM and Spm<sub>0.02</sub> mM under cutoff irrigation at flowering stage increased of spikes number per plant ( $16.53 \pm 1.50$ ) in compared other treatments. Also, under cutoff irrigation condition at seed filling stage, SA<sub>0.8</sub> mM treatment had a better effect on this trait than other treatments and the highest of spikes number per plant obtained at  $17.73 \pm 0.90$  (Table 2). Mousavinick (2012) reported that the number of spikes per plant was reduced under stress due to reduced irrigation frequencies. In a study conducted on isabgol the highest spikes number stemmed from irrigation based on 100% FC and application of 1 mM salicylic acid and the lowest from 25% FC and without using salicylic acid or at lower concentrations (0.01 and 0.5 mM) (Shekofteh et al., 2015).

**Table 1.** Variance Analysis effect of cutoff irrigation, salicylic acid and spermine foliar application on some morphological and physiological traits of isabgol

Source of variation	Rep.	Irrigation (IR)	Error (Ea)	Salicylic acid (SA)	Spermine (Spm)	IR×SA	IR×Spm	SA×Spm	IR×SA×Spm	Error (Ebc)	C.V (%)
df	2	2	4	2	1	4	2	2	4	30	-
Plant height	Ns	**	2.42	**	Ns	*	Ns	*	*	1.013	3.54
Number of spike per plant	Ns	**	1.37	**	Ns	**	Ns	Ns	**	0.67	5.41
Number of seed per spike	Ns	**	5.08	*	*	**	Ns	Ns	**	15.67	5.46
Spike length	*	**	0.03	**	**	**	**	**	**	0.01	2.49
1,000-seed weight	*	**	0.001	Ns	Ns	**	Ns	Ns	**	0.001	2.13
Biological yield	Ns	**	26,594	*	Ns	*	*	**	**	33,289	7.75
Seed yield	*	**	964	**	**	**	**	**	**	855.5	7.26
Harvest index	Ns	**	3.16	**	**	**	**	Ns	**	3.1	10.18
Seed mucilage percent	Ns	**	0.10	**	Ns	**	Ns	Ns	**	1.37	6.90
Seed mucilage yield	Ns	**	25.63	**	**	**	**	**	**	41.44	9.39
Membrane stability index	Ns	**	0.56	**	Ns	**	Ns	Ns	*	0.65	0.86
Electrolyte leakage	Ns	**	0.49	**	Ns	**	Ns	Ns	*	0.65	12.65
Relative water content	Ns	**	13.51	**	Ns	**	Ns	Ns	**	9.91	4.38

Ns, \* and \*\* are Non-Significance and Significance at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively.

**Table 2.** Comparison Mean effect of cutoff irrigation × salicylic acid × spermine foliar application on some morphological characteristic of isabgol

Irrigation regime	Salicylic acid	Spermine	Plant height (cm)	Number of spike plant <sup>-1</sup>	Number of seed spike <sup>-1</sup>	Spike length (cm)	1,000 seed weight (gr)	Biological yield (kg.ha <sup>-1</sup> )	Seed yield (kg.ha <sup>-1</sup> )	Harvest index (%)
Normal	control	control	29.20 ± 0.40bcd	18.60 ± 0.60a	85 ± 4.36a	3.40 ± 0.02e-h	1.66 ± 0.04c-f	1,820 ± 50g	513.50 ± 10ab	28.21 ± 1.2a
		0.02 Mm	28.37 ± 1.16cde	15.73 ± 0.11c-f	76.67 ± 4.04bc	3.53 ± 0.40cde	1.61 ± 0.05fg	2,255 ± 82ef	321.83 ± 39e	14.30 ± 2.01fgh
	0.4 mM	control	28.25 ± 1.85cde	12.60 ± 0.40i	55.33 ± 4.93g	3.43 ± 0.11d-g	1.67 ± 0.01b-e	2,315 ± 180c-f	360.67 ± 19de	15.63 ± 1.15e-h
		0.02 Mm	28.10 ± 2.10cde	14.93 ± 0.61d-g	72.00 ± 7.00cde	3.45 ± 0.02d-g	1.68 ± 0.03bcd	2,151.7 ± 245f	343.83 ± 56e	15.96 ± 1.42efg
	0.8 mM	control	29.20 ± 1.81bcd	13.60 ± 0.72ghi	66.67 ± 6.66ef	3.54 ± 0.19cde	1.71 ± 0.03bc	2,613.3 ± 73abc	332.50 ± 9e	12.73 ± 0.60hi
		0.02 Mm	30.55 ± 0.25ab	13.27 ± 50hi	63.67 ± 3.21f	3.00 ± 0.08k	1.66 ± 0.04c-f	1,601.7 ± 45g	214.33 ± 6f	13.39 ± 0.24gh
Irrigation cutoff at flowering stage	control	control	25.70 ± 1.75f	13.07 ± 0.83hi	62.67 ± 2.08f	3.16j ± 0.09jk	1.65 ± 0.03def	2,451.7 ± 148b-e	367.83 ± 21de	15.05 ± 1.50fgh
		0.02 Mm	26.04 ± 0.55f	14.13 ± 0.58gh	67.67 ± 2.08def	3.25 ± 0.07hij	1.58 ± 0.04g	2,398.3 ± 253b-f	433.50 ± 51c	18.17 ± 2.42cde
	0.4 mM	control	25.67 ± 0.83f	14.47 ± 1.30fgh	68.33 ± 4.73def	3.18 ± 0.16ij	1.64 ± 0.02d-g	2,386.7 ± 5.77b-f	319.50 ± 8e	13.39 ± 0.37gh
		0.02 Mm	27.42 ± 1.12def	13.82 ± 0.42ghi	67.67 ± 4.04def	3.35 ± 0.17fgh	1.66 ± 0.04c-f	2,513.3 ± 291a-e	342.83 ± 54e	13.75 ± 2.64gh
	0.8 mM	control	27.02 ± 0.89ef	16.23 ± 0.93cd	68.33 ± 1.53def	3.50 ± 0.12c-f	1.62 ± 0.03efg	2,453.3 ± 281b-e	405.67 ± 16cd	16.72 ± 2.46def
		0.02 Mm	26.70 ± 1.13ef	16.53 ± 1.50bc	71.67 ± 3.21cde	3.33 ± 0.09ghi	1.65 ± 0.03def	2,578.3 ± 103a-d	256 ± 35f	9.90 ± 0.97i
Irrigation cutoff at seed filling stage	control	control	29.72 ± 0.85abc	15.00 ± 1.11d-g	73.67 ± 1.15cd	3.58 ± 0.07cd	1.68 ± 0.07bcd	2,311.7 ± 173def	497.33 ± 48ab	21.58 ± 2.43b
		0.02 Mm	26.87 ± 0.64ef	14.87 ± 0.94d-g	80.33 ± 2.08ab	3.24 ± 0.06hij	1.79 ± 0.01a	2,285 ± 121def	493 ± 31ab	21.64 ± 2.09b
	0.4 mM	control	29.82 ± 0.02abc	16.00 ± 1.00cde	80.00 ± 2.00ab	3.63 ± 0.12bc	1.73 ± 0.04b	2,226.7 ± 178ef	485.83 ± 45b	21.94 ± 2.94b
		0.02 Mm	31.37 ± 0.70a	14.73 ± 1.17efg	80.67 ± 1.53ab	3.74 ± 0.01ab	1.67 ± 0.06b-e	2,578.3 ± 211a-d	494.33 ± 22ab	19.30 ± 2.35bcd
	0.8 mM	control	31.13 ± 0.40a	17.73 ± 0.90ab	82.00 ± 1.00ab	3.91 ± 0.06a	1.72 ± 0.04b	2,795 ± 263a	540.83 ± 3a	19.47 ± 1.97bcd
		0.02 Mm	29.90 ± 0.87abc	16.20 ± 1.40cd	81.67 ± 3.21ab	3.53 ± 0.13cde	1.63 ± 0.03d-g	2,631.7 ± 25ab	528 ± 5ab	20.07 ± 0.32bc

Means within each column followed by the same letter are not statistically different  $\alpha = 0.05$  by LSD test.

### **Number of seed per spike**

The comparison of mean of treatments showed that highest number of seed per spike observed in the normal irrigation and non-use of SA and Spm treatment ( $18.60 \pm 0.60$ ) (Table 2). Hayat et al. (2010) reported that at higher concentrations, SA itself may cause a high level of stress in plants. The number of seed per spike was not significantly affected by spray treatments under cutoff irrigation condition at flowering stage. Also foliar application of SA and Spm increased the number of seed per spike (except non-use of SA and Spm treatment) under cutoff irrigation condition at seed filling stage, although they were not significantly different from each other (Table 2). Ramroudi et al. (2011) reported that the number of spikes per plant and the number of seeds per spike on isabgol decreased in the irrigation delay at flowering stage. It is inferred that salicylic acid and spermine spraying in different concentrations were able to compensate part of the negative effects of moderate drought stress on yield and yield components.

### **Spikes length**

Based on the comparison of means, the spikes length (except SA<sub>0.8</sub> mM and Spm<sub>0.02</sub> mM treatment) increased under normal irrigation in compared to control (Table 2). We can infer that, the reduction of the spike length under SA<sub>0.8</sub> mM and Spm<sub>0</sub> mM treatment is probably due to the high concentrations of phenolic acid salicylic compound. The spikes length (except SA<sub>0.8</sub> mM and Spm<sub>0</sub> mM treatment with  $3.50 \pm 0.12$ ) was not significantly affected by spray treatments under cutoff irrigation condition at flowering stage. Also the spikes length (except SA<sub>0</sub> mM and Spm<sub>0.02</sub> mM treatment) increased by spray treatments under cutoff irrigation condition at seed filling stage. The longest spike length observed in SA<sub>0.8</sub>+Spm<sub>0</sub> mM and SA<sub>0</sub>+Spm<sub>0.02</sub> mM treatments (with  $3.74 \pm 0.01$  and  $3.91 \pm 0.06$ , respectively) (Table 2). The reduction of spike length under stress conditions can be as a result of reduced photosynthesis and subsequently reduced production and transfer of assimilates for plant growth (Pirasteh-Anosheh et al., 2012). It is inferred that the use of salicylic acid and spermine, as a result of increased water use efficiency (Kumar et al., 2000; Fariduddin et al., 2003; Farooq et al., 2009) and reduction of oxidative stress (Mittler, 2002; Todorova et al., 2015), could prevent reduced spike lengths under interrupting irrigation condition.

### **1,000 seed weight**

Comparison of mean of treatments showed that Spm<sub>0.02</sub> spraying treatment under cutoff irrigation condition at seed filling and flowering stage had the highest and lowest seed weight with respectively  $1.79 \pm 0.01$  and  $1.58 \pm 0.04$  grams (Table 2). Mousavinick (2012) stated that under drought stress conditions, due to the reduction of photosynthesis, the amount of dry matter production and accumulation, thereby reducing the amount of dry matter transferred to the isabgol seeds and the weight of the seeds decreased.

### **Biological yield**

The biological yield in some treatments (except SA<sub>0.8</sub> mM and Spm<sub>0.02</sub> mM treatment) increased in compared to control under normal irrigation and spraying salicylic acid and spermine (Table 2). The biological yield was not significantly affected by foliar application of SA and Spm under cutoff irrigation condition at flowering stage. Increasing the concentrations of salicylic acid and spermine under cutoff irrigation at

seed filling stage significantly increased the biological yield and the heaviest biological yield was related to SA<sub>0.8</sub>+Spm<sub>0</sub> treatment with 2,795 ± 263 kg ha<sup>-1</sup> respectively. However, this treatment had no significant difference with some treatments (Table 2). Rahimi et al. (2011) showed that water stress (irrigation terminated at the start of flowering) increased the biological yield of isabgol and psyllium. These compounds reduce the adverse effects of drought stress preventing the reduction of plant height and leaf loss as a result of and strongly affects on photosynthetic apparatus and photochemical enhancement of light energy is absorbed.

### **Seed yield**

The highest seed yield observed in cutoff irrigation at seed filling stage and SA<sub>0.8</sub>+Spm<sub>0</sub> treatment with 540.83 ± 3 kg ha<sup>-1</sup>. However, no significant difference observed between irrigation cuttings at seed filling stage at different levels of salicylic acid and spermine spraying. The lowest seed yield was allocated to foliar application SA<sub>0.8</sub>+Spm<sub>0.02</sub> at normal irrigation and cutoff irrigation at flowering stage treatments (214.33 ± 6 and 256 ± 35 kg ha<sup>-1</sup>, respectively) (Table 2). We can infer that salicylic acid and spermine spraying could increase the grain yield of isabgol by stimulating the physiological processes that cause active transfer of photosynthetic products from source to sink. Rezaichianah & Pirzad (2014) reported a 13% increase in black cumin grain yield under drought stress condition with 0.5 mM salicylic acid application.

### **Harvest index**

Comparison of mean between treatments showed that normal irrigation and non-use of SA and Spm treatment had the highest harvest index (28.21 ± 1.2). In this regard, no significant difference was obtained between irrigation cuttings in the seed filling stage at different levels of salicylic acid and spermine spraying. Under intense stress conditions, the seed yield decreased by 35% in compared to the control (Table 2). The results are in agreement with the observations of Ramroudi et al. (2011) on isabgol. The results showed that both grain yield and biological yield were reduced under the integrated salicylic acid and spermine treatments at cutoff irrigation condition at flowering stage, which led to a reduction in the harvest index. In contrast, the treatments under irrigation condition at seed filling stage, despite the high level of grain yield; the harvest index was reduced due to the high biological yield function.

### **Seed mucilage percentage**

Exogenous application of SA and Spm increased the seed mucilage percentage (except SA<sub>0.8</sub> and Spm<sub>0.02</sub> mM treatment) under normal irrigation, although they were not significantly different with each other treatments. The highest amount of seed mucilage percent observed in SA<sub>0.8</sub>+Spm<sub>0.02</sub> mM with 19.67 ± 1.53% (Table 3). In some treatments under moderate and intense stress, spraying salicylic acid and spermine increased the seed mucilage percent, but had no effect on other treatments. The lowest amount of seed mucilage percent was obtained from SA<sub>0.8</sub>+Spm<sub>0.02</sub> mM with 13 ± 1% (Table 3). The results are in agreement with the observations of Koochehi et al. (2011) on isabgol and psyllium. They reported that the maximum amount of mucilage and swelling factor were obtained for both species were obtained at irrigation level of 2,000 m<sup>3</sup> ha<sup>-1</sup>.

**Table 3.** Comparison Mean effect of cutoff irrigation × salicylic acid × spermine foliar application on some physiological characteristics of isabgol

Irrigation regime	Salicylic acid	Spermine	Seed mucilage (%)	Seed mucilage yield (kg.ha <sup>-1</sup> )	Membrane stability index (%)	Electrolyte leakage (%)	Relative water content (%)
Normal	control	control	18 ± 1.50ab	92 ± 5.20ab	92.97 ± 0.89fg	7.03 ± 0.89bc	77.95 ± 1.64bc
		0.02 Mm	18.67 ± 1.53ab	60 ± 8.18g	92.08 ± 0.15gh	7.92 ± 0.15ab	75.50 ± 1.28cd
	0.4 mM	control	18 ± 1.00ab	65 ± 3.46fg	93.85 ± 0.67def	6.15 ± 0.67cde	71.13 ± 3.01de
		0.02 Mm	19.67 ± 1.53a	67.33 ± 5.86efg	94.82 ± 0.58cd	5.18 ± 0.58ef	75.37 ± 5.01cd
	0.8 mM	control	18 ± 1.00ab	60 ± 3.46g	96.57 ± 1.17ab	3.43 ± 1.17gh	87.69 ± 3.01a
		0.02 Mm	16 ± 1.50cd	34.33 ± 0.58i	95.40 ± 0.87bc	4.60 ± 0.87fg	81.37 ± 1.46b
Irrigation cutoff at flowering stage	control	control	18 ± 1.00ab	66.33 ± 3.51fg	92.04 ± 0.52gh	7.96 ± 0.52ab	69.56 ± 4.00e
		0.02 Mm	15 ± 1.00d	65.33 ± 10.97fg	91.44 ± 0.604h	8.56 ± 0.60a	63.26 ± 2.33f
	0.4 mM	control	14.33 ± 1.15de	45.33 ± 4.04h	91.42 ± 0.85h	8.58 ± 0.85a	59.82 ± 2.52f
		0.02 Mm	18 ± 2.00ab	61.67 ± 11.59g	91.44 ± 0.94h	8.56 ± 0.94a	59.66 ± 3.87f
	0.8 mM	control	19 ± 1.00ab	77.33 ± 6.66cde	91.89 ± 0.64gh	8.11 ± 0.64ab	61.50 ± 2.34f
		0.02 Mm	17.67 ± 0.58bc	45 ± 5.57h	92.78 ± 0.72fg	7.22 ± 0.72bc	69.39 ± 4.63e
Irrigation cutoff at seed filling stage	control	control	16 ± 2.00cd	78.67 ± 4.73cd	92.62 ± 1.11fgh	7.38 ± 1.11abc	69.11 ± 5.32e
		0.02 Mm	17.67 ± 0.58bc	86.33 ± 8.08bc	93.51 ± 0.50def	6.45 ± 0.50cde	72.03 ± 3.24de
	0.4 mM	control	15 ± 1.00d	73 ± 7.00def	94.71 ± 0.72cde	5.29 ± 0.72def	70.31 ± 2.77de
		0.02 Mm	13 ± 1.00e	64.33 ± 7.50fg	93.46 ± 1.11ef	6.54 ± 1.11cd	72.13 ± 2.56de
	0.8 mM	control	17.67 ± 1.53bc	94.67 ± 8.50ab	97.63 ± 1.31a	2.37 ± 1.31h	80.06 ± 3.42bc
		0.02 Mm	18.67 ± 0.58ab	97.67 ± 3.51a	96.56 ± 0.75ab	3.44 ± 0.75gh	77.52 ± 3.33bc

Means within each column followed by the same letter are not statistically different  $\alpha = 0.05$  by LSD test.

### **Seed mucilage yield**

The highest seed mucilage yield observed in the normal irrigation and non-use of SA and Spm treatment ( $92 \pm 5.20 \text{ kg ha}^{-1}$ ). The seed mucilage yield (except SA<sub>0.8</sub> mM and Spm<sub>0</sub> mM treatment with  $77.33 \pm 6.66 \text{ kg ha}^{-1}$ ) was not significantly affected by spray treatments under cutoff irrigation condition at flowering stage. Also the seed mucilage yield increased at foliar application of SA<sub>0.8</sub> mM and with/without Spm under cutoff irrigation condition at seed filling stage (with  $94.67 \pm 8.50$  and  $97.67 \pm 3.51 \text{ kg ha}^{-1}$ , respectively) in compared to control (Table 3). In the research conducted by Ramroudi et al. (2011), the highest yield of mucilage of isabgol was related to irrigation regime, and the least was obtained from discontinuation of irrigation after flowering. Shekofteh et al. (2015) reported that with an application of 1 mM salicylic acid and without it, the highest and lowest percentages of mucilage of isabgol were 41.16 and 41.03, respectively.

### **Membrane stability index (MSI)**

The interactions between irrigation levels and spray treatments showed that SA<sub>0.08</sub> and Spm<sub>0.02</sub> application increased membrane cell stability in plants under normal irrigation and cutoff irrigation at seed filling stage. The highest amount of MSI demonstrated in SA<sub>0.8</sub>+Spm<sub>0</sub> mM with  $97.63 \pm 1.31\%$ . Also there was not significantly different in MSI between SA and Spm spraying under cutoff irrigation condition at flowering stage (Table 3). In a study Naghashzadeh (2014); MSI as affected by different irrigation regimes was decreased by increasing drought stress. Researcher reported that well-watered had the highest MSI of all irrigation regimes and severe drought stress was 28% lower than well-watered conditions. A similar result was reported that exogenous salicylic acid and spermine was effective in enhancing the cell membrane stability under drought stress. Bandurska & Stroinski (2005) reported that plant treatment with SA before drought stress reduced a damaging action of water deficit on cell membrane in leaves. The increase of cell membrane stability with 300 ppm salicylic acid under drought stress conditions was reported by Sibi et al. (2012). Application of spermine and putrescine increased drought tolerance through reducing the electrolyte leakage, increasing compatibility osmolytes and antioxidant enzyme activity (Amraee et al., 2016).

### **Relative water content (RWC)**

The highest relative water content was related to SA<sub>0.8</sub>+Spm<sub>0</sub> treatment with  $38.97 \pm 1.33$  percent under normal irrigation. The RWC in leaves was not significantly affected by spray treatments under cutoff irrigation condition at flowering stage. Foliar application of SA and Spm increased the relative water content in leaves in compared to control under cutoff irrigation condition at seed filling stage (Table 3). Relative water content is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance (Barahuyi Nikju, 2017). A similar result Naghashzadeh (2014) reported that there was not significantly different in RWC between well-watered (70% field capacity) and moderate drought stress (50% field capacity). Severe drought stress (30% field capacity) was 26% lower than well-watered conditions. When osmotic stress occurs, the solution metabolites on cells increase to prevent water deficit and turgor pressure reduction, which include nitrogen ingredients, such as proline and other amino acids, poly amines and ammonium (Tamura et al., 2003; Naghashzadeh, 2014). In a study, Kumar et al. (2000) reported an increase in water use efficiency, transpiration rate and internal CO<sub>2</sub> concentration in

response to foliar application of salicylic acid. Also Hayat et al. (2008) reported that the treatment of under stressed plants with lower concentrations of salicylic acid significantly enhanced the photosynthetic parameters, membrane stability index, leaf water potential and relative water contents thereby improved tolerance of the plants to drought stress. In fact, foliar spraying of salicylic acid moderated the damaging effects of water deficit on cell membranes plant through increased the ABA content in leaves, which might have contributed to the enhanced tolerance of plants to drought stress (Bandurska & Stroinski, 2005).

### Electrolyte leakage (EL)

Comparison of means between treatments showed that the Electrolyte leakage percentage (except SA<sub>0</sub>mM and Spm<sub>0.02</sub> mM treatment) under normal irrigation and spraying salicylic acid and spermine decreased in compared to control (Table 3). Also the Electrolyte leakage percentage was not significantly affected by foliar application of SA and Spm under cutoff irrigation condition at flowering stage (Table 3). The highest amount of EL observed in this stage however, had no significant difference with some treatments. Exogenous application of salicylic acid and spermine decreased the Electrolyte leakage percentage in leaves in compared to control under cutoff irrigation condition at seed filling stage. The lowest amount of EL obtained in SA<sub>0.8</sub>+Spm<sub>0</sub> mM with  $2.37 \pm 1.31\%$  (Table 3).

Masoumi et al. (2010) stated drought stress causes a significant decrease RWC in the *Kochia Scoparia* leaves and increase electrolyte leakage compare with control.

### Regression

Based on the results of stepwise regression analysis, the number of spikes per plant was the first trait that entered the model and justified 0.36 of grain yield changes. In the resulting model, the number of seed per spike (x1) and spike length (x2) remained at the end. The above-mentioned traits explained 0.44% of the total variation (Table 4), therefore, it can be stated that these traits are the most important traits affecting the yield of isabgol (*Plantago ovata* Forssk.) in the studied treatments and possibly selecting to increase grain yield through these traits, will be effective; therefore, the final model of yield with the dependent traits was obtained as follows:

$$\text{Yield} = -457.23 + 5.22 (x1) + 140.43(x2) \quad (7)$$

**Table 4.** Stepwise regression analysis for grain yield per unit area as the dependent variable and the other independent variables

Variables added to the model	Constants	Regression coefficients		Coefficient of Determination (R <sup>2</sup> )
		b1	b2	
Number of seed per spike	-106.98ns	7.038**		0.357
Spike length	-457.23**	5.22**	140.43**	0.439

## CONCLUSION

We can conclude, the application of salicylic acid and spermine moderate the drought stress and it lead to increase plant height, number of seed per spike, biological yield, seed yield, seed mucilage percent, membrane stability index, electrolyte leakage

and relative water content. The best dose for salicylic acid and spermine was it 0.08 mM and 0.02 mM, respectively. They were more effect when we applied in cutoff irrigation at seed filling stage condition.

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