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ROLE OF FOLIAR SPRAY OF SALICYLIC ACID AND SPERMINE ON SOME CHARACTERISTICS OF ISABGOL (*Plantago ovata* Forssk)

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Abstract

In order to investigate the effect of drought stress on some physiological and biochemical characteristics of isabgol, a study was carried out as a split-plot factorial experiment based on a randomized complete block design with 18 treatments and three replications, at Iran, during growing seasons of 2016 and 2017. Irrigation treatment included; normal irrigation, cutoff irrigation at the flowering stage and cutoff irrigation at seed filling stage which considered as main-plots. Salicylic acid with three levels (0, 0.4 and 0.8 mM) and Spermine with two levels (0 and 0.02 mM) was assigned in sub-plots. The results of experiment showed that the highest grain yield under irrigation conditions was obtained by foliar spraying of 0.8 mM Salicylic acid and 0.02 mM Spermine at seed filling stage. Application of Salicylic acid at concentration of 0.8 mM along with or without Spermine improved the amount of the cell membrane stability index under different irrigation levels. The highest catalase activity in both years of the study was attributed to normal irrigation treatment with the foliar application of Salicylic acid at 0.4 mM concentration and with or without Spermine. Application of $SA_{0.8}$ *mM and* $Spm_{0.02}$ *mM under cutoff irrigation condition increased the most of the measured features compared to control, which indicates the positive effect of these compounds.*

Key words: *antioxidant defense system, drought stress, electrolyte leakage, herbal plant, osmotic compounds.*

INTRODUCTION

Isabgol (*Plantago ovata* forssk) of the family *Plantaginaceae* is an important medicinal plant, which is widely used in the textile, military, food, cosmetics and pharmaceutical industries (Mardani Karani, 2013). Drought stress is an important environmental factor affecting crop productivity worldwide. Drought stress affects mainly through disturbance of the balance between the productions of reactive oxygen species (ROS) and antioxidant defense mechanism and cause oxidative stress (Nasibi, 2011). Under such condition, the high activity of antioxidant enzymes and high levels of non-enzyme antioxidants are very important for plant tolerance to stress (Nasibi, 2011). Cell membrane is the first site of damage under stress conditions. By reducing the potential of cellular water, the accumulation of compatible osmolytes involved in osmoregulation like proline and soluble sugars allows additional water to be taken up from the environment and for a short time; the balance of water is maintained within plant cells. Thus, osmotic adjustment is an adaptation mechanism to increase drought tolerance (Inze and Montagu, 2000; Kumar et al., 2003). An alternative approach is to apply exogenous phytohormones (polyamines, salicylic acid and gibberellic acid), plant growth promoting rhizobacteria or other effective compounds that can protect plants under limited moisture (Hara et al., 2012).

Salicylic acid (SA) and Spermine (Spm) are important phytohormone and are involved in responding to biotic stresses. Moradi and Pourghasemian (2018) reported that contents of carotenoid, soluble sugars, proline and phenol in Marigold were significantly increased by decreasing amount of irrigation water, while foliar application of salicylic acid with 1.5 mM concentration; increased the plant dry weight, carotenoid contents and soluble sugars relative to the control conditions. In a study on exogenous application of salicylic acid under water stress conditions, the amount of secondary metabolites, chlorophyll, anthocyanin, protein, phenol and flavonoid in the *Melissa officinalis* L. increased (Jamal Omidi et al., 2018). In another study on the Centipedegrass Mutant, the increased activity of antioxidant enzymes such as catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and superoxide dismutase (SOD) was reported in after external application of putrescine, spermidine and spermine (Liu et al., 2017). Combined application of 500 µM salicylic acid and 1 µM spermine increased seed protein content, oil content and proline concentration of castor bean leaves under drought stress conditions (Tadayon and Izadi, 2015). The aim of current study was (a) to investigate the effect of Salicylic acid and Spermine application on seed yield and (b) to understand physiological mechanism/s involved under water stress condition.

MATERIALS AND METHODS

Experiment

To evaluate the response of some physiological and biochemical characteristics of isabgol response to water deficit and application of salicylic acid and spermine, a two-year experiment (2016 and 2017) was conducted in the research field of 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 meteorological information during the experiments are shown in Table 1.

The land soil texture was silt-loam, with bulk density of 1.5 g/cm^3, pH of 7.92, electrical conductance of 1.2 dS.m^{-1}, field slope of ≤0.2, organic carbon of 1.11%, total N of 0.11%, available P of 21.2 mg.kg^{-1} and K of 504 mg.kg^{-1}. The experiment was arranged as a split plot factorial based on randomized complete block design with 18 treatments and three replications. Treatments include: three irrigation levels (Control (non-stress), irrigation cutoff at flowering stage (severe stress) and irrigation cutoff at seed filling stage (moderate stress)), three Salicylic acid level (SA_0 = sprayed with distilled water, $SA_{0.4}$ = sprayed 0.4 mM of Salicylic acid, $SA_{0.8}$ = sprayed 0.8 mM of Salicylic acid) and two Spermine levels (Spm_0 = sprayed with distilled water, $Spm_{0.02}$ = sprayed 0.02 mM of Spermine). Irrigation was used as main-plot, Salicylic acid application and Spermine spraying was as sub-plot. Isabgol (with 98% viability and seed purity) was hand sown in 0.5-1 cm soil depth on 7 March 2016 and 2017. In this experiment nitrogen and phosphorus fertilizers were added respectively with a dose of 75 kg.ha^{-1} urea and 10 kg.ha^{-1} triple super phosphate, based on soil test and fertilizer recommendations for isabgol. The exogenous Salicylic acid (molecular weight 138.1, Sigma) and Spermine (molecular weight 202.3, Sigma) were applied during plant budding (flowering stem production), flowering and seed filling stages.

Table 1. Metrological statistics of Gonbad Kavous in 2016 and 2017

	Precipitation (mm)		Average temperature (°C)		Relative humidity (%)	
	2016	2017	2016	2017	2016	2017
March	23	35.6	9.3	12.8	79	75.2
April	65.1	37.2	15.2	14.8	77	76
May	27.8	30.4	22.1	21.4	72	70
Jun	3.2	0.3	23.3	25.2	72	59

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 and Gardener, 1986).

The depth of irrigation was determined based on the average soil water content that was calculated by following equation (Allen et al., 1998):

$$dw = \frac{(\theta m1 - \theta m2)}{100} \times \rho b \times ds \qquad (1)$$

In this equation; dw (cm) represents depth of irrigation, θm_1 represents initial weight moisture (FC) (%), θm_2 represents secondary weighs moisture (WP) (%), ρb represents bulk density (g/cm^3) and ds represents depth of soil (cm).

Irrigation (with furrow irrigation system) was carried out on all plots until the complete plant establishment (four-leaf stage) as needed. Then, soil moisture content was maintained before the application of stress treatments for all experimental plots.

Measurements

After biological maturity, 10 plants were randomly sampled from each plot to measure membrane stability and electrolyte leakage.

We harvested two square meters of three central rows from each plot to determine the seed yield.

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

$$MSI = [1 - (EC1/EC2)] \times 100 \qquad (2)$$

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

$$EL = EC1/EC2 \times 100 \qquad (3)$$

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. In order to investigate the uniformity of variances between two year Levene test was used.

RESULTS AND DISCUSSIONS

Analysis of variance (ANOVA) (Table 2) of the first year of the experiment revealed that the two-way interaction effects of irrigation and Salicylic acid were statistically significant on all characteristics ($P \leq 0.01$ and $P \leq 0.05$). The two-way interaction irrigation and Spermine had a significant effect on some characters for example seed yield and catalase activity. Salicylic acid and Spermine had a significant effect on seed yield and catalase activity. Analysis of variance (ANOVA) revealed that the three-way interaction effects of irrigation and Salicylic acid and Spermine were statistically significant on all characteristics ($P \leq 0.01$ and $P \leq 0.05$). Second year results (Table 3) revealed that the two-way interaction effects of irrigation and Salicylic acid plus the three-way interaction effects of irrigation and Salicylic acid and Spermine were statistically significant on all characteristics ($P \leq 0.01$ and $P \leq 0.05$). The two-way interaction irrigation and Spermine had a significant effect on measured characteristics except for membrane stability index and electrolyte leakage.

Table 2. Variance analysis effect of cutoff irrigation, salicylic acid and spermine foliar application on some morphological and physiological traits of isabgol in 2016

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	-
Seed yield	*	**	960.5	**	**	**	**	**	**	854	7.25
Membrane stability index	Ns	**	0.56	**	Ns	**	Ns	Ns	*	0.65	0.86
Electrolyte leakage	Ns	**	0.56	**	Ns	**	Ns	Ns	*	0.65	12.65
Catalase activity	Ns	**	0.44	**	**	**	**	**	*	0.12	4.38

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

Table 3. Variance analysis effect of cutoff irrigation, salicylic acid and spermine foliar application on some morphological and physiological traits of isabgol in 2017

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	-
Seed yield	*	**	960.5	Ns	**	**	*	**	**	364	4.22
Membrane stability index	Ns	**	6.00	**	Ns	**	Ns	**	**	10.15	3.80
Electrolyte leakage	Ns	**	6.00	**	Ns	**	Ns	**	**	10.15	19.70
Catalase activity	Ns	**	0.93	*	**	**	**	**	**	0.44	6.37

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

Membrane stability index (MSI)

The interactions between irrigation levels and spray treatments in the first year of the experiment showed that $SA_{0.08}$ mM+Spm_0 mM application increased membrane cell stability in plants under normal irrigation and cutoff irrigation at seed filling stage. The highest amount of MSI was obtained in $SA_{0.8}$ mM+Spm_0 mM with 97.63%. There was no significant difference in MSI between SA and Spm spraying under cutoff irrigation condition at the flowering stage (Table 4). The results of the comparison of the mean of second-year data also indicated that the cell membrane stability of some of the treatments was more than control under different levels of irrigation and foliar application of Salicylic acid and Spermine (Table 4). The highest and lowest cell membrane stability under normal irrigation and non-foliar application under severe stress conditions were assigned to foliar spray $SA_{0.8}$ mM+Spm_0 mM with 94.68 and 71.06 percent, respectively (Table 4).

Table 4. Interaction of irrigation × Salicylic acid × Spermine in the first and second year of experiment, respectively, on membrane stability index percentage of isabgol

Treatments	Control (non-stress)						Irrigation cutoff at flowering stage						Irrigation cutoff at seed filling stage					
	SA_0		$SA_{0.4}$		$SA_{0.8}$		SA_0		$SA_{0.4}$		$SA_{0.8}$		SA_0		$SA_{0.4}$		$SA_{0.8}$	
Year	Spm_0	$Spm_{0.02}$	Spm_0	$Spm_{0.02}$	Spm_0	$Spm_{0.02}$	Spm_0	$Spm_{0.02}$	Spm_0	$Spm_{0.02}$	Spm_0	$Spm_{0.02}$	Spm_0	$Spm_{0.02}$	Spm_0	$Spm_{0.02}$	Spm_0	$Spm_{0.02}$
2015-2016	92.97 fg	92.08 gh	93.85 def	94.82 cd	96.57 ab	95.40 bc	92.04 gh	91.44 h	91.42 h	91.44 h	91.89 gh	92.78 fg	92.62 fgh	93.51 def	94.71 cde	93.46 ef	97.63 a	96.56 ab
2016-2017	80.97 f-i	86.73 b-e	88.86 bc	81.83 e-h	94.68 a	90.80 ab	71.06 j	78.02 hi	75.86 ij	83.48 d-g	84.61 c-g	76.45 i	88.11 bcd	89.18 bc	79.89 ghi	83.48 d-g	88.90 bc	85.99 b-e

Means with similar letters did not show statistically significant differences at 5% level of probability according to LSD

SA_0= No salicylic acid (water spray), $SA_{0.4}$= Spraying 0.4 mM of salicylic acid, $SA_{0.8}$= Spraying 0.8 mM of salicylic acid. Spm_0= not using spermine (water spray), $Spm_{0.02}$= Spraying 0.02 Mm of spermine.

Seed yield

We can infer that Salicylic acid and Spermine spraying could increase the grain yield of isabgol by stimulating the physiological processes that cause an active transfer of photosynthetic products from source to sink. In a study, Afsharmanesh et al. (2008) stated that the yield of isabgol under severe stress conditions (irrigation after 25% field capacity) was 43% lower than that of moderate stress (irrigation after 75% field capacity). Rezaichianah and Pirzad (2014) reported a 13% increase in black cumin grain yield under water stress condition with 0.5 mM salicylic acid application. The results of this study are also consistent with the results of Ramroudi et al. (2011).

Membrane stability index (MSI)

Water stress causes a disruption between the production of reactive oxygen species and antioxidant defense, which will cause oxidative stress. As soon water availability of plants reduces then stomata would be closed and thus the flow of carbon dioxide reduces. Reducing carbon dioxide does not only directly reduce the activity of rubisco carboxylase in the Calvin cycle; it also increases the production of reactive oxygen species by incomplete oxygen recovery (Farooq et al., 2009). In a study Naghashzadeh (2014); MSI, as affected by different irrigation regimes, was decreased by increasing drought stress. He 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 water stress. Bandurska and Stroinski (2005) reported that plant treatment with SA before drought stress reduced a damaging action of water deficit on the 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).

Electrolyte leakage (EL)

The positive effect of the use of polyamines on the reduction of ion cell membrane leakage has also been reported in other studies (Kubis et al., 2014). Masoumi et al. (2010) stated that drought stress causes a significant decrease RWC in the *Kochia Scoparia* leaves and increase electrolyte leakage compare with control.

CONCLUSIONS

Application of salicylic acid in case of moderate and severe water stress can increase seed yield, membrane stability index. Application of salicylic acid at appropriate concentrations can alleviate adverse effects of water deficit stress on growth and performance of the isabgol plants when applied at a proper time which can be determined taking into account the climatic conditions of the production area.

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