

Seed Treatment with Salicylic Acid Enhance Drought Tolerance in Capsicum

Deepti Prabha^{1,*}, Yogesh kumar Negi²

¹Department of Seed Science & Technology HNB Garhwal University, Srinagar Garhwal, Uttarakhand, India

²Department of Basic Science Uttarakhand University of Horticulture & Forestry College of Forestry & Hill Agriculture Ranichauri, Tehri, Garhwal, Uttarakhand, India

*Corresponding author: deepti_prabha@rediffmail.com

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Abstract Salicylic acid is a natural chemical used to enhance biotic and abiotic stress tolerance in plants. In our experiment we studied about the effect of salicylic acid on the growth of the capsicum under water stress condition. Four concentrations (0.25, 0.50, 1.00 and 2.00 mM) of salicylic acid (SA) were applied through seed soaked method. Pretreatment with salicylic acid improved the majority of physiological and morphological parameters measured in capsicum seedling and plants. Results showed that SA treatment reduced the injury rating value, electrolyte leakage and increased proline content in seedlings, the dry matter of plants and ultimately yield. The best drought protection observed when the seeds were soaked in 0.50 mM SA.

Keywords: capsicum, salicylic acid, seed treatment, tolerance, water stress

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1. Introduction

Plant growth is greatly affected by a combination of environmental stresses such as extreme temperatures, drought, high salinity etc. From agriculture point of view, such stresses are among the most significant factor responsible for substantial and unpredictable losses in crop production. As sessile organism plants had to develop various biochemical and physiological mechanism to respond and adapted to these stresses and thus acquire stress tolerance. Adaptation to these stresses has been suggested to be mediated by both preexisting and induces defenses [4,9,19]. Now the scientists are working on the increase in stress tolerance in plants. Generally approaches are using to increase stress tolerance in plants are genetic engineering, traditional breeding, development of somaclonal variants and use of growth regulators.

Salicylic acid is naturally occurring plant hormone, which increase the capacity of plant to fight against biotic and abiotic stresses [20,21]. SA influences various physiological and biochemical functions in plants. Exogenous application of SA effects on a wide range of physiological processes including cold tolerance, chilling tolerance, Ozone, heat and heavy metal stress and salinity tolerance [5,15,14,21] has been reported by several reporters. Drought is one of the most important factor limit plant growth and ultimately production. Drought is claimed to reduce production upto 25 % throughout the world [7]. In the literature we found salicylic acid also increases the drought tolerance in plants [10,13]. The

exogenous application of salicylic acid depends upon various factors, like developmental stage, mode of application, concentration of application etc. [2,11].

Sweet pepper belongs to the family solanaceae. Sweet pepper is native to Tropical South America. It is now widely cultivated in Central and South America, Peru, Bolivia, Costa Rica, Mexico, in almost all the European countries, Honkong and India. Sweet pepper is a common crop in this region and sown in August and December. So for the proper growth of the crop irrigation is necessary because there is no rain at this time. If water is deficient then the production is badly affected. In respect to drought stress, relevant work is limited in case of Sweet pepper, which is an important vegetable crop. So there is lot of scope since increase in irrigated land is difficult, water is scarce and the only way to improve crop performance is to increase drought tolerance. There is no information about response of capsicum (cv. California wonder) to salicylic acid under water stress. So the present study was conducted to investigate the effect of salicylic acid on Sweet pepper grown under water stress. The Objectives of this work were to standardize the optimum concentration of salicylic acid, which will be most effective against drought for Sweet pepper.

2. Materials and Methods

2.1. Plant Material and Treatment

Sweet pepper (cv. California wonder) seeds were used in this experiment. For surface sterilization seeds were

washed with 1% sodium hypochlorite solution to remove seed borne pathogens then washed with running water and dried. Seeds were soaked in SA at 0.25, 0.50, 1.00 and 2.00 mM concentration at room temperature for 24 hours, and then the seeds were immediately planted in the pots. Three replications were taken. Initially six seeds per pot were sown, 6 days after emergence the seedlings were thinned to four seedlings per pot. Seeds were kept with average temperature 25.5°C/ 15.5°C (day/ night) under natural light.

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. There were 12 unit pots in total. The experiment included 4 treatments and 3 replications. The complete randomized design with three replications and four plants per pots were used in the experiment.

2.2. Imposition of Drought Stress

30 days after seed soaking treatment, all the seedling were watered thoroughly, then they are subjected to drought stress. Drought stress was imposed by holding water until all four seedlings were severely wilted. Then the plants were irrigated thoroughly and the first data were recorded after 72 hours of irrigation, then the plants were allowed to grow to the full maturity and rest of the data were taken after about 136 days after sowing.

2.3. Injury Rating Value

All the plants were visually examined to determine the drought injury. 1-5 numbering was given according to the scale: no visible symptom, Small necrotic area on leaf (< 5 % of leaf area necrotic), large necrotic area on leaf (>50 % of leaf area necrotic), plant is severely injured but alive and plant died. By assigning the values 1,2,3,4 and 5 respectively, to each of treatment an average IRV calculated for each treatment [15].

2.4. Electrolyte Leakage

Electrolyte leakage is used to assess membrane permeability was determined according to Lutt et al. [16]. Five leaf discs were taken from the youngest fully expanded leaf on one randomly chosen plant per replicate. The leaves were washed thoroughly with distilled water to remove surface contamination. Then the leaves were placed in a test tube containing 20 ml of distilled water and incubated for 24 hr on a shaker at room temperature. The electrical conductivity (EC1) of the bathing solution was determined. Then the samples were kept at 121°C for 20 min and second reading (EC2) was determined after cooling the solution at room temperature. The electrolyte leakage was calculated as EC1/EC2 and expressed in percentage.

2.5. Proline Determination

Proline determination was done according to the method of Bates et al. [3]. 0.5 g plant tissue were taken and homogenized in 5 ml of 3% sulphosalicylic acid using pre washed mortar and pestle. Filter the homogenate through Whatman No. 1 filter paper and filtrate was used for the estimation of proline content. 2 ml of extract was taken in test tube and 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent were added. Reaction mixture was

heated in a boiling water bath at 100°C for 1 hour. Brick red color was developed. After cooling the reaction mixtures, 4 ml of toluene was added and then transferred to a separating funnel. After thorough mixing, the chromospheres containing toluene is separated and its absorbance read at 520 nm in spectrophotometer against toluene blank. Appropriate proline standard were included in order to calculate the concentration of proline in each shoot tissue sample.

2.6. Morphological Values

After taking the injury rating values (IRVs), the shoot and root lengths were taken. Plants were carefully uprooted and the soil was washed under running water. Data were collected from twelve plants. Data were collected on growth and yield characteristics during the growth of plants and at harvesting time of the crop. These were plant height (cm), number of branches per plant, number of leaves per plant, number of fruits per plant, yield per plant (g), root length, fresh weight and dry weight of root and shoot.

2.7. Analysis

The recorded data for different characters were analyzed statistically using 'MSTAT-C' program to find out the level of significance of variation among the treatments. The analysis of variance was performed by F-test, while the significance of difference between the pairs of treatment means were evaluated by the Duncan's Multiple Range Test (DMRT) test at 5% and 1% level of probability.

Table 1. Effect of salicylic acid concentration on visual symptom damage (IRV), electrolyte leakage (EC1/EC2) and proline content of Sweet pepper seedlings subjected to 14 d of drought stress

Con of SA (mM)	Injury rating value	EC1/EC2	Proline content ($\mu\text{g/g}$)
0.25	1.64**	25.32**	208.70**
0.50	1.46**	21.10**	265.80**
0.75	1.77**	22.31**	230.70**
1.0	1.78**	23.08**	218.96**
Control	3.58	39.25	165.93

** - Values are significant at 1% cd

* - Values are significant at 5% cd

3. Results

Our results showed that after exposure to drought, those seedlings that did not receive SA treatment exhibited severe symptom of drought injury, while the SA treated seedlings were slightly damaged. However the lowest injury rating value (IRVs) were obtained after the application of 0.50mM SA followed by 0.75mM SA concentration (Table 1). An increase in electrical conductivity indicates elevated leakiness of ions due to loss of membrane integrity. The application of SA significantly decreased electrolyte leakage in leaf discs. The greatest decrease in leaf electrolyte leakage occurred when seeds were soaked in 0.50 mM SA followed by 0.25 mM SA concentration (Table 1). Same as a positive effect was also found on the proline concentration of plants. SA treatment significantly increases the concentration of proline in shoots. Plants obtained from the seeds treated with 0.50 mM concentration of SA showed the highest

proline content that was 265.80 $\mu\text{g/g}$, the value was around 1.6 times higher over control.

Salicylic acid treatment also shows the significant increase in the other morphological characters like plant height (cm), number of branches per plant, number of leaves per plant, yield per plant (g) (Table 2). However, 0.50 mM concentration was found more effective than the other concentrations used.

In case of plant height the seed treated with 0.25 mM SA concentration was not significant while all the other concentrations were significant. Seeds soaked in 0.5mM and 0.75 Mm concentration were found significant while the other two concentration were not significant for No. of branches per plant and No. of leaves per plant. Seeds treated with SA (all four concentrations) again showed the significant increase in the yield (Table 2).

Table 2. The effect of salicylic acid concentration on plant height, No. of branches per plant, No. of leaves per plant and yield per plant after drought stress

Con of SA (mM)	Plant Hight (m)	No. of branches per plant	No. of leaves per plant	Yield per plant (gm)
0.25	1.02	4.16	185.11	195.47**
0.50	1.30**	5.71**	204.93**	275.71**
0.75	1.15**	5.15**	192.09**	238.02**
1.0	1.12**	4.51	186.96	212.59**
Control	1.00	4.18	185.12	189.35

** - Values are significant at 1% cd

* - Values are significant at 5% cd

SA application also significantly increased both fresh weight and dry weight of shoot and root (Table 3). Fresh weight and dry weight of shoots shows approximately two fold increase over control. 0.1 mM concentration of SA

was not found significant in case of root length, root fresh weight and root dry weight. The largest increase in root and shoot fresh weight and dry weight occurring when the seeds were soaked in 0.50 mM SA Concentration.

Table 3. The effect of salicylic acid concentration on root length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight after drought stress

Con of SA (mM)	Root length (cm)	Shoot fresh weight (gm)	Shoot dry weight (gm)	Root fresh weight (gm)	Root dry weight (gm)
0.25	28.35*	279.05**	102.38**	56.92	24.82*
0.50	32.93**	418.01**	146.16**	61.38**	26.74**
0.75	32.36**	407.67**	144.59**	58.45*	24.50*
1.0	25.39	396.84**	140.69**	57.78	24.30
Control	25.5	232.05	99.06	55.50	23.31

** - Values are significant at 1% cd

* - Values are significant at 5% cd

4. Discussion

There was a decrease in drought IRVs when the seeds were soaked in 0.5 mM SA concentration. These results were supported by Bandurska and Stroinski [1] in barley and Korkmaz et al. [15]. Similar results were also reported

by bray et al. [4], who found that seed treated with SA caused a rapid accumulation of abscisic acid (ABA) and prevented decrease in indol 3- acetic acid (IAA) and cytokinin content which in turn reduces the inhibitory effects of water and salinity stress on plant growth. SA may influence the activity of certain.

Table 4. Correlation between different physiological and morphological parameters

Parameter s	IRV	EC1/EC2	PC	PH	BPP	LPP	YPP	RL	SFW	SDW	RFW	RDW
IRV	1											
EC1/EC2	0.9778**	1										
PC	-0.8702**	0.9057**	1									
PH	-0.6116**	0.6950**	0.9172*	1								
BPP	-0.5355*	0.6305**	0.8732*	0.9621*	1							
LPP	-0.4550*	-0.5093*	0.8248*	0.9445*	0.9535*	1						
YPP	-0.5924**	0.6698**	0.9107*	0.9819*	0.9921*	0.9694*	1					
RL	-0.8248**	0.9234**	0.9011*	0.8177*	0.7875*	0.6220*	0.7915*	1				
SFW	-0.7455**	0.8642**	0.8791*	0.8567*	0.8129*	0.6576*	0.8143*	0.9866*	1			
SDW	-0.6391**	0.7788**	0.8359*	0.8736*	0.8408*	0.6883*	0.8312*	0.9542*	0.9883*	1		
RFW	-0.7198**	0.7680**	0.9659*	0.9795*	0.9428*	0.939**	0.9757*	0.8194*	0.8292*	0.8192*	1	
RDW	-0.7398**	0.7119**	0.9086*	0.8551*	0.7992*	0.8763*	0.8628*	0.6386*	0.6188*	0.5774*	0.9334*	1

** - Values are significant at 1% cd

* - Values are significant at 5% cd

Abbreviation- Injury rating value (IRV), Electrolyte leakage (EC1/EC2), Proline content ($\mu\text{g/g}$) (PC), Plant Height (m) (PH), No. of branches per plant (BPP), No. of leaves per plant (LPP), Yield per plant (gm) (YPP), Root length (cm) (RL), Shoot fresh weight (gm) (SFW), Shoot dry weight (gm) (SDW), Root fresh weight (gm) (RFW) and Root dry weight (gm) (RDW) different physiological and morphological parameters. Enzymes directly or may induce expression of the genes responsible for these stresses protective mechanisms [11]. Electrolyte leakage was also decreased after the seed treatment with SA. The study was supported by Bandurska and Stroinski [1] that barley seedling whose seed treated with SA showed far less leakage than the seedlings of control. Wang et al. [21] in their experiment found that SA causes increase in activities of antioxidant enzymes which in turn protect plant from membrane injury or may results in the synthesis of other substance which have a protection effect on the plant growing under stress. Concentration of proline was also increased in the plants whose seeds were treated with SA. The same results were also reported by Misra and Saxena [18], who showed that SA treatment increased the proline content of lentil seedling grown under stress conditions. Proline has multiple functions in plants such as regulation of osmotic pressure, protection of membrane integrity, stabilization of enzymes/proteins, maintenance appropriate $\text{NADP}^+/\text{NADPH}$ ratios and as a scavenger of free radicals [8]. The accumulation of proline under stress conditions has been correlated with increased stress tolerance in plants [17].

Control plants did not receive application of SA treatment. Water stress reduced the plant height, No. of branches, No. of leaves and yield per plant. Exogenous SA application significantly improved these attributes under water stress. The study was supported by Hussain et al. [12] who found SA application significantly increased the head diameter, number of achene, 1000-achene weight, achene yield and oil yield in sunflower under water stress conditions.

SA treatment also significantly increased shoots and roots fresh weight and dry weight. A stimulation of shoot and root growth by the SA treatment under water stress was also reported by Baninasab [2] in cucumber. El-Tayeb [5] found that the application of SA increased the dry weight of the barley seedlings under water stressed conditions. Korkmaz et al. [15] also reported that SA increased fresh weight and dry weight of shoots and roots in water stress conditions in muskmelon plants. From the IRV and electrolyte leakage results it can be interfered that lower the IRV and electrolyte leakage higher will be the vigour of plants, which in trans will increase the fresh weight and dry weight of the shoots and roots.

In this study, we found the correlation between and among various physiological data (visual symptom damage (IRV), electrolyte leakage (EC1/EC2) and proline content) and morphological parameters (plant height, number of branches per plant, number of leaves per plant, yield per plant, root length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight) in capsicum plants subjected to water stress conditions. There is a significant correlation among these physiological and morphological parameters (Table 4). The results of correlation state that there is a positive correlation of IRV with leaf electrolyte leakage and negative correlation with

proline content. Lower IRV may results in the increase of other morphological characters.

Finally we found that seeds soaked in 0.50 mM concentration of salicylic acid were found most effective in providing drought tolerance. We can easily use the salicylic acid to prevent the crop from losses due to water stress and may have other significant practical applications also.

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