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Effects of salicylic acid on growth and germination parameters in *in-vitro* raised Indian mustard (*Brassica juncea* L.) genotypes under water stress

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ABSTRACT

Drought is one of the most challenging problems that adversely affects growth and development of plants. Salicylic acid (SA) has been involved in reducing the adverse effect of water scarcity. The research work was conducted in the Plant tissue culture laboratory, Department of Life Sciences, Jaipur National University, Jaipur (Rajasthan). To explore the individual effects of salicylic acid on the seedling growth parameters of Brassica juncea L. (cv. Pusa Agrani and CS-52) study was conducted under different concentrations of water stress conditions. Under in vitro conditions, two sets of 7-d-old seedling, were grown with three levels of drought induced by PEG 6000 concentrations (5, 10 , and 15 %), including stress free concentration (control) with three replicates, Another duplicate set of seedlings were supplemented with 8 µM salicylic acid (SA) to study its ameliorative action. Application of SA was most effective in alleviating the harmful effects of water stress on germination percentage, plumule length, radicle length, seedling height, fresh and dry weight of seedling. These parameters were drastically reduced with increasing drought conditions. The drought stress tolerance also improved with the application of salicylic acid. It was minimum under 15% PEG concentration.

Keywords: Germination parameters; drought tolerance; *Brassica juncea*; salicylic acid

INTRODUCTION

Agriculture is the largest consumer of water in the world, and in the drier areas of the world, the use of water for agriculture can exceed 90 per cent of consumption. Global warming is also predicted to affect most severely. The agricultural systems are most vulnerable to climatic conditions. Small increases in temperature are very detrimental to productivity. Among the major oilseed crops, *Brassica* crops are the most affected by drought and salinity, due to the fact that they are mainly grown in arid and semiarid areas. The role of SA in seed germination, fruit yield (Hayat *et al.,* 2010), enzymatic activity (Dolatabadian *et al.,* 2008), photosynthetic rate (Khan *et al.,* 2003), uptake and transport of ions (Afzal *et al.,* 2005), and plant growth and yield (Hussein *et al.,* 2007) have been well addressed. Exogenous application of SA induces activation of the antioxidant enzyme system in Indian mustard (*Brassica juncea* L.) resulting in a reduction of detrimental effects of water stress (Dolatabadian *et al.,* 2008), However, some information is available regarding drought tolerance of pumpkin (Cucurbita pepo L.) in response to exogenously applied SA (Rafique *et al.,* 2011). It is proved that if SA is applied exogenously, it might enhance the drought tolerance ability of Indian mustard. Hence, the study was carried out to find out the effects of SA on germination, early seedling growth and stress tolerance index of Indian mustard under drought stress.

MATERIALS AND METHODS

The experiment was conducted in the Plant tissue culture laboratory, Department of Life Science, Jaipur National University, Jaipur (Rajasthan). Various concentrations of PEG 6000 ((0), 5 % PEG, 10 % PEG and 15 % PEG) were used and distilled water used as control (0). Another set of seedlings of both varieties of mustard was treated with 8 µM SA simultaneously. The varieties of *B. juncea* were tolerance (PUSA-AGRANI) and sensitive (CS-52) under different concentrations of PEG. The seeds were first surface sterilized with 0.1 % $HgCl_2$ solution for two minutes and repeatedly washed with sterilized distilled water to prevent contamination. Prior to experiment, petri dishes of 10 cm diameter were thoroughly washed and sterilized in hot air oven at 70ºC for 2 hours. After sterilization, approximately 10-15 seeds were inoculated in petri dishes lined with Whatman filter paper No. 1 and treated daily with four concentrations of different stresses. The

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petri dishes were incubated in a growth chamber at 25 ± 2ºC, 70 % relative humidity (HR) and 16 hour photoperiod. Each replicate was inspected intensively and at the last day (7th day) traits such as: germination percentage, shoot length, root length, total plant height and seedling fresh and dry weight were measured. Germination started after two days of inoculation and germination count was continued until 7th day. A seed was considered germinated when both plumule and radicle had emerged > 0.05 cm through the seed coat. The germination percentage calculated as:
Germination Percentage = Number of germinated seeds/ total number of seeds ×100
After 7th days of inoculation, plumule length, radicle length and plant height of 10 randomly picked seedlings from each petri dish was measured. The fresh and dry weight of seedlings were measured by picking 10 seedlings randomly from each petri dish and drying in oven at 80ºC for 24 hours and weighting them using sensitive balance. Stress tolerance index was calculated as total plant (shoot and root) fresh weight obtained from 10 randomly selected seeds grown on different concentrations PEG, compared to total plant fresh weight obtained from non-stress concentration. Drought tolerance indices were calculated using the following formulas respectively:

Drought Tolerance Index = $(Ys \times Yp)/Yp^{-2}$ (Fernandez, 1992)

where Ys, Yp and represent yield under stress and yield under non stress for each cultivar, and yield mean in non-stress conditions for all cultivars respectively.
All data obtained were subjected to analysis of variance (ANOVA) and the mean differences were compared by a Duncan's multiple range test (DMRT) using the INDOSTAT software. The differences at P ≤ 0.01 were considered highly significant.

RESULTS AND DISCUSSION

Germination Percentage: The results revealed that the marginal mean of varieties, treatments, concentrations and interaction of varieties × concentrations were highly significant (Table 1).

Table 1: Effect of drought stress with salicylic acid on seedling growth parameters of *Brassica junceas* genotypes

Germination parameter	Concentration	PUSA AG.	PUSA AG (SA)	CS-52	CS-52 (SA)
Germination Percentage	Control	83.85	90.00	75.00	90.00
	5% PEG	66.14	75.00	66.14	71.57
	10% PEG	61.21	68.85	61.21	68.85
	15% PEG	52.77	66.14	54.78	66.14
	SEm± = 3.91	CD (P = 0.05) = NS		C.V % = 9.53	
Seedling Plumule Length	Control	3.15	3.78	3.32	3.77
	5% PEG	2.26	3.40	2.73	3.07
	10% PEG	2.20	2.49	2.13	2.68
	15% PEG	1.31	1.63	0.71	1.86
	SEm± = 0.13	CD (P = 0.05) = 0.36		C.V % = 7.76	
Seedling Radicle Length	Control	10.04	3.78	9.14	3.70
	5% PEG	9.62	7.37	8.98	7.62
	10% PEG	8.83	7.05	7.73	6.41
	15% PEG	7.03	4.02	6.60	4.65
	SEm± = 0.31	CD (P = 0.05) = 0.88		C.V % = 8.3	
Seedling Plant Height	Control	13.04	7.56	12.40	7.46
	5% PEG	11.89	10.63	11.71	10.70
	10% PEG	11.03	9.55	9.86	9.10
	15% PEG	8.34	5.66	7.32	6.50
	SEm± = 0.35	CD (P = 0.05) = 0.98		C.V % = 6.48	
Seedling Fresh Weight	Control	0.37	0.51	0.35	0.46
	5% PEG	0.20	0.21	0.18	0.20
	10% PEG	0.14	0.16	0.12	0.15
	15% PEG	0.06	0.07	0.05	0.07
	SEm± = 0.03	CD (P = 0.05) = NS		C.V % = 17.89	
Seedling Dry Weight	Control	0.044	0.051	0.036	0.039
	5% PEG	0.041	0.045	0.032	0.034
	10% PEG	0.024	0.041	0.029	0.034
	15% PEG	0.016	0.035	0.026	0.029
	SEm± = 0.03	CD (P = 0.05) = NS		C.V % = 16.29	

Application of SA showed higher percentage of germination under stressed conditions and control. Osmotic stress causes decrease in percentage of germination with increasing the drought concentration because germination is one of the most sensitive traits in the early stage of most plants. Similar findings have been reported in by Tesfaye *et al.,* (2014). When osmotic stress is induced by drought it causes a significant reduction in water uptake hence low water contents in germinating embryos and endosperm (Pratap and Sharma, 2010), indicating that these tissues were under stress conditions. GP was greatly influenced by SA under stress condition, because it significantly stimulated many enzymes activities. Lakzayi *et al.,* (2014) reported that exogenous application of SA enhanced the activities of antioxidant enzymes (APX, SOD, CAT) involved in seed germination such as transkelolase, enolase, malate dehydrogenase, phosphoglycerate kinase, glyceraldeyde 3-phosphate, fructose 1, 6-diphosphatase, dehydrogenase and pyruvate decarboxylase.

Seedling Plumule Length (cm): The SA produced significantly higher shoot length under stressed conditions and control. The findings of present study were conformity with those of Hayat *et al.,* (2008). Under water scarcity due to water stress, the reduction in plumule length probably because of genetic variation between two varieties of *B. juncea* and excessive accumulation of Na^{+} and Cl^{-} ions in the cell wall elasticity, thus secondary cell appears sooners and cell wall become rigid as a result the turgid pressure efficiency in cell enlargement reduces plumule length.

The increment in plumule length by the addition of SA may be due to certain supply of metabolites to young developing tissue. The accumulation of glycine betaine in plants helps to enhance the maintenance of water availability to sustain growth and redevelopment under osmotic stress conditions (Lakzayi *et al.* 2014).

Seedling Radicle Length (cm): The variety PUSA-AGRANI gave significantly highest radicle length of 7.54 cm followed by CS-52 (6.76 cm). Among the treatments, significantly highest radicle length was observed by (7.03 cm) while among the concentration, gave significantly highest radicle length (8.08 cm) in comparison to control (Table..1). This means that SA did not increase radicle length of both cultivars under non stressed conditions, but it improved radicle length significantly at each stress level. These investigations were consistent with the findings of Luan *et al.,* (2014) in *Helianthus annus* under water stress. The results elucidate that radicle length of plants under water deficit conditions was significantly higher in comparison to other stresses. In contrast to our findings, results of Sanadhya *et al.,* (2013) indicated that when increasing the levels of water stress, there was a dramatic increase in radicle length in comparison with drought and other abiotic stresses in *Vigna radiata*.

Table 2: Effect of drought stress with salicylic acid on seedling stress tolerance indices of *Brassica juncea* genotype

Concentration	PUSA AG.	PUSA AG (SA)	CS-52	CS-52 (SA)
Control	0.26	0.31	0.23	0.25
5 % PEG	0.14	0.13	0.12	0.11
10% PEG	0.09	0.09	0.08	0.088
15% PEG	0.04	0.04	0.03	0.38
	S.Em± = 0.08	C.D. (P=0.05) = NS	C.V. % = 28.02	

Seedling Plant Height (cm): The variety PUSA-AGRANI produced significantly highest total plant height of 10.02 cm followed by variety CS-52 (9.38 cm) (Table 1). Among the concentration, 5% PEG (11.89 cm) gave significantly highest plant height followed by control (10.05 cm). Exogenous SA treatments increased, these parameters as compared to control under abiotic stresses (Jazi and Oregani, 2014; Chauhan *et al.,* 2015).

Seedling Fresh and Dry Weight (g): For fresh weight, the marginal mean of second set that was treated with SA produced significantly highest fresh weight under stressed conditions compared with control (PUSA-AGRANI (0.361 g) followed by variety CS-52 (0.329 g). For the dry weight, the marginal mean of second set that was treated with SA produced significantly highest dry weight under stressed conditions and control (PUSA-AGRANI (0.043 g) followed by variety CS-52 (0.036 g)). Among the concentrations, control (0.044 g) noted significantly highest seedling dry weight followed by con. 1 (0.039 g) (Table 1).

Stress Tolerance Index (STI) (%): The maximum tolerance was observed by 5% PEG concentration; it was followed by the 10 % and 15% PEG concentration respectively. The significantly highest tolerance was remarked with the application of SA with no stress that is control of SA (Table 2).

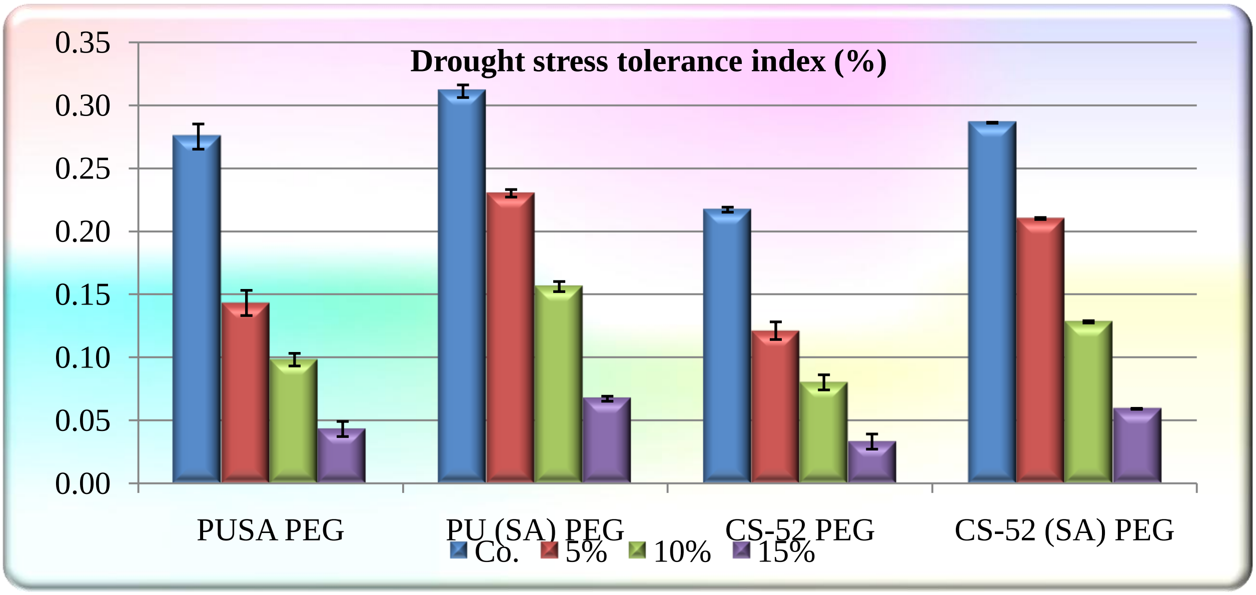

Figure 1: Effect of Drought levels on drought stress tolerance (%) with and without SA treatment on mustard genotype

It can be concluded from the present study that the application of salicylic acid countered the direct as well as indirect effects of water stress and them improved the germinating efficiency, metabolic and growth in mustard plants.

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