CIBTech Journal of Biotechnology ISSN: 2319–3859 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjb.htm
2015 Vol. 4 (4) October-December, pp.38-54/Chauhan et al.
Research Article

SALICYLIC ACID MEDIATED MODULATION OF GROWTH AND GERMINATION PARAMETERS IN THE *IN-VITRO* RAISED PLANTLETS OF *B. JUNCEA* GENOTYPES

Rajani Chauhan, Mohd. Shahnawaz and *Dheera Sanadhya
School of Life Sciences, Jaipur National University, Jaipur, Rajasthan, India
**Author for Correspondence*

ABSTRACT
In order to study the effect of salicylic acid on seed germination and seedling growth parameters in two varieties of *Brassica juncea* species under drought, salinity and their combination conditions. Present research was carried out under *in vitro* conditions to observe two sets of 7-d-old seedling, subjected to three levels of drought induced by PEG 6000 concentrations (5 %, 10 %, 15 %), including stress free concentration with three replicates, salinity was imposed using 50 mM, 100 mM, 150 mM concentrations of Sodium Chloride (NaCl) and combinations of drought and salinity were as follows: Combination 1 (2.5 % PEG + 25 mM NaCl), Combination 2 (5 % PEG + 50 mM NaCl), Combination 3 (7.5 % PEG + 75 mM NaCl), another duplicate set of seedlings were supplemented with 8 µM salicylic acid (SA) to study its ameliorative action. The results elucidate that the exogenous application of SA not only mitigated the inhibitory effects of drought, salinity and their combination in both genotypes, but also in some cases induced a stimulatory effect greater than that estimated in the control plants.

Keywords: *Germination Seedling Parameters; Polyethylene Glycol; Sodium Chloride; Drought Tolerance; Salt Tolerance; Combination Tolerance; Brassica Juncea; Salicylic Acid*

***Abbreviations*:** *B. juncea*, *Brassica juncea*; cm, Centimeter; g, Gram; GP, Germination Percentage; HR, Relative Humidity; NaCl, Sodium Chloride; PEG, Polyethylene Glycol; SA, Salicylic Acid; STI, Salinity Tolerance Index; DTI, Drought Tolerance Index; CTI, Combination Tolerance Index.

INTRODUCTION

Indian mustard *Brassica juncea* (L.) Czen and Cross are economically important agricultural commodities which are grown in more than 50 countries of the world (Bhardwaj *et al.*, 2015). It is a winter oilseed crop grown across the northern Indian agricultural land. There are so many distribution areas which are centered in the north-west agro-climatic region, where either maximum ground water sources are highly saline or water available is not required to sustain growth of plants.

Water scarcity is the main cause of desertification or salinization of cultivated land due to water and salt stresses. Abiotic stresses like light, heat, cold, drought, salinity, UV rays and also heavy metals are responsible for disruption of osmotic and ionic homeostasis, ultimately, damage to structural and functional proteins of plant cells thus plant growth and productivity are greatly affected (Bohra and Sanadhya 2015; Sanadhya *et al.*, 2013). Theses abiotic stresses are the most vital restraining factor in crop establishment and are becoming an increasingly relentless problem in several regions of the world. However, plant responses to drought and salinity have much in common. Extent and nature of both stresses varies with the developmental stages of plants particularly during germination, reproductive and maturation stage (Chauhan *et al.*, 2015) that leads to decline in yield. The germination stage of plants is affected first of all because at this stage plants are more sensitive to abiotic stages than other growth and developmental stages (Luan *et al.*, 2014). Both stresses and their combination sometimes occur together that are the main factors which limit the seed germination and seed establishment of plants growing in arid and semi-arid areas and hence, is one of the most important factors for successful crop establishment (James *et al.*, 2002). In the past few decades, among many strategies used to combat the deleterious effects of drought and salinity stresses, exogenous application of plant growth regulators has received considerable attention.

Salicylic acid (SA) or ortho hydroxyl benzoic acid is known as an endogenous growth regulator of phenolic type distributing in a whole plant species, which induces biotic and abiotic stress tolerance in crops (Joseph *et al.,* 2010; Ghorbani *et al.,* 2011). Among abiotic stresses it has been reported to counter drought (Singh and Usha, 2003), low temperature (Tasgin *et al.,* 2003), high temperature (He *et al.,* 2005) and salinity (EI Tayeb, 2009) etc.

The role of SA is evident 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 Canola (*Brassica napus* L.) resulting in a reduction of detrimental effects of salt stress (Dolatabadian *et al.,* 2008). However, some information is available regarding salt and drought tolerance of pumpkin (*Cucurbita pepo* L.) in response to exogenously applied SA (Rafique *et al.,* 2011).

Comparative studies of drought, salinity and their combinations on germination and seedling growth parameters of 15 varieties of *Brassica juncea* species have been investigated. According to the statistical analysis of germination data two varieties were selected out of 15 showing the maximum tolerance (PUSA-AGRANI) and maximum susceptible (CS-52) characteristics under stress conditions (Data already published) (Chauhan *et al.,* 2015). Now the selected varieties are under further study to combat the deleterious effects of stresses with the exogenous application of Salicylic acid (SA) using germination and seedling growth parameters. Considering the above mentioned literature about the effects of SA it is proved that if SA is applied exogenously, it might enhance the drought and salinity tolerance ability of Indian mustard. Hence, the study was carried out to find out the effects of SA on two varieties of *B. juncea* germination, early seedling growth and stress tolerance index under drought, salinity and their combination. The results of the present study can be helpful to create tolerance capacity of Indian mustard under abiotic stress conditions and also elucidate the role of exogenously applied SA in drought, salinity and their combination stresses tolerance.

MATERIALS AND METHODS

Description of the Study Area and Plant Genetic Material

The experiment was conducted in the plant tissue culture laboratory, department of life science, Jaipur National University, Jaipur (Rajasthan). Seeds of two varieties of *B. juncea* species were procured from S.K.N. Agriculture University, Jobner.

Treatment and Experimental Design

In present work, to impose drought, various concentrations of PEG 6000 were used like (0), 5 % PEG, 10 % PEG, 15 % PEG, to impose salinity three potential levels of NaCl were used like 50 mM, 100 mM and 150 mM and distilled water used as control. Studies of drought and salinity Polyethylene glycol and NaCl were used in combination. Three potential levels of combination were used like combination 1 (2.5 % PEG + 25 mM NaCl), combination 2 (5 % PEG + 50 mM NaCl) and combination 3 (7.5 % PEG + 75 mM NaCl). Two sets of seedlings were subjected to two different varieties of *B. juncea*. Another set of seedlings of both varieties was treated with 8 µM SA simultaneously.

Experiment Procedure

In order to assess the response of the two varieties of *B. juncea* species in which one is tolerance (PUSA-AGRANI) and another is susceptible (CS-52) under different concentration of PEG, NaCl and their combination. The seeds were first surface sterilized with 0.1 % $HgCl_2$ solution for two minutes and repeatedly washed with sterilized distilled water to present 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 paper no. 1 and treated daily with four concentrations of different stresses. The 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 (7^{th} day) traits such as: germination percentage, shoot length, root length, total plant height and seedling fresh and dry weight were measured.

Germination Percentage

Germination started after two days of inoculation and germination count was continued until 7^{th} day. A seed was considered germinated when both plumule and radicle had emerged > 0.05 cm through the seed coat (Abdul *et al.,* 2006). The germination percentage calculated as:

Germination Percentage = [Number of germinated seeds/ total number of seeds] ×100

Seedling Plumule Length (cm)

7^{th} days after inoculation, a plumule length of 10 randomly picked seedlings from each petri dish was measured in centimeters.

Seedling Radicle Length (cm)

7^{th} days after inoculation, radicle length of 10 randomly picked seedlings from each petri dish were measured in centimeters.

Total Plant Height (cm)

7^{th} days after germination, total plant height (plumule + radicle length) of 10 randomly picked seedlings from each petri dish were also measured in centimeters.

Seedling Fresh and Dry Weight (g)

Seedlings fresh and dry weight was 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

Stress tolerance index was calculated as total plant (shoot and root) fresh weight obtained from 10 randomly selected seeds grown on different PEG, NaCl and their combination concentrations compared to total plant fresh weight obtained from non-stress concentration. Drought, Salt and their Combination tolerance indices were calculated using the following formulas respectively:

DTI = $(Ys \times Yp) / \bar{Yp}^2$ (Fernandez, 1992)

STI = $(Ys + Yp) / \bar{Yp}^2$ (Fernandez, 1992)

CTI = $(Ys \times Yp) / \bar{Yp}^2$

In the given formula Ys, Yp and $\bar{Yp}$ represent yield under stress and yield under non stress for each cultivar, and yield mean in non-stress conditions for all cultivars respectively.

Statistical Analysis

All data obtained was 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 $P \leq 0.01$ were considered highly significant.

RESULTS AND DISCUSSION

Germination Percentage

The three way analysis of variance (ANOVA) for germination percentage reveals that the marginal mean of varieties, treatments, concentrations and interaction of varieties × concentrations are highly significant ($P < 0.001$) (Table 1. A). The results elucidate that between two set of both cultivars, with the application of SA set of each cultivars shows high percentage germination under stressed conditions compared with control (PUSA-AGRANI (77.15) and CS-52 (76.86)) (Table 1. B). Among treatments NaCl (73.53) has given significantly highest germination percentage while among the concentrations Control has given significantly highest germination percentage of 85.16 compared to other levels of concentrations that is shown under section Table 1. (B). Drought and salinity are physiologically related, because they induce osmotic stress and all most metabolic responses of the affected plants are similar to some extent (Djibril *et al.,* 2005). Osmotic stress causes decrease in percentage of germination with increasing the salt concentration because germination is one of the most sensitive traits in the early stage of most plants. Similar findings in seed germination have been addressed in *Eastern ethiopia* 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 was observed (Pratap and Sharma, 2010), indicating that these tissues were under stress conditions. In the present work GP was greatly influenced by SA under all stresses (Figure 1) because it significantly stimulated many enzymes activities. Furthermore, 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. The results of present study indicated conformity with the previous investigations on Fox berry (*Sanguisorba minor*) (Zare *et al.,* 2009) and in Wheat (*Triticum aestivum*) (Movaghatian and Khorsandi, 2013).

Seedling Plumule Length (cm)

The ANOVA results for seedling plumule length data indicated that the effect of SA among the marginal mean of varieties, treatments and concentrations along with their all possible interactions was highly significantly different ($P < 0.001$) (Table 2 A). The marginal mean of second set that was treated with SA was given significantly highest shoot length under stressed conditions compared with control (CS-52 (3.18) followed by PUSA-AGRANI (3.08) (Table 2 B). Among the treatments mean significantly highest shoot length was observed by NaCl (3.12) while among the concentrations Control (3.53) noticed the significantly highest shoot length. The results elucidate that addition of SA has shown the highest tolerance capacity (shoot length) in comparison to control and the same trend was observed at each concentration level of stresses that is shown under section Table 2 (B). The finding of present study showed conformity with the previous research, observation of Hayat *et al.,* 2008; Mazaheri and Kalantari, 2006 who stated that an increase in plumule length of tomato, barley and canola as a result of application with SA under water deficit condition. On the other hand, the same result was observed under salinity in canola (Razavizadeh and Rostami, 2013).

Under water scarcity due to water stress, saline water and sometimes due to both stresses, the reduction in plumule length probably because 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 that result in short plumule length. The increment in plumule length by the addition of SA may be due to certain supply of metabolites to young developing tissue. As metabolic production takes place within the leaves and is significantly interrupted at high saline water, either due to lower water uptake or toxic effect of Na^+ and Cl^- ions (Hussain *et al.,* 2008). Increasing osmotic stress levels due to drought and combination significantly reduced mean plumule length of Indian mustard seedlings (Figure 1). Several studies have demonstrated that exogenous application of SA enhances plant growth and development. Mahmudirad *et al.,* (2014) reported that with the addition of SA significantly promoted growth of canola seedlings, because of the exogenous application of SA to the water stressed plants enhanced glycine betaine content as compared to control plants (Lakzayi *et al.,* 2014). The accumulation of glycine betaine in plants helps to enhance the maintenance of water availability to sustain growth and development under osmotic stress conditions.

Seedling Radicle Length (cm)

The ANOVA results for seedling radicle length data indicated that the effect of SA among the marginal means of varieties, treatments and concentrations along with their all possible interactions was highly significantly different ($P < 0.001$) (Table 3 A). The marginal mean of variety PUSA-AGRANI was given significantly highest radicle length of 7.54 followed by CS-52 (6.76). Among the treatments mean significantly highest radicle length has been observed by PEG (7.03) while among the concentration mean conc.1 was given significantly highest radicle length (8.08) in comparison to control (Table 3 B). 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 (Figure 1). These investigations were consistent with 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 in *Vigna radiata* while increasing the levels of water stress, there was a dramatic increase in radicle length in comparison with salt and other abiotic stresses. Inhibition of root growth under saline water is a result of accumulation of Na^+ and Cl^- ions induced inhibition of cell division in root tips and elongation rate of cells that mainly occurs by an irreversible of proton pump that is responsible for the process (Jazi and Oregani, 2014). The application of SA in response to osmotic stresses, plants produce osmolytes like trehalose or protein, glycine beanie, which protect them from dehydration or protein denaturation therefor it shows highest tolerance capacity (Hapani and Marjadi, 2015). The finding of this study indicated conformity with the previous investigations on *Lycopersicon esculentum* Mill (Arbaoui *et al.*, 2015) under saline water.

Seedling Plant Height (cm)

The ANOVA results for seedling plant height data showed that the effect of SA among the marginal mean of varieties, treatments and concentrations along with their all possible interactions was highly significantly different ($P < 0.001$) (Table 4 A). The marginal mean of variety PUSA-AGRANI was given significantly highest total plant height of 10.02 followed by variety CS-52 (9.38). Among the treatments mean significantly higher total plant height was observed by PEG (9.55) while among the concentration 1 (11.25) has given significantly highest plant height followed by control (10.05) (Table 4 B). The toxic effects of NaCl ions commonly reduced the absorption of CO_2 due to reduced leaf surface area. It means the presence of excess NaCl ions in the nutrient medium reduce water uptake and transport which may affect the photosynthesis phenomenon of plants as results growth and development of plants are interrupted (Azmat *et al.*, 2006). Exogenous SA treatments increased, most of these parameters as compared to control under abiotic stresses (Jazi and Oregani, 2014).

Seedling Fresh and Dry Weight (g)

The results of ANOVA for seedling fresh and dry weight data indicated that the effect of SA among the marginal mean of varieties, treatments and concentrations along with their all possible interactions was highly significantly different ($P < 0.001$) (Table 5, 6 A).

For fresh weight, the marginal mean of second set that was treated with SA was given significantly highest fresh weight under stressed conditions compared with control (PUSA-AGRANI (0.361) followed by variety CS-52 (0.329)) (Table 5 B).

Among the treatment mean significantly highest fresh weight was obtained by combination (NaCl + PEG) (0.347) while among the concentrations control (0.443) was noticed significantly highest seedling fresh weight followed by control 1 (0.302) (Table 5 B).

Table 1 (A): Comparative Effect on Germination Percentage of PUSA-AGRANI (Tolerance Variety) and CS-52 (Susceptible Variety) of *Brassica juncea* species grown under Drought, Salinity and their Combination with the application of Salicylic Acid (SA)

Salicylic Acid Concentration: (8 µM)

Treatment	Concentration	PUSA-AG	PUSA-AG (SA)	CS-52	CS-52 (SA)
PEG	Control	83.857	90.000	75.000	90.000
	5% PEG	66.143	75.000	66.143	71.570
	10% PEG	61.217	68.857	61.217	68.857
	15% PEG	52.777	66.143	54.783	66.143
NaCl	Control	77.713	90.000	83.857	90.000
	50 mM	66.143	83.857	66.143	83.857
	100 mM	63.430	77.713	63.930	77.713
	150 mM	54.783	68.857	56.997	71.570
Combination	Control	83.857	90.000	77.713	90.000
	Comb.1	61.217	77.713	66.143	77.713
	Comb.2	61.217	68.857	59.003	68.857
	Comb.3	50.853	68.857	52.860	66.143
				S.Em±	3.918
				C.D.5%	NS
				C.V.%	9.539

Table 1 (B): The Marginal Mean of Varieties, Treatments and Concentration for Germination Percentage

Variety	Variety Mean	Treatment	Treatment Mean	Concentration	Conc. Mean
PUSA-AG.	65.267	PEG	69.857	Control	**85.166**
PUSA-AG. (SA)	**77.154**	NaCl	**73.535**	Conc.1	71.804
CS-52	65.316	Comb.	70.063	Conc.2	66.739
CS-52 (SA)	**76.869**			Conc.3	60.897
S.Em ±	1.131		0.980		1.131
C.D. 5%	3.175		2.750		3.175

Each data is replicated thrice. Value in a column with different letters is significantly different at P ≤ 0.01 applying DMRT; NS = Non-Significant.
Conc.1, Conc.2 and Conc.3 are indicating of every treatment's concentration

Table 2 (A): Comparative Effect on Seedling Plumule Length of PUSA-AGRANI (Tolerance Variety) and CS-52 (Susceptible Variety) of *Brassica juncea* species grown under Drought, Salinity and their Combination with the application of Salicylic Acid (SA)
Salicylic Acid Concentration: (8 µM)

Treatment	Concentration	PUSA-AG	PUSA-AG (SA)	CS-52	CS-52 (SA)
PEG	Control	3.150	3.780	3.250	3.773
	5% PEG	2.263	3.400	2.733	3.073
	10% PEG	2.207	2.497	2.133	2.687
	15% PEG	1.317	1.637	0.710	1.860
NaCl	Control	3.630	3.667	3.283	3.703
	50 mM	3.283	3.590	3.233	3.910
	100 mM	2.933	3.363	2.820	3.310
	150 mM	1.813	2.447	2.030	3.047
Combination	Control	3.277	3.757	3.310	3.787
	Comb.1	2.553	3.400	3.287	3.413
	Comb.2	1.967	3.220	2.373	3.017
	Comb.3	1.717	2.227	2.223	2.670
				S.Em±	0.128
				C.D.5%	0.358
				C.V%	7.765

Table 2 (B): The Marginal Mean of Varieties, Treatments and Concentration for Seedling Plumule Length

Variety	Variety Mean	Treatment	Treatment Mean	Concentration	Conc. Mean
PUSA-AG.	2.509	PEG	2.529	Control	**3.531**
PUSA-AG. (SA)	**3.082**	NaCl	**3.129**	Conc.1	3.178
CS-52	2.616	Comb.	2.887	Conc.2	2.711
CS-52 (SA)	**3.188**			Conc.3	1.975
S.Em ±	0.037		0.980		0.037
C.D. 5%	0.103		2.750		0.103

Each data is replicated thrice. Value in a column with different letters is significantly different at P ≤ 0.01 applying DMRT; NS = Non-Significant.
Conc.1, Conc.2 and Conc.3 are indicating of every treatment's concentration

CIBTech Journal of Biotechnology ISSN: 2319–3859 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjb.htm
2015 Vol. 4 (4) October-December, pp.38-54/Chauhan et al.
Research Article

Table 3 (A): Comparative Effect on Seedling Radicle Length of PUSA-AGRANI (Tolerance Variety) and CS-52 (Susceptible Variety) of *Brassica juncea* species grown under Drought, Salinity and their Combination with the application of Salicylic Acid (SA)
Salicylic Acid Concentration: (8 µM)

Treatment	Concentration	PUSA-AG	PUSA-AG (SA)	CS-52	CS-52 (SA)
PEG	Control	10.047	3.780	9.147	3.703
	5% PEG	9.627	7.373	8.980	7.627
	10% PEG	8.830	7.057	7.730	6.417
	15% PEG	7.030	4.020	6.607	4.653
NaCl	Control	9.330	3.863	9.143	3.550
	50 mM	7.863	8.553	6.990	7.747
	100 mM	6.883	7.343	5.690	6.490
	150 mM	2.793	4.967	1.943	3.830
Combination	Control	9.727	3.717	9.040	3.630
	Comb.1	8.477	8.970	6.617	8.217
	Comb.2	5.643	6.910	5.930	7.083
	Comb.3	4.287	5.083	3.410	5.477
				S.Em±	0.314
				C.D.5%	0.881
				C.V.%	8.369

Table 3 (B): The Marginal Mean of Varieties, Treatments and Concentration for Seedling Radicle Length

Variety	Variety Mean	Treatment	Treatment Mean	Concentration	Conc. Mean
PUSA-AG.	**7.545**	PEG	**7.039**	Control	6.556
PUSA-AG. (SA)	5.970	NaCl	6.061	Conc.1	**8.087**
CS-52	6.769	Comb.	6.389	Conc.2	6.834
CS-52 (SA)	5.702			Conc.3	4.508
S.Em ±	0.091		0.078		0.091
C.D. 5%	0.254		0.220		0.254

Each data is replicated thrice. Value in a column with different letters is significantly different at P ≤ 0.01 applying DMRT; NS = Non-Significant.
Conc.1, Conc.2 and Conc.3 are indicating of every treatment's concentration

Table 4 (A): Comparative Effect on Seedling Plant Height of PUSA-AGRANI (Tolerance Variety) and CS-52 (Susceptible Variety) of *Brassica juncea* species grown under Drought, Salinity and their Combination with the application of Salicylic Acid (SA)
Salicylic Acid Concentration: (8 µM)

Treatment	Concentration	PUSA-AG	PUSA-AG (SA)	CS-52	CS-52 (SA)
PEG	Control	13.040	7.563	12.400	7.460
	5% PEG	11.890	10.637	11.713	10.700
	10% PEG	11.037	9.553	9.863	9.103
	15% PEG	8.347	5.663	7.320	6.510
NaCl	Control	12.797	7.533	12.427	7.253
	50 mM	11.150	12.120	10.230	11.657
	100 mM	9.820	10.710	8.513	9.800
	150 mM	4.607	7.417	3.977	6.880
Combination	Control	12.910	7.477	12.350	7.417
	Comb.1	11.030	12.340	9.910	11.633
	Comb.2	7.613	10.133	8.303	10.100
	Comb.3	6.010	7.310	5.637	8.147
				S.Em±	0.349
				C.D.5%	0.980
				C.V.%	6.482

Table 4 (B): The Marginal Mean of Varieties, Treatments and Concentration for Seedling Plant Height

Variety	Variety Mean	Treatment	Treatment Mean	Concentration	Conc. Mean
PUSA-AG.	**10.021**	PEG	**9.550**	Control	10.052
PUSA-AG. (SA)	9.038	NaCl	9.181	Conc.1	**11.251**
CS-52	9.387	Comb.	9.270	Conc.2	9.546
CS-52 (SA)	8.888			Conc.3	6.485
S.Em ±	0.101		0.087		0.101
C.D. 5%	0.283		0.245		0.283

Each data is replicated thrice. Value in a column with different letters is significantly different at $P \leq 0.01$ applying DMRT; NS = Non-Significant.
Conc.1, Conc.2 and Conc.3 are indicating of every treatment's concentration

CIBTech Journal of Biotechnology ISSN: 2319–3859 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjb.htm
2015 Vol. 4 (4) October-December, pp.38-54/Chauhan et al.
Research Article

Table 5 (A): Comparative Effect on Seedling Fresh Weight of PUSA-AGRANI (Tolerance Variety) and CS-52 (Susceptible Variety) of *Brassica juncea* species grown under Drought, Salinity and their Combination with the application of Salicylic Acid (SA)
Salicylic Acid Concentration: (8 µM)

Treatment	Concentration	PUSA-AG	PUSA-AG (SA)	CS-52	CS-52 (SA)
PEG	Control	0.378	0.517	0.359	0.466
	5% PEG	0.206	0.217	0.183	0.202
	10% PEG	0.142	0.160	0.123	0.159
	15% PEG	0.062	0.077	0.051	0.070
NaCl	Control	0.378	0.544	0.392	0.502
	50 mM	0.317	0.403	0.272	0.392
	100 mM	0.273	0.399	0.219	0.331
	150 mM	0.224	0.318	0.204	0.316
Combination	Control	0.394	0.517	0.406	0.461
	Comb.1	0.232	0.428	0.386	0.389
	Comb.2	0.182	0.393	0.345	0.370
	Comb.3	0.130	0.357	0.270	0.290
				S.Em±	0.031
				C.D.5%	NS
				C.V.%	17.896

Table 5 (B): The Marginal Mean of Varieties, Treatments and Concentration for Seedling Fresh Weight

Variety	Variety Mean	Treatment	Treatment Mean	Concentration	Conc. Mean
PUSA-AG.	0.243	PEG	0.211	Control	**0.443**
PUSA-AG. (SA)	**0.361**	NaCl	0.343	Conc.1	0.302
CS-52	0.268	Comb.	**0.347**	Conc.2	0.258
CS-52 (SA)	**0.329**			Conc.3	0.197
S.Em ±	0.009		0.008		0.009
C.D. 5%	0.025		0.022		0.025

Each data is replicated thrice. Value in a column with different letters is significantly different at P ≤ 0.01 applying DMRT; NS = Non-Significant.
Conc.1, Conc.2 and Conc.3 are indicating of every treatment's concentration

Table 6 (A): Comparative Effect on Seedling Dry Weight of PUSA-AGRANI (Tolerance Variety) and CS-52 (Susceptible Variety) of *Brassica juncea* species grown under Drought, Salinity and their Combination with the application of Salicylic Acid (SA)
Salicylic Acid Concentration: (8 µM)

Treatment	Concentration	PUSA -AG	PUSA-AG (SA)	CS-52	CS-52 (SA)
PEG	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
NaCl	Control	0.047	0.052	0.039	0.042
	50 mM	0.037	0.047	0.036	0.040
	100 mM	0.032	0.038	0.032	0.039
	150 mM	0.024	0.036	0.028	0.025
Comb	Control	0.048	0.051	0.042	0.042
	Comb.1	0.038	0.047	0.035	0.038
	Comb.2	0.031	0.038	0.032	0.036
	Comb.3	0.021	0.029	0.025	0.029
				S.Em±	0.003
				C.D.5%	NS
				C.V.%	16.295

Table 6 (B): The Marginal Mean of Varieties, Treatments and Concentration for Seedling Dry Weight

Variety	Variety Mean	Treatment	Treatment Mean	Concentration	Conc. Mean
PUSA-AG.	0.034	PEG	0.035	Control	**0.044**
PUSA-AG. (SA)	**0.043**	NaCl	**0.037**	Conc.1	0.039
CS-52	0.033	Comb.	0.036	Conc.2	0.034
CS-52 (SA)	**0.036**			Conc.3	0.027
S.Em ±	0.001		0.001		0.001
C.D. 5%	0.003		NS		0.003

Each data is replicated thrice. Value in a column with different letters is significantly different at P ≤ 0.01 applying DMRT. NS = Non-Significant.
Conc.1, Conc.2 and Conc.3 are indicating of every treatment's concentration

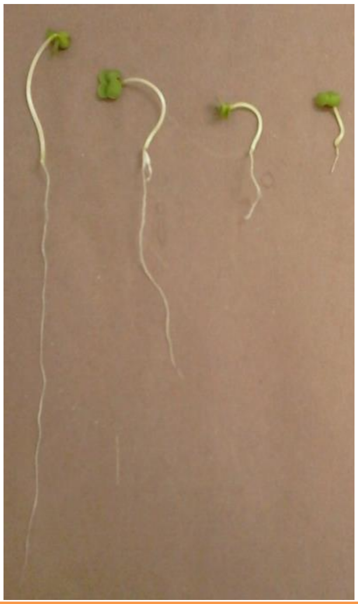
Table 7 (A): Comparative Effect on Seedling Stress Tolerance Indices of PUSA-AGRANI (Tolerance Variety) and CS-52 (Susceptible Variety) of *Brassica juncea* species grown under Drought, Salinity and their Combination with the application of Salicylic Acid (SA).
Salicylic Acid Concentration: (8 µM)

Treatment	Concentration	PUSA-AG	PUSA-AG (SA)	CS-52	CS-52 (SA)
PEG	Control	0.269	0.312	0.237	0.258
	5% PEG	0.144	0.130	0.121	0.110
	10% PEG	0.098	0.097	0.080	0.088
	15% PEG	0.043	0.047	0.033	0.038
NaCl	Control	1.280	1.473	1.317	1.333
	50 mM	1.180	1.227	1.157	1.100
	100 mM	1.103	1.243	1.063	1.083
	150 mM	1.010	1.120	1.030	1.070
Combination	Control	0.244	0.315	0.256	0.297
	Comb.1	0.145	0.262	0.244	0.255
	Comb.2	0.114	0.242	0.218	0.240
	Comb.3	0.081	0.216	0.171	0.181
				S.Em±	0.082
				C.D.5%	NS
				C.V.%	28.025

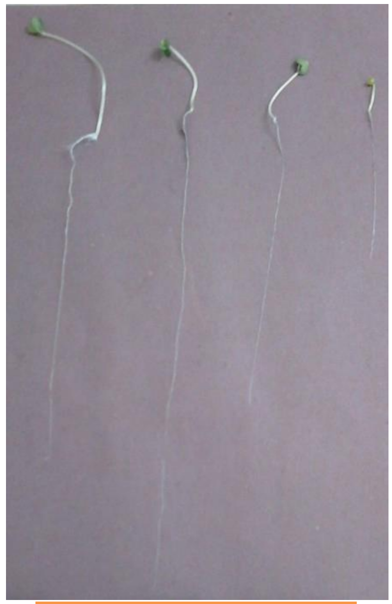
Table 7 (B): The Marginal Mean of Varieties, Treatments and Concentration for Seedling Stress Tolerance Indices

Variety	Variety Mean	Treatment	Treatment Mean	Concentration	Conc. Mean
PUSA-AG.	0.476	PEG	0.132	Control	**0.633**
PUSA-AG. (SA)	**0.557**	NaCl	**1.174**	Conc.1	0.506
CS-52	0.494	Comb.	0.218	Conc.2	0.472
CS-52 (SA)	0.504			Conc.3	0.420
S.Em ±	0.024		0.021		0.024
C.D. 5%	NS		0.058		0.067

Each data is replicated thrice. Value in a column with different letters is significantly different at P ≤ 0.01 applying DMRT; NS = Non-Significant.
Conc.1, Conc.2 and Conc.3 are indicating of every treatment's concentration



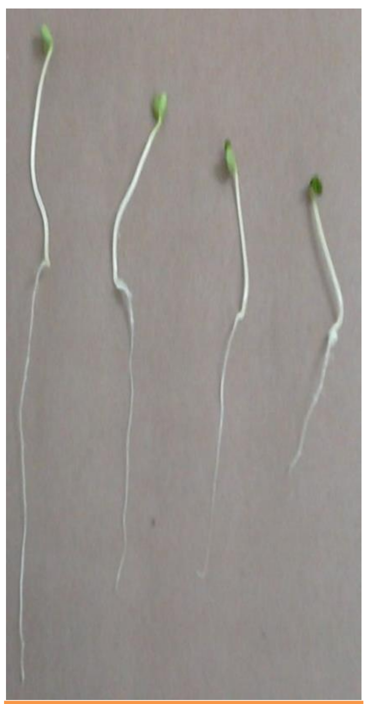
(A) Grown under Salt stress conditions



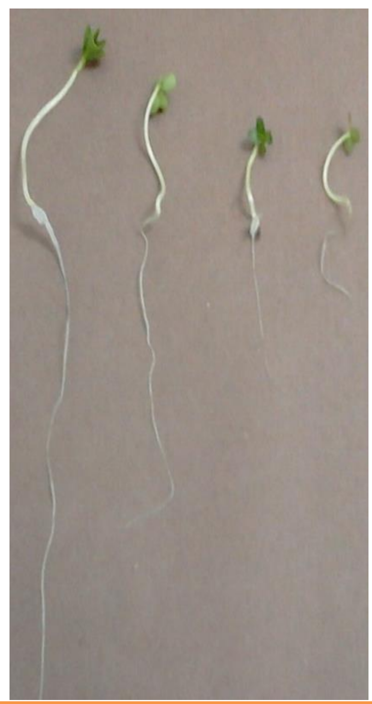
(B) Grown under Drought stress conditions

(C) Grown under Combination conditions

Grown under Salt stress conditions and it's amelioration by SA



Grown under Drought stress conditions and it's amelioration by SA



Grown under Combination conditions and it's amelioration by SA

Figure 1: The Effects of Drought, Salinity and their Combination on Susceptible Variety (CS-52) of *B. juncea* species and its amelioration using the application of Salicylic Acid (SA)

Research Article

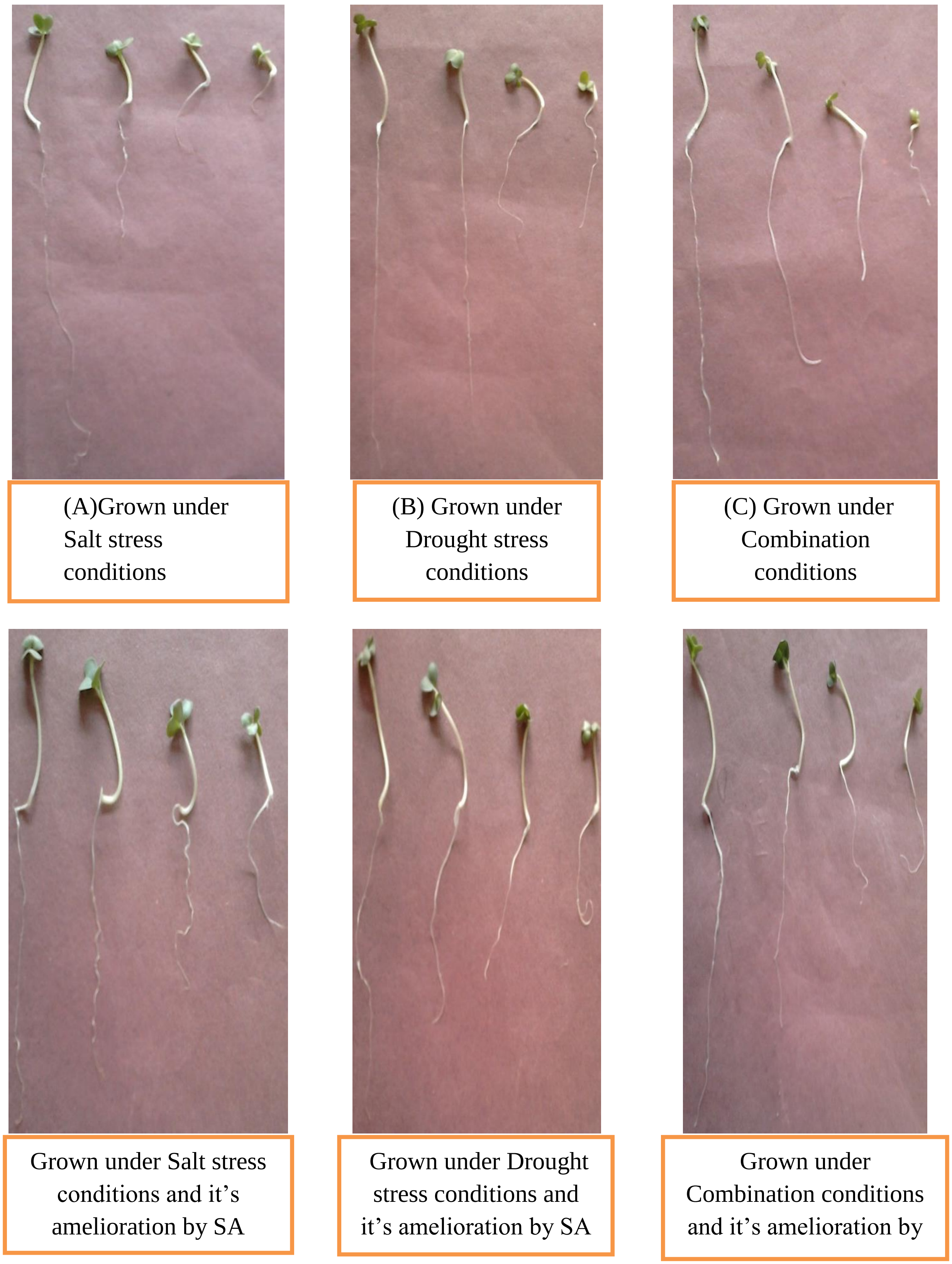


Figure 2: The Effects of Drought, Salinity and their Combination on Susceptible Variety (CS-52) of *B. juncea* species and its amelioration using the application of Salicylic Acid (SA)

-Where for both varieties:
(A) Salt Concentration (For Salinity): 50 mM NaCl, 100mM NaCl and 150 mM NaCl respectively (including Control).
(B) PEG Concentration (For Drought): 5 % PEG, 10 % PEG and 15 % PEG respectively (including Control).
(C) Combination Concentration (For Combination): Com.1, Com.2 and Com.3 respectively (including Control).
➢ Salicylic Acid (SA) Concentration: 8µM

For the dry weight, the marginal mean of second set that was treated with SA was given significantly highest dry weight under stressed conditions compared with control (PUSA-AGRANI (0.043) followed by variety CS-52 (0.036)) (Table 6 B). Among the treatment mean significantly dry matter was obtained by NaCl (0.037) while among the concentrations control (0.044) was noticed significantly highest seedling dry weight followed by con. 1 (0.039) (Table 6 B).
Water scarcity due to drought, salinity and sometime due to both is characterized by wilting, closure of stomata and reduces in cell enlargement and growth due to reduction of water content, turgor and total water potential. Cell division, enlargement and differentiation, are the main processes that determine the quality and quantity of plant growth, affected by various abiotic factors such as drought, salinity and low temperature (Sayyari *et al.,* 2013). Reduction in fresh and dry weight of both cultivars under osmotic stresses might be associated with suppression of cell expansion and cell growth due to the lower turgor pressure and also more leaf senescence. The results elucidate that exogenous application of SA exhibited significant response to improve growth attributes as compared to untreated SA plants. The result shows, full agreement with previous research, observation reported by Delavari *et al.,* (2010) on sweet basil plants under water deficit conditions. In contrast, Senaratna *et al.,* (2000) have observed a similar mechanism to be responsible for SA induced multiple stress tolerance in bean and tomato plants. The ability of SA to increase plant fresh and dry mass, ameliorating the adverse effect of abiotic stresses, it may have significant implications in improving the plant growth and combat the yield barrier arising from conditions of limited water availability.

Stress Tolerance Index (STI)

The difference among the tolerance mean of two varieties, three stresses and four concentrations may be due to these factors or their combinations. The results of ANOVA for seedling stress tolerance indices reveal that the three treatments ($P=1.92\times10^{-60}$) and their five levels of concentration (6.99×10^{-08}) are marginally performing highly significant difference. The mean tolerance was observed by NaCl (1.174) it was followed by the stress combination (0.218). Similarly, among the concentration, the significantly highest tolerance was remarked with the application of SA (0.633) with no stress that is control of SA (Table 7 B).
According to ANOVA results the non-significant results of any interaction table reflect that the variations are in the same direction either we may study row wise or column wise. The stress tolerance index (DTI and STI) was defined by Fernandez (1992), which can be used to identify genotypes and tolerance capability that produces high yield under both stressed and non-stressed conditions. In present investigation all the deleterious effects of stresses were combat by the exogenous application of SA because it is a potent signaling molecule in plants is included in eliciting specific responses to biotic and abiotic stresses (Lakzayi *et al.,* 2014).
The results were in line with previous studies of winter wheat plants (Tasgin, 2003) against low temperature stress, induces thermo tolerance in mustard seedlings (Dat *et al.,* 1998) or modulates plant responses to salt and osmotic stresses (Borsani, 2001) water stress (Senaratna. 2000) and herbicides (Ananieva, 2004).

Conclusion

The present study and available literature reviewed in the discussion suggested that exogenous treatment of SA improves salinity, combination and drought stress tolerance respectively. In plants, SA accumulates

in trace amounts but functions a lot (Alam *et al.*, 2013). Although a number of reports indicated the positive effects of SA under abiotic stress condition, but subjected to germination tolerance indices under drought, salinity and their combination conditions are scarce.
Therefore, further studies are required keeping in mind for the elucidation of the role of SA in providing the stress tolerance of plants. Complete elucidation of the seedling growth roles of SA with its detailed protective mechanisms will be helpful for developing stress tolerance in plants which is still in high demand for researches.

REFERENCES

Abdul WI, Ahmad H, Ghulam Q, Ghulam M, Tariq M and Muhammad M (2006). Effect of Salinity on Germination, Growth, Yield, Ionic Balance and Solute Composition of Pigeon Pea (*Cajanus Cajan* (L.) Millsp. *Pakistan Journal of Botany* **38**(4) 1103-1117.

Afzal I, Basra SMA, Ahmad N and Farooq M (2005). Optimization of hormonal priming techniques for alleviation of salinity stress in wheat (*Triticum aestivum* L.) *Caderno de Pesquisa série Biologia Santa Cruz do Sul* **17**(1) 95-109.

Alam MM, Hasanuzzaman M, Nahar K and Fujita M (2013). Exogenous salicylic acid ameliorates short-term drought stress in mustard (*Brassica juncea* L.) seedlings by up regulating the antioxidant defense and glyoxalase system. *Australian Journal of Crop Science* **7**(7) 1053–1063.

Ananieva EA, Christov KN and Popova LP (2004). Exogenous treatment with salicylic acid leads to increased antioxidant capacity in leaves of barley plants exposed to paraquat. *Journal Plant Physiology* **161**(3) 319–328.

Arbaoui M, Yahia N and Belkhodja M (2015). Germination of the tomato (Lycopersicon esculentum Mill.) in response to salt stress combined with hormones. *International Journal of Agronomy and Agricultural Research* **7**(3) 14-24.

Azmat R, Haider S and Askari S (2006). Phytotoxicity of Pb: Effect of Pb on germination, growth, morphology and histomorphology of *Phaseolus mungo* and *Lens culinaris*. *Pakistan Journal of Biological Sciences* **9**(5) 979-984.

Bhardwaj AR, Joshi G, Kukreja B, Malik V, Arora P, Pandey R, Shukla RN, Bankar KG, Agarwal SK, Goel S, Jagannath A, Kumar A and Agarwal M (2015). Global insights into high temperature and drought stress regulated genes by RNA-Seq in economically important oilseed crop *Brassica juncea. BMC Plant Biology* **15**(9) 1-15.

Bohra A and Sanadhya D (2015). Phytotoxic effects of cadmium on seed germination and seedling growth of Indian mustard (*Brassica juncea L*) czern coss. *International Research Journal of Biological Sciences* **4**(4) 1–7.

Borsani O, Valpuesta V and Botella MA (2001). Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. *Plant Physiology* **126**(3) 1024–1030.

Chauhan R, Bohra A and Sanadhya D (2015). Comparative studies of drought, salinity and their combinations on germination and seedling growth parameters of Indian mustard (Brassica juncea) cv**.** *International Journal of Life Sciences* **4**(3) 158-164.

Chauhan R, Bohra A and Sanadhya D (2015). Morphological, Physiological and Biochemical Responses in Plants Subjected to Drought and Salinity. *Journal of Plant Science Research* **31**(2) 197-213.

Dat JF, Foyer CH and Scott JM (1998). Changes in salicylic acid and antioxidants during induction of thermo-tolerance in mustard seedlings. *Plant Physiology* **118**(4) 1455-1461.

Delavari PM, Baghizadeh A, Enteshari SH, Kalantari KHM, Yazdanpanah A and Mousavi EA (2010). The effects of salicylic acid on some of biochemical and morphological characteristic of *Ocimum basilicum* under salinity stress. *Australian Journal of Basic and Applied Science* **4**(10) 4832–4845.

Dijibril SOK, Mohamed D, Diaga D, Diegane BF, Abaya S Maurice and Alain B (2005). Growth and development of date palm (*Phoenix dactylifera* L.) Seedlings under drought and salinity stresses. *African Journal of Biotechnology* **4**(9) 968-972.

Dolatabadian A, Sanavy SAMM and Chashmi NA (2008). The effects of foliar application of ascorbic acid (vitamin C) on antioxidant enzymes activities, lipid peroxidation and proline accumulation of canola (*Brassica napus* L.) under conditions of salt stress. *Journal of Agronomy and Crop Science* **194**(3) 206-213.

El-Khallal SM, Hathout TA, Ashour AEA and Kerrit AA (2009). Brassinolide and salicylic acid induced antioxidant enzymes, hormonal balance and protein profile of *Zea maize* plants grown under salt stress. *Research Journal of Agriculture and Biological Science* **5** 391-402.

Fernandez GCJ (1992). Effective selection criteria for assessing plant stress tolerance. In: *Proceeding of a Symposium, Taiwan* **13**-18 257-270.

Ghorbani Javid M, Sorooshzadeh A, Moradi F, Modarres Sanavy SAM and Allahdadi I (2011). The role of phytohormones in alleviating salt stress in crop plants. *Australian Journal of Crop Science* **5**(6) 726 – 734.

Hapani P and Marjadi (2015). Salt tolerance and biochemical responses as a stress indicator in plants to salinity: a review. *CIB Tech Journal of Biotechnology* **4**(1) 33-46.

Hayat QS, Hayat M, Irfan and Ahmad A (2010). Effect of exogenous salicylic acid under changing environment: A review. *Environmental and Experimental Botany* **68**(1) 14-25.

Hayata S, Aiman Hasana SA, Fariduddina Q and Ahmadb A (2008). Growth of tomato (*Lycopersicon esculentum*) in response to salicylic acid under water stress. *Journal of Plant Interactions* **3**(4) 297-304.

He YL, Liu YL, Cao WX, Huai MF, Xu BG and Huang BG (2005). Effects of salicylic acid on heat tolerance associated with antioxidant metabolism in Kentucky bluegrass. *Crop Science* **45**(3) 988-995.

Hussain M, Malik MA, Farooq M, Ashraf MY and Cheema MA (2008). Improving drought tolerance by exogenous application of glycine betaine and salicylic acid in sunflower. *Journal of Agronomy and Crop Science* **194**(3) 193-199.

Hussein MM, Balbaa LK and Gaballah MS (2007). Salicylic acid and salinity effect on growth of *maize* plants. *Journal of Agriculture and Biological Science* **3**(4) 321-328.

James RA, Rivelli AR, Munns R and Caemmerer S (2002). Factors affecting CO_2 assimilation, leaf injury and growth in salt-stressed durum wheat. *Functional Plant Biology* **29**(12) 1393–1403.

Jazi SB and Oregani KE (2014). Impact of salicylic acid on the growth and photosynthetic pigment of canola (*Brassica napus* L.) under lead stress. *International Journal of Biosciences* **4**(10) 290-297.

Joseph B, Jini D and Sujatha S (2010). Insight into role of exogenous salicylic acid on plants growth under salt environment. *Asian Journal of Crop Science* **2**(4) 226–235.

Khan W, Prithiviraj B and Smith DL (2003). Photosynthetic responses of corn and soyabean to foliarapplication of salicylates. *Journal of Plant Physiology* **160**(5) 485-492.

Lakzayi M, Sabbagh E, Rigi K and Keshtehgar A (2014). Effect of Salicylic acid on activities of antioxidant enzymes, flowering and fruit yield and the role on reduce of drought stress. *International Journal of Farming and Allied Science* **3**(9) 980-987.

Luan Z, Xiao M, Zhou DH, Tian Y, Wu Y, Guan B, and Song Y (2014). Effects of salinity, temperature, and polyethylene glycol on the seed germination of sunflower (*Helianthus annuus* L.) *The Scientific World Journal* **2014** 9.

Mahmudirad S, Rokhzadi A and Mohammadi K (2014). Effects of superabsorbent polymer application and salicylic acid spraying on chickpea productivity. *International Journal of Biosciences* **5**(5) 203-208.

Mazaheri M. and Kalantari K (2006). Effect of three factors salicylic acid, ethylene, drought stress and their interaction on seed germination of canola (*Brassica napus* L.). *Journal of Biology* **9** 408-418.

Movaghatian A and Khorsandi F (2013). Effects of Salicylic Acid on Wheat Germination Parameters under Drought Stress. *American-Eurasian Journal of Agriculture & Environmental Science* **13**(12) 1603-1608.

Pratap V and Sharma YK (2010). Impact of Osmotic Stress on Seed Germination and Seedling Growth in Black Gram (*Phaseolus Mungo*). *Journal of Environmental Biology* **31**(5) 721-726.

Rafique N, Raza SH, Qasim M and Iqbal N (2011). Pre-sowing application of ascorbic acid and salicylic acid to seed of pumpkin and seedling response to salt. *Pakistan Journal of Bot*any **43**(6) 2677-2682.

Razavizadeh R and Rostami F (2013). Changes in growth and antioxidant capacity of canola by salinity and salicylic acid under in vitro. *International Research Journal of Applied and Basic Sciences* **4**(12) 4093-4101.

Sanadhya D, Kathuria E and MaliK CP (2012). Effect of drought stress and its interaction with two phytohormones on *Vigna radiata* seed germination and seedling growth. *International Journal of life Sciences* **1**(3) 201-207.

Sayyari M, Ghanbari F, Fathhi S and Bavandpour F (2013). Chilling tolerance improving of watermelon seedling by salicylic acid seed and foliar application. *Notulae Scientia Biology* **51**(1) 67-73.

Senaratna T, Touchell D, Bunn E and Dixon K (2000). Acetyl salicylic acid (aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. *Plant Growth Regulation* **30**(2) 157–161.

Singh B and Usha K (2003). Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regulation* **39**(2) 137-141.

Tasgin E, Atici O and Nalbantoglu B (2003). Effect of salicylic acid and cold on freezing tolerance in winter wheat leaves. *Plant Growth Regulation* **41**(3) 231-236.

Tesfaye A, Petros Y and Zeleke H (2014). Screening Some Accessions of Lentil (*Lens Culinaris* M.) For Salt Tolerance at Germination and Early Seedling Stage in Eastern Ethiopia. *International Journal of Technology Enhancements and Emerging Engineering Research* **2**(8) 106-113.

Zare SA, Tavili A, Shahbazi and Akbari A (2009). Effect of different levels of salicylic acid on improvement of germination parameters in fox berry (*Sanguisorba minor* L.) under salinity and drought conditions. *Iranian Journal Natural Research Range and Watershed Management* **63**(1) 29-39.