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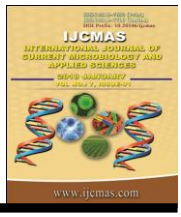

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Curative Effect of Ascorbic Acid and Gibberellic Acid on Wheat (*Triticum astivum* L.) Metabolism under Salinity Stress

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A B S T R A C T

An alternative strategy to ameliorate salt stress could be to use exogenous application of plant growth stimulators. The present investigation was carried out to study the effects of hormonal priming with ascorbic acid and gibberellic acid on wheat (*Triticum astivum* L.) metabolism during germination phase under saline conditions. Seeds of wheat var. GW-496 were pre-soaked in three levels each of ascorbic acid (AsA) *viz*., (50, 100 and 150 mg L^{1}) and gibberellic acid (GA_3) *viz*., (150, 200 and 250 mg L^{-1}) for 2 hrs under salinity stress given by treating with NaCl @ 50, 75 and 100 mM with four replications. The result showed that seeds primed with 100 mg L^{-1} AsA and 250 mg L^{-1} GA_3 effectively enhances seed moisture content, germination percentage, germination index, root/shoot length ratio, root/ shoot fresh weight ratio and vigour index I. However, among biochemical parameters application of 100 mg L^{-1} AsA and 150 mg L^{-1} GA_3 enhanced proline content. Seeds primed with 100 mg L^{-1} AsA and 250 mg L^{-1} GA_3 effectively enhanced polyphenol oxidase activity. It could be concluded that, pretreatment of wheat cultivar with AsA and GA_3 could partially alleviate the harmful effect of salinity by increasing vigour, antioxidative enzymes activity and accumulation of osmolytes.

Introduction

Wheat is a major renewable resource for food and industrial raw materials, and among major crops grown on the largest area worldwide. Wheat is the most widely grown crop worldwide grown over 200 million ha and the second most abundant staple crop grown worldwide providing globally 20% of all food calories. Salinity is one of the major and increasing problems in irrigated agriculture in India and world, particularly in wheat grown areas. Approximately 7% of worlds land area, 20% of the worlds cultivated land and nearly 50 % of the irrigated land is affected by salt stress as reported by Abdelfattah *et al.*, (2009). In India, total salt affected area is 12 million ha Abdul *et al.*, (1973). Salinity affects almost

every aspect of the physiology and biochemistry of plants and significantly reduces yield. The effect of salinity on plant may cause disturbance in plant metabolism as reported by El-Tayeb *et al.,* (2005). It was also reported that seed germination, one of the most critical phases in plant life, is greatly affected by salinity Abo-Kassem (2007), which either induces a state of dormancy at low levels or completely inhibits germination at higher levels Iqbal *et al.,* (2006).

Plant hormones are active members of the signal cascade involved in the induction of plant stress responses (Pedranzani *et al.,* 2003). The exogenous application of gibberellic acid (GA_3) improved tolerance under abiotic stress by induction and increasing of the endogenous levels of salicylic acid (Alonso- Ramírez *et al.,* 2009). Gibberellic Acid (GA_3) is the most important growth hormone which increases cell growth and elongation, cell division in cambial zone, breaks seed dormancy, promotes seed germination, intermodal length, hypocotyls growth, increases the size of leaves, enable greater photosynthesis and plant metabolism and ultimately increases plant or crop yield under normal as well stress condition. Gibberellic acid has been reported to increase germination percentage and seedling growth and overcome the preventive effects of the salt stress on germination (Kabar and Baltepe, 1987). Hence, the present investigation was carried out in order to investigate the extent of effectiveness of these two in ameliorating the adverse effect of salinity stress. Ascorbic acid (AsA) is regarded as one of the most effective growth regulators against abiotic stresses Batool *el al.,* (2012). Azooz *et al.,* (2013) showed that application of ascorbic acid through seed soaking enhanced plants growth by increased germination percentage, root and shoot fresh and dry weights, chlorophyll content and higher accumulation osmolytes. Experimental studies on different plants have shown that pretreatment with AsA reduced salt induced adverse effects and resulted in a significant increment of growth and yield Batool *el al.,* (2012).

Materials and Methods

Experimental materials

Seed of wheat cultivar GW-496, ascorbic acid (AsA), gibberellic acid (GA_3) and sodium chloride (NaCl)

Treatment

The experiment was carried out with four replications, 100 (25 seeds in each replica) seeds were soaked in distilled water (control), three concentrations of ascorbic acid (AsA) viz., (50, 100 and 150 mg L^{-1}) and three concentrations of gibberellic acid (GA_3) viz., (150, 200 and 250 mg L^{-1}) solutions for 2 hrs and then the same were treated with 2.5 g/l thiram for about 2 minutes. For germination, 25 seeds from each sample were spread in Petri dishes over Whatman No.1 filter paper. The sufficient volume (10 ml from 1st to 5th day and 20 ml from 5th to 11th) of NaCl concentrations (50 mM, 75 mM and 100 mM) were added to induce salinity stress, whereas distilled water was provided as control.

Physiological Analysis

Germination percentage

Germination percentage was recorded at 24, 48, 72, 96 and 120 h after pre-soaking treatment. It was calculated as per ISTA (International Seed Testing Association) rules 1985 with the following formula:

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated (At a particular time)}}{\text{Total number of seeds taken for germination}} \times 100$$

Germination index

Germination index was recorded at 24 h after pre-soaking treatment. It was calculated as per formula given by Maguire (1962), using average value derived from germination trial and finally the replication means are presented.

$$\text{Germination index} = \frac{\text{Increase in germination \% over time}}{\text{Change in time in day}}$$

Seed moisture percentage

Seed moisture was recorded at 24 h after pre-soaking treatment.

$$\text{Seed moisture \%} = \frac{\text{(Actual sample weight – oven dry weight)}}{\text{Actual sample weight}} \times 100$$

Root to shoot length ratio

Root / shoot length ratio was recorded at 11th day after germination (DAG).

$$\text{Root / shoot length ratio} = \frac{\text{Root length in cm}}{\text{Shoot length in cm}}$$

Root/ shoot fresh weight ratio

Root/shoot fresh weight ratio was recorded at 11th day after germination (DAG).

$$\text{Root / shoot fresh weight ratio} = \frac{\text{Root fresh weight in g}}{\text{Shoot fresh weight in g}}$$

Vigour index I

Vigour index- I was recorded at 11th day after germination. A combination of standard germination percent with seedling length provides evaluation of seedling vigour index. It was calculated as per procedure prescribed by Abdul-Baki and Anderson (1973), as under

Vigour index - I = Germination percent × Seedling mean length

Biochemical Analysis

Polyphenol oxidase activity

The estimation of Peroxidase activity was determined as per the methods suggested by Malik and Singh, (1980): Leaves (0.5 g) were extracted in mortar and pestle motor pastor and homogenized in 50 mM sodium phosphate buffer (pH 7.0) containing 1 % soluble polyvinyl pyrolidine (PVP). The homogenate was centrifuged at 13,000 x g for 15 min at 4°C and the supernatant used for assays of the activities of POD. The activity of POD was assayed by adding aliquot of the tissue extract (100 µL) to 3 mL of assay solution, consisting of 3 mL of reaction mixture containing 13 mM guaiacol, 5 mM H_2O_2 and 50mM Na-phosphate (pH 6.5). An increase in optical density at 470 nm for 1 min at 250c was recorded using spectrophotometer. POD activity was expressed as change in absorbance min/mg/protein.

Statistical analysis

Data analysis was performed using techniques of analysis of variance (ANOVA) with the statistical software ―DSAASTAT (Version 1.101). Mean separations were performed by Duncan‘s Multiple Range Test (DMRT) at 5% level.

Results and Discussion

Germination percentage

The germination percentage decreased remarkably with increasing salinity levels. Salinity stress at the mild level posed a little

reduction on the germination percentage however; a drastic reduction was observed at moderate and severe stress conditions. Similarly, the increasing salt stress levels with substantial decrease in overall germination percentage was reported by Akbarimoghaddam *et al.*, (2011) and Datta *et al.*, (2009) in wheat. The observed decrease in germination percentage may be attributed to the decrease in osmotic potential, increasing toxic ions, changing the remobilization balance of seeds reservoirs, loss of viability at higher salinity level and reduced water imbibitions. In addition high salinity delayed radical emergence and decreased germination percentage.

A marked increase in germination percentage was found after pretreatment of wheat seeds with ascorbic acid and gibberellic acid under saline condition. In case of AsA treated wheat seeds at 24, 48, 72 and 96 h under all three salt stress levels its concentration of 100 mg L^{-1} exhibited the highest germination percentage.. At 120 h all treatment of AsA performed equally as 100% (data in parenthesis) germination was achieved. Similar observation was proposed by Afzal *et al.*, (2006) in wheat and Behairy *et al.*, (2012) in fenugreek.

Treatment of wheat seeds with GA_3 at 24, 48, and 72 h under mild, moderate and severe stress condition treatment with GA_3 @ 250 mg L^{-1} showed maximum enhancement in germination percentage. At 120 h 100% (data in the parenthesis) germination was achieved under all three salinity stress levels. Similar result was reported in wheat by Ozhan and Hajibabaei (2013) that GA_3 treatments increased germination percentage under salinity stress condition. GA_3 involved in biosynthesis and secretion of α-amylase enzyme in plants hydrolyzing starch into simpler sugar thereby enhancing germination percentage.

Germination index

Germination index was negatively affected by salt (NaCl) stress, a general trend of decrease in germination index was found with increase in salinity. It was found that NaCl at the mild (50 mM) and moderate (75) levels showed less reduction with the tune of (5%) and (14%) respectively as compared to and severe (100 mM) concentration with the reduction of (30%). The result is in agreement with that obtained by Behairy *et al.*, (2012) who reported that in fenugreek seeds speed of germination was significantly reduced in direct relation to the increasing salinity level, an effect that could possibly be due to reduced seeds metabolism and water absorption.

Application of wheat seeds with AsA and GA_3 significantly increased the speed of germination under saline condition. In case of AsA treated seeds under all the three salinity levels, the application of 100 mg L^{-1} AsA showed maximum increase. Similar result was presented by Mohsen *et al.*, (2014) that soaking of seeds in ascorbic acid alleviates the adverse effects of the higher salinity levels on the speed of germination.

This was due to its possible utility as an organic substrate for respiratory energy metabolism that helps in stress tolerance and increased speed of germination. Under all three stress condition seeds treated with GA_3 @ 250 mg L^{-1} was the best in nullifying the adverse effect of salt stress on speed of germination. Stimulation of germination index by GA_3 might be due to GA_3-induced inhibition of Na^+ accumulation with a concomitant increase in K^+ accumulation in radicle and plumule. GA_3 plays crucial role in imbibition by decreasing the density of cytoplasm and hence, increasing water absorption and increasing germination percentage as evident in the present investigation also.

Seed moisture percentage

It sharply decreased with increasing salinity levels. The higher concentration (100 mM) showed more reduction (24%) as compared to mild (50 mM) and moderate (75 mM) concentrations where the reduction was to the tune of (7%) and (13%) respectively, as compared to control. El Goumi *et al.,* (2011) also stated that increasing salinity caused the diminution of water content in the seeds, roots and the shoots for all cultivars of wheat; and the reduction of water content was the most noticeable with highest NaCl level. The highest seed moisture content of AsA treated seeds were found with its moderate concentration and treatment of wheat seeds with GA_3, its highest concentration showed greatest seed moisture content. Abou-Leila *et al.,* (2012) also revealed that when jatropha plants were treated with AsA under different salt concentrations (4000, 8000 and 12000 ppm) there was an increase in relative water content and osmotic pressure through osmotic adjustment. Relative water content percentage (RWC) and osmotic potential also increased as the concentration of ascorbic acid increased.

This increase in water content may be attributed to the increase in accumulation of osmolytes under the influence of added AsA which maintained the water potential, thereby moisture content of seed. The result may also be due to the fact that hormones generally decrease the viscosity of cytoplasm and increase diffusion of water into the cell.

Root to shoot length ratio

A progressive increase in root to shoot length ratio was found with increase in salinity levels. Severe stress forced more increase of root/shoot length ratio with the tune of (10%), as compared to moderate (22%) and mild stress (25%) conditions. The result is in accordance with Hameed *et al.,* (2008). Application of AsA and GA_3 were found to increase the root to shoot length ratio under salinity stress levels. For the seeds pretreated with AsA under all stress levels, the maximum increment was found with the application of highest level of AsA. Rafique *et al.,* (2011) also reported that application of 15 mg L^{-1} AsA showed enhanced root/shoot length ratio under saline condition.

Under mild and moderate stress condition, GA_3 @ 150 and 250 mg L^{-1} were effective in nullifying the adverse effect of salt stress on root to shoot length ratio. However, under severe stress GA_3 @ 200 mg L^{-1} showed best result. This result was justified by the fact that both AsA and GA_3 regulates physiological and biochemical activities in plants and can be used as a potential growth promoter to improve root growth more than the shoot growth under saline conditions, thereby increasing root to shoot length ratio.

Root/shoot fresh weight ratio

Salinity (NaCl) stress had an overall adverse effect on root to shoot fresh weight ratio as the same decreased progressively with increasing salinity levels. Khavarinegad *et al.,* (2014) and James *et al.,* (2006) also observed similar results in lentil and *Sarcobatus vermiculatus* respectively. This damage could be attributed to reduction in water availability, toxicity of specific ions, and nutritional imbalance caused by such ions. These effects might inhibit the root growth more which was in direct contact with salt then the shoot growth. Remarkable increase in root to shoot fresh ratio was found after pretreatment of wheat seeds with ascorbic acid and gibberellic acid under saline condition. It was found that seeds pretreated with AsA @ 50 mg L^{-1} showed best increment under mild and severe stress conditions. Under moderate stress level treatment with AsA at its highest concentration (150 mg L^{-1}) was more effective.

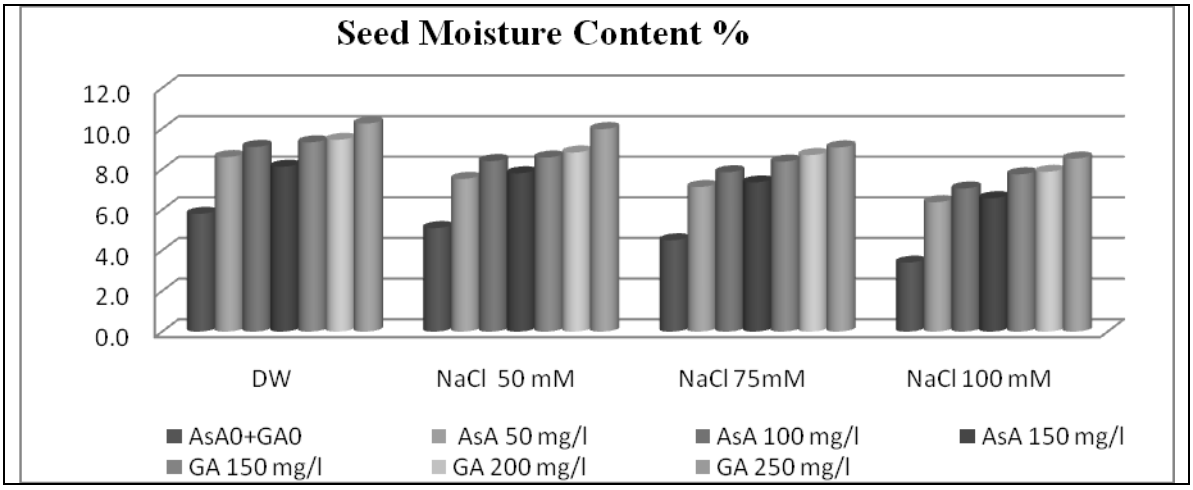
Table.1 Effect of NaCl induced salinity stress, ascorbic acid (AsA) and gibberellic acid (GA_3) on seed germination (arc sign) at 24, 48, 72, 96 and 120 h after germination of wheat. The data were transformed using arc sign transformation

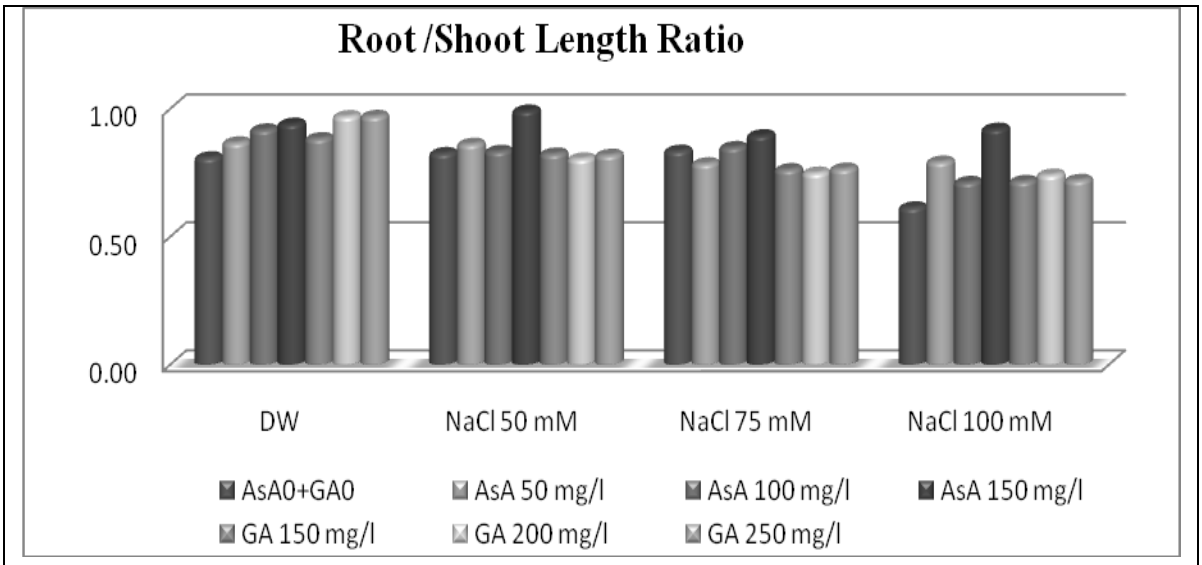
Treatment	Germination Percentage (%)									
	24 hr		48 hr		72 hr		96 hr		120 hr	
Distilled Water (DW)	71.8 ghi	(90 hij)	73.9 ijk	(92efgh)	75.0 efg	(93.25 f)	76.1 fg	(94 hi)	90 a	(100 a)
DW + AsA 50 mg L^{-1}	76.4 d	(95 de)	77.8 fg	(96 abc)	79.3 cd	(96.5 cd)	81.5 cd	(98 ef)	90 a	(100 a)
DW +AsA 100 mg L^{-1}	88.5 a	(99 ab)	88.6 ab	(100 a)	90.0 a	(100 a)	90.0 a	(100 a)	90 a	(100 a)
DW + AsA 150 mg L^{-1}	82 b	(98 abc)	85.1 cd	(99 a)	88.6 ab	(99.75 a)	88.6 ab	(100 a)	90 a	(100 a)
DW + GA_3 150 mg L^{-1}	79.8 bc	(97 bc)	81.9 e	(98 abc)	85.7 b	(98.75 ab)	86.5 b	(99 abcde)	90 a	(100 a)
DW + GA_3 200 mg L^{-1}	82.2 b	(98 abc)	86.5 bc	(99 a)	88.6ab	(99.75 a)	90.0 a	(100 a)	90 a	(100 a)
DW + GA_3 250 mg L^{-1}	90 a	(100 a)	90.0 a	(100 a)	90.0 a	(100 a)	90.0 a	(100 a)	90 a	(100 a)
Mild Stress										
NaCl 50 mM	67.4 jk	(85 m)	69.1 mn	(87 ijk)	70.0 hi	(88.25 i)	70.7 ijk	(89 lm)	90 a	(100 a)
NaCl 50 mM + AsA 50 mg L^{-1}	73 efgh	(92 fgh)	76.5 fghi	(95 abcde)	77.8 cde	(95.5 de)	79.7 de	(97 fg)	90 a	(100 a)
NaCl 50 mM + AsA 100 mg L^{-1}	80 bc	(97 bc)	83.7 de	(99 a)	88.6 ab	(99.75 a)	90.0 a	(100 ab)	90 a	(100 a)
NaCl 50 mM + AsA 150 mg L^{-1}	74.3 def	(93 ef)	76.8 fghi	(95 abcd)	78.1 cde	(95.75 de)	80.5 cd	(97 f)	90 a	(100 a)
NaCl 50 mM + GA_3 150 mg L^{-1}	75.2 de	(93 efg)	76.5 fghi	(95 abcde)	77.8 cde	(95.5 de)	83.6 c	(98 acdef)	90 a	(100 a)
NaCl 50 mM + GA_3 200 mg L^{-1}	76.4 d	(95 de)	79.2 f	(97 abc)	80.9 c	(97.5 bc)	88.0 ab	(100abcd)	90 a	(100 a)
NaCl 50 mM + GA_3 250 mg L^{-1}	90.5 a	(100 a)	90.0 a	(100 a)	90.0 a	(100 a)	90.0 a	(100 ab)	90 a	(100 a)
Moderate Stress										
NaCl 75mM	61.3 l	(77 o)	64.9 p	(82 l)	65.7 k	(83 j)	66.4 l	(84 o)	90 a	(100 a)
NaCl 75 mM + AsA 50 mg L^{-1}	66.4 k	(85 m)	70.7 lmn	(89 hijk)	71.6 hi	(90 gh)	73.3 ghi	(92 jk)	90 a	(100 a)
NaCl 75 mM + AsA 100 mg L^{-1}	70.9 hi	(89 jk)	75.0 ghi	(93 cdef)	76.2 de	(94.25 ef)	82.2 cd	(98 def)	90 a	(100 a)
NaCl 75 mM + AsA 150 mg L^{-1}	67.8 jk	(86 m)	72.1jkl	(91 fghi)	73.1 fgh	(91.5 g)	74.2 gd	(93 j)	90 a	(100 a)
NaCl 75 mM + GA_3 150 mg L^{-1}	71.5 ghi	(90 ijk)	74.7 hij	(93 defg)	75.8 ef	(94 ef)	77.5 ef	(95 gh)	90 a	(100 a)
NaCl 75 mM + GA_3 200 mg L^{-1}	72.3 fgh	(91 ghij)	76.8 fgh	(95 abcd)	78.2 cde	(95.75 de)	88.6 ab	(100 abc)	90 a	(100 a)
NaCl 75 mM + GA_3 250 mg L^{-1}	78.8 c	(96 cd)	82.6 de	(98 ab)	86.5 b	(99.25 a)	90.0 a	(100 abc)	90 a	(100 a)
Severe Stress										
NaCl 100 mM	52.7 m	(63 p)	56.9 q	(70 m)	57.6 l	(71.25 k)	58.2 m	(72 p)	80.5 b	(97 b)
NaCl 100 mM + AsA 50 mg L^{-1}	63.4 l	(80 n)	65.7 op	(83 kl)	66.4 jk	(84 j)	67.8 kl	(86 n)	90 a	(100 a)
NaCl 100 mM + AsA 100 mg L^{-1}	71.1 hi	(89 k)	71.1 klm	(90 hijk)	72.1 ghi	(90.5 g)	73.9 gh	(92 j)	90 a	(100 a)
NaCl 100 mM + AsA 150 mg L^{-1}	68.5 jk	(85 m)	69.3 lmn	(88 ijk)	70.2 hi	(88.5 hi)	72.1 hij	(91 kl)	90 a	(100 a)
NaCl 100 mM + GA_3 150 mg L^{-1}	67.3 k	(87 lm)	68.1 no	(86 jkl)	68.9 ij	(87 i)	69.6 jk	(88 m)	90 a	(100 a)
NaCl 100 mM + GA_3 200 mg L^{-1}	69.7 ij	(88 kl)	71.6 klm	(90 ghij)	72.6 gh	(91 g)	74.8 fgh	(93 ij)	90 a	(100 a)
NaCl 100 mM + GA_3 250 mg L^{-1}	73.9 efg	(92 fghi)	76.2 ghi	(92efgh)	77.5 de	(93.25 f)	81.2 cd	(98 f)	90 a	(100 a)
Note: The original data are presented in the parenthesis										

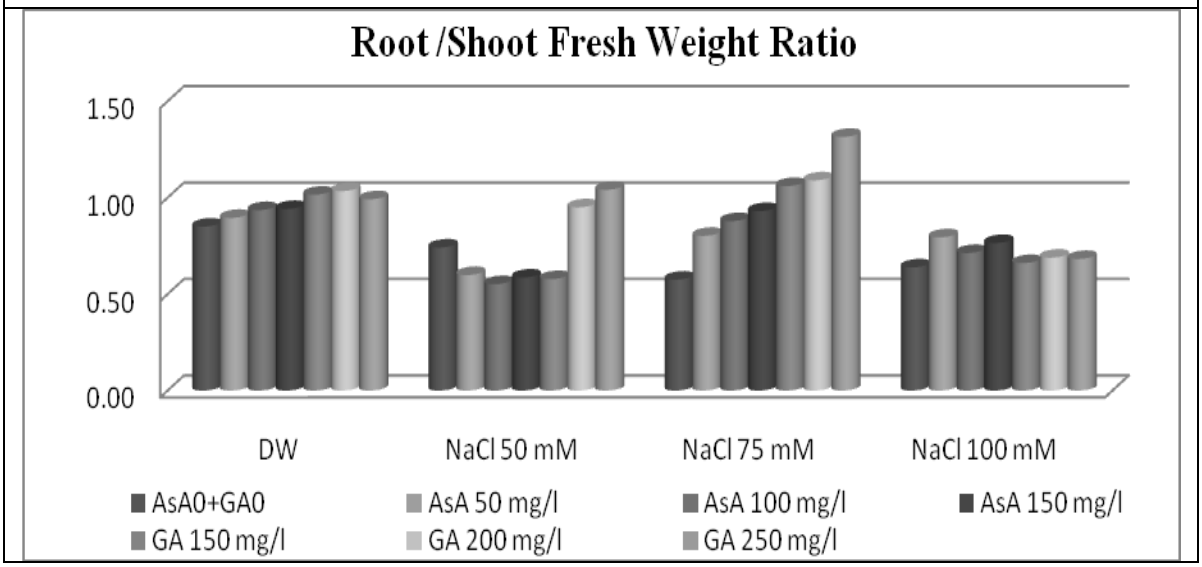
Table.2 Effect of NaCl induced salinity stress, ascorbic acid (AsA) and gibberellic acid (GA_3) on speed of germination at 24 h after germination of wheat

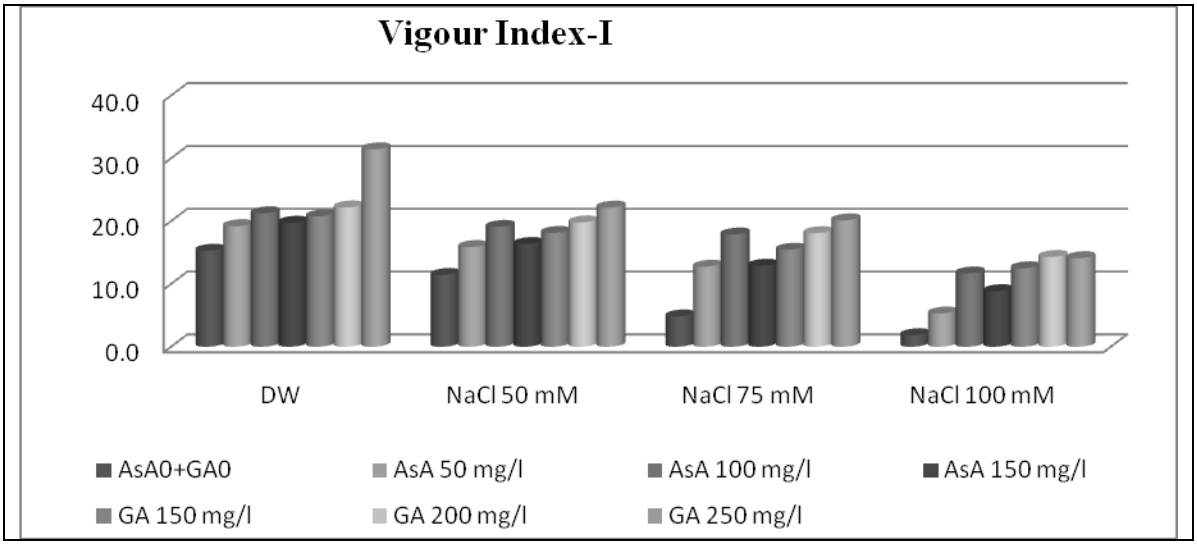
Treatments	Speed of Germination	Treatments	Speed of Germination
Distilled Water	**24 h**	**Mild Stress**	**24 h**
Distilled Water (DW)	3.8 hij	NaCl 50 mM	3.6 m
DW + AsA 50 mg L^{-1}	3.9 de	NaCl 50 mM + AsA 50 mg L^{-1}	3.8 fghi
DW +AsA 100 mg L^{-1}	4.1 ab	NaCl 50 mM + AsA 100 mg L^{-1}	4.1 bc
DW + AsA 150 mg L^{-1}	4.1 abc	NaCl 50 mM + AsA 150 mg L^{-1}	3.9 efg
DW + GA_3 150 mg L^{-1}	4.0 c	NaCl 50 mM + GA_3 150 mg L^{-1}	3.9 ef
DW + GA_3 200 mg L^{-1}	4.1 abc	NaCl 50 mM + GA_3 200 mg L^{-1}	3.9 de
DW + GA_3 250 mg L^{-1}	4.2 a	NaCl 50 mM + GA_3 250 mg L^{-1}	4.2 a
Treatments	**Speed of Germination**	**Treatments**	**Speed of Germination**
Moderate Stress	**24 h**	**Severe Stress**	**24 h**
NaCl 75mM	3.2 o	NaCl 100 mM	2.6 p
NaCl 75 mM + AsA 50 mg L^{-1}	3.5 m	NaCl 100 mM + AsA 50 mg L^{-1}	3.3 n
NaCl 75 mM + AsA 100 mg L^{-1}	3.7 jk	NaCl 100 mM + AsA 100 mg L^{-1}	3.7 jk
NaCl 75 mM + AsA 150 mg L^{-1}	3.6 m	NaCl 100 mM + AsA 150 mg L^{-1}	3.6 im
NaCl 75 mM + GA_3 150 mg L^{-1}	3.8 ijk	NaCl 100 mM + GA_3 150 mg L^{-1}	3.5 m
NaCl 75 mM + GA_3 200 mg L^{-1}	3.8 ghij	NaCl 100 mM + GA_3 200 mg L^{-1}	3.7 kl
NaCl 75 mM + GA_3 250 mg L^{-1}	4.0 bcd	NaCl 100 mM + GA_3 250 mg L^{-1}	3.8 fgh

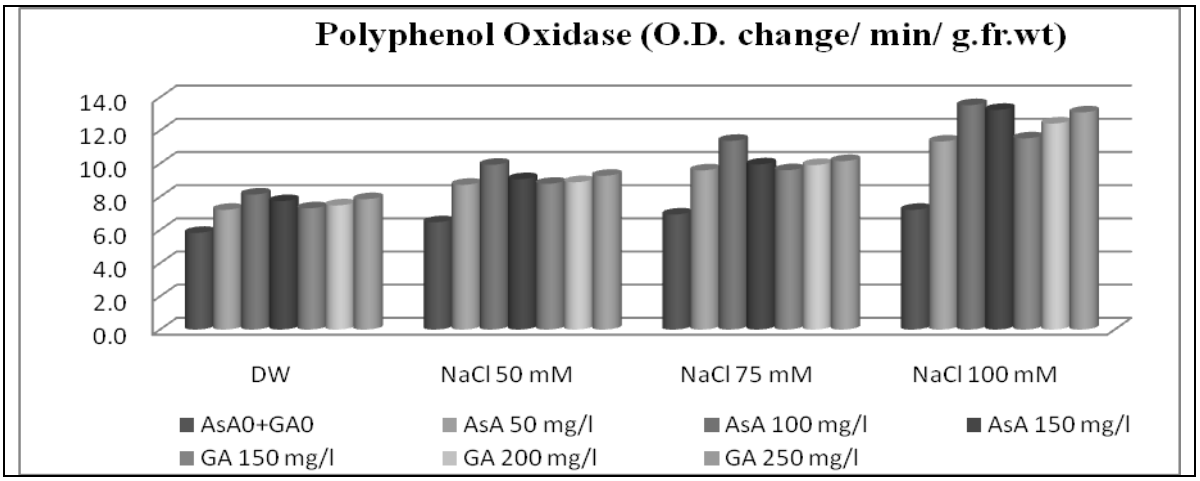
Effect	C.V. (%)	S.Em. ±	CD at (0.05)
SALT x GRT	8.5	0.003	0.011




Root /Shoot Length Ratio
1.00
0.50
0.00
DW
NaCl 50 mM
NaCl 75 mM
NaCl 100 mM
AsA0+GA0
AsA 50 mg/l
AsA 100 mg/l
AsA 150 mg/l
GA 150 mg/l
GA 200 mg/l
GA 250 mg/l


Root /Shoot Fresh Weight Ratio
1.50
1.00
0.50
0.00
DW
NaCl 50 mM
NaCl 75 mM
NaCl 100 mM
AsA0+GA0
AsA 50 mg/l
AsA 100 mg/l
AsA 150 mg/l
GA 150 mg/l
GA 200 mg/l
GA 250 mg/l


Vigour Index-I
40.0
30.0
20.0
10.0
0.0
DW
NaCl 50 mM
NaCl 75 mM
NaCl 100 mM
AsA0+GA0
AsA 50 mg/l
AsA 100 mg/l
AsA 150 mg/l
GA 150 mg/l
GA 200 mg/l
GA 250 mg/l



With respect to the treatment of wheat seeds with GA_3 under mild and moderate stress condition, the maximum increment was shown by its treatment at highest concentration (250 mg L^{-1}). Seeds treatment with mild concentration of GA_3 (200 mg L^{-1}) performed well under severe stress condition. Similar report was reported by Khavarinegad *et al.*, (2014) who showed that pre-treatment of lentil seeds with GA_3 increased the root to shoot fresh weight ratio under saline condition.

Vigour index I

A progressive increase in salinity levels effectively reduced the vigour index I. NaCl, at the moderate and severe levels showed maximum reduction of (31%) and (34%) respectively as compared to mild stress (59%) levels. The result is supported by Mosavian and Eshraghi (2013) and Elouaer *et al.*, (2012). The reduction in seedling vigour may be due to reduced germination percent observed in the present investigation. An increase in vigour index I was found after pretreatment of wheat seeds with ascorbic acid and gibberellic acid under saline condition. Application of AsA @ 100 mg L^{-1} was the best under all levels of salinity. The result is supported by Ghoohestani *et al.*, (2012) who reported that seeds priming of tomato with salicylic acid and ascorbic acid resulted in increased vigour index I under salt stress concentration. Ascorbic acid protects metabolic processes against H_2O_2 and other toxic derivatives of oxygen which affect many enzyme activities, minimizes the damage caused by oxidative processes through synergistic function with other antioxidants, and stabilizes membranes (Agarwal and Pandey, 2004 and Sairam *et al.*, 2005). All salinity levels under application of 250 mg L^{-1} GA_3 exhibited increased vigour index. Enhanced germinative parameters in response to GA_3 application have led to increased seedling vigour.

Polyphenol oxidase activity

PPO activity increased considerably with increasing salinity levels. Slight induction (15%) in the given parameter was observed under mild salt stress level. However, at moderate and severe stress levels more than double increase of 36% and 38% were observed. Similar result was reported by Sairam *et al.*, (2005) in wheat.

A noticeable increase in polyphenol oxidase activity was observed after pretreatment of wheat seeds with ascorbic acid and gibberellic acid under saline condition. Seeds pretreated with AsA @ 100 mg L^{-1} was numerically the

best and treatment of AsA @ 150 mg L^{-1} was at par with it under all three stress condition. The result is in agreement with Elhamid *et al.*, (2014) in wheat cultivar.

AsA-induced enhanced salt tolerance in wheat plants was due to having a better antioxidant system as found in the present investigation for the effective removal of ROS plants, and maintenance of ion homeostasis. Application of the highest concentration GA_3 (250 mg L^{-1}) under all three stress conditions, showed maximum increase in PPO activity. Thus, it could be inferred that PPO plays a vital role in plant defense against oxidative stress by scavenging H_2O_2 in chloroplast, cytosol, mitochondria and peroxisome of plant cells. Among the two biostimulators, seeds treatment with AsA @ 100 mg L^{-1} was the best in ameliorating the adverse effect of salinity and increased polyphenol oxidase activity.

Salinity stress (NaCl) at moderate and severe levels (75 and 100 mM) showed more reduction in almost all germination parameters and some biochemical parameters *viz.*, seed moisture content, germination percentage, germination index, root/shoot length ratio, root/shoot fresh weight ratios and vigour index-I as compared to salinity stress at mild level (50 mM) during all the periods of germination. However, salinity stress at all three levels (50, 75 and 100 mM) enhanced root/shoot length ratio and antioxidants *viz.*, polyphenol oxidase activity. Thus, distinct favorable effect of ascorbic acid and gibberellic acid in alleviation of salinity stress could be discerned as evidenced by activated physiological parameters and metabolism of germinating seedlings of wheat after pretreatment under saline condition. Hence, this study proves the role of these bio-stimulators in ameliorating the deleterious effect of salinity stress (Table 1 and 2).

Future prospects

The present study paves the way for use of Ascorbic acid and Gibberellic acid at germination stage of crop to overcome the low productivity of crops under salinity stress. The present work throws light on the development of physiological and biochemical resistance in the crop as a result of presoaking treatment of the growth stimulator Ascorbic Acid and growth hormone Gibberellic acid which counter the effect of salinity stress. The yield losses could be minimized by conferred resistance against salinity stress and the physiological and biochemical markers conferring resistance could be used in further crossing programs to select resistant lines.

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