

Gibberlic Acid and Salicylic Acid Effects on Seed Germination and Seedlings Growth of Wheat (*Triticum aestivum* L.) Under Salt Stress Condition

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Abstract: Seedlings establishment at early growth stages of crop plants is severely affected by soil salinity. Therefore, high germination rate and vigorous early growth under salty soils is preferred. In this study germination and seedling growth of a wheat (*Triticum aestivum* L.) cultivar was assessed using three replicates of 50 seeds in a factorial laid out in two separate experiments as Completely Randomized Design (CRD) testing combinations of three levels of salinity (0, 50, 100 and 200 mMol NaCl) and four levels of salicylic acid (0, 1.5, 3 and 4.5 m gr Lit) in the first experiment and the same salinity levels with three levels of gibberellic acid (0, 0.5 and 1 m Mol) in the second experiment. SA increased germination percentage and germination rate to 3 m gr Lit, but applying more decrease it. However, GA decreased germination percentage and germination rate to about 38 and 41% in 1 m Mol than control, respectively. Germination percentage and germination rate was significantly increased by SA and GA under salinity conditions compared to non treatment of SA and GA. Priming with 1.5 m gr Lit SA showed maximum radicle and hypocotyl length and higher amount decreased both traits. Priming with SA could not improve radicle length and radicle dry weight in all salinity levels. However, treated seeds with SA produced the higher hypocotyl length in all salinity levels than untreated seeds. GA decreased radicle length, while increased hypocotyl length relatively. Application of GA enhanced radicle and hypocotyl length in all salinity levels compared to untreated seeds with treatment. Dry weight of hypocotyl was decreased due to salinity stress but seedlings raised from seeds primed with SA improved dry weight of seedlings as compared to non treatment of SA under non salinity and salinity conditions. Application of GA decreased both radicle and hypocotyls dry weight. GA decreased seedling fresh and dry weight to about 20 and 35% in 1 m Mol than control.

Key words: Wheat Salicylic acid • Gibberellic acid • Germination rate • Seedling • Radicle • Hypocotyl

INTRODUCTION

Salinity is one of the most important factors that limit crop production in arid and semi-arid regions [1]. Salinity affects about 7% of the world's total lands area [2]. The percentage of cultivated land affected by salt is even greater, comprises 19% of 2.8 billion hectares of arable land on the earth [3-5]. Furthermore there is also a dangerous trend of a 10% per year increase in the saline area throughout the world. Data collected at CIMMYT suggest that 8-10% of the area planted to wheat in India, Pakistan, Iran, Egypt, Libya and Mexico is affected by salinity [6]. Wheat is a mandatory salt tolerant crop and serves as a staple food in 43 countries [7, 8]; including Iran, where it is grown on a large area. On the other hand, Iran is one of the countries that suffer from sever salinity

problems. For example 18 million ha or 10% of total land area in Iran is salinity or sodicity soil. [9].

Germination of seeds is one of the most crucial and decisive phases in the growth cycle of plant species since it determines plant establishment and final yield of the crops. Poor germination and seedling establishment are the results of soil salinity. It is an enormous problem adversely affecting growth and development of crop plants and results in to low agricultural production [10]. Therefore, any treatment which could be used to improve seed germination and subsequent seedling establishment under saline conditions would be highly desirable. Pre-sowing seed treatments have been shown to enhance stand establishment in non-saline areas [11] and have potential in saline areas as well [12, 13]. Prior to selecting these alternatives, it seems necessary to examine seed

vigor enhancement techniques leading to better and synchronized stand establishment under stress conditions. Physiological treatments to improve seed germination and seedling emergence under various stress conditions have been intensively investigated in the past two decades [14]. It is thought that the depressive effect of salinity on germination could be related to a decline in endogenous levels of hormones [15]. However, incorporation of plant growth regulators during presoaking, priming and other pre-sowing treatments in many vegetables crops have improved seed performance. Typical responses to priming are faster and closer spread of times to germination and emergence over all seedbed environments and wider temperature range of germination, leading to better crop stands and hence improved yield and harvest quality, especially under suboptimal and stress condition growing conditions in the field [16].

Presoaking seeds with optimal concentration of phytohormones has been shown to be beneficial to growth and yield of some crop species growth under saline conditions by increasing nutrient reserves through increased physiological activities and root proliferation [17]. Concerted attempts have been made to mitigate the harmful effects of salinity by application of plant growth regulators [18]. Thus the detrimental effects of high salts on the early growth of wheat seedlings may be reduced to some extent by treating seeds with the proper concentration of a suitable hormone [19].

Salicylic acid (SA) and Gibberellic Acid (GA) play an important role in the defense response to stresses in plant species [20, 21]. Several studies also supported a major role of SA and GA in modulating the plant response to several abiotic stresses including salt and water stress [22-24].

Salicylic acid (SA), a plant Phenolic is now considered as a hormone-like endogenous regulator and its role in the defense mechanisms against biotic and abiotic stress has been well documented [22, 25]. It was found that inhibition of catalase, a H₂O₂ scavenging enzyme, by SA plays an essential role in the generation of reactive oxygen species [26]. By increasing H₂O₂ concentration of the tissues, moderate doses of SA may activate the antioxidative mechanisms. Application of exogenous SA enhanced the drought and salt stress resistance of plants [23, 27], but the results were contradictory and depended on the developmental phase of plants [28] or on the experimental conditions [29]. Both high salinity and drought give rise to ionic and osmotic effects combined with oxidative damage in an important role in determining the sensitivity of plants to various abiotic stresses [30, 31], notably at the seedling stage

[28]. The inclusion of SA at 0.5 mM in the germination medium was associated with increase germination percentage of tomato [32].

Gibberellic Acid (GA), which comes under the naturally occurring growth hormone which regulates the growth and development of plants [33]. The GA are associated with various plant growth and development processes such as seed germination, hypocotyls elongation, leaf expansion, floral initiation, uniform flowering, floral organ development, reduced time to flowering, increased flower number and size and induction of some hydrolytic enzymes in the aleurone of cereal grains [34-36].

Growth regulators like GA₃ are reported to alleviate the inhibitory effect of salinity on germination [37-40]. Kaur *et al.* [41] found that GA₃ at 6 μM concentration induces increased germination and seedling growth under salt stress. Ashraf *et al.* [42] showed that GA₃ application increased the nutrient uptake, dry weight, plant height, leaf area and yield of wheat under saline conditions. There is also evidence that GA₃ can significantly relieve NaCl-induced growth inhibition in rice [43]. Starck and Kozinska [44] reported that the GA₃ caused more absorption of P and Ca²⁺ and less absorption of Na⁺, while it partially adjusted the ion ratios in bean. Bejaoui [45] concluded that the effects of exogenously applied GA₃ in alleviation of salt stress may be caused by activation of special enzymes which participate in RNA and protein synthesis. Aloni and Pressman [46] discussed possible interaction between salinity and the GA₃ effect on petiole elongation, cellular breakdown and bolting in celery.

Seed germination and stand establishment in wheat farms is very often poor due to high level of salinity of irrigation water in Iran. Therefore, the aim of this experiment was to study the effect of seed pre-sowing treatment with SA and GA on germination and seedling growth of a wheat cultivar under salinity stress conditions.

MATERIAL AND METHODS

Plant Material: The experiment carried out in Islamic Azad University of Ramhormoz, Khuzestan, Iran in March 2012. Similar seed size and weight of a wheat cultivar (*Triticum aestivum* L.) was selected to exclude effect of that on the seedling establishment. Seeds were surface sterilized in 1.5% (v/v) sodium hypochloride for 10 min and thoroughly washed with sterile tap water. Seeds were germinated in covered, sterilized, disposable petri dishes containing Whatman No. 1 filter paper moistened with either distilled water (control), or different treatment

solutions. Germination was assessed using three replicates of 50 seeds in a factorial laid out in two separate experiments as Completely Randomized Design (CRD) testing combinations of three levels of salinity (0, 50, 100 and 200 mMol NaCl) and four levels of salicylic acid (0, 1.5, 3 and 4.5 m gr Lit) in the first experiment and the same salinity levels with three levels of gibberellic acid (0, 0.5 and 1 m Mol) in the second experiment. The seeds were kept for 6 hours in the SA and GA solutions, after which the solution was eliminated and the seeds were dried lightly by depositing them on filter paper that absorbed most of the solution left on the seeds and then finally they were deposited in separate Petri dishes between two filter papers.

Growth Conditions: Seeds were incubated in a growth chamber (Type 8194, VINDON) and were considered germinated with the emergence of the radical. Temperature was maintained during the 10-d duration of the germination tests at 25°C (±0.5). In order to maintain adequate moisture, 5 mL of the original salt solutions were added to each petri dish every three days. Germination was scored when a 2 mm radical emerged from the seed coat [47]. Every three days, the germinated seeds were removed from the petri dishes. The seeds to germinate in each replicate were retained for measurements of radical and hypocotyl lengths at the end of the experiment. After 240 h, final germination percentages were recorded and seedling fresh weight immediately determined. To determine the impact of the treatments on seed germination, all seedlings were separated from the remaining seeds. Seedlings were harvested after ten days and washed with deionized water after harvest.

Five washed seedlings from each replication were separated into root and shoot for the determination of their fresh and dry weight. Dry weight was determined after oven drying the samples at 65°C. Stem diameter was measured above the first real leaf by using caliper ruler with 0.001 mm.

Growth Parameters: Germination percentage, germination rate, radicle and hypocotyl length, seedling fresh and dry weight, radicle and hypocotyls dry weight were measured. A germination index was calculated for each subpopulation as Germination Rate GR:

$$GR = X1/Y1+(X2-X1)/Y2+...+(Xn-Xn-1)/Yn$$

Where Xn is the germination percentage on Nth day and Yn in the number of day from first day experiment [48].

Data Analysis: Data were analyzed using the GLM procedure of SAS program [49]. Significant differences between treatments were determined using Duncans multiple range test at 0.05 level.

RESULTS AND DISCUSSION

Germination Percentage: Variance analysis results of germination percentage are shown in Table 1 and 2. According to Table 1 and 2, salinity (P<0.01), salicylic acid (P<0.05) and gibberellic acid (P<0.05) affected germination percentage significantly. In addition, a significant interaction was obtained between salicylic acid and gibberellic acid to salinity (P<0.05). Germination percentage was reduced by salinity compared to non

Table 1: Analysis of variance for the traits investigated in a wheat cultivar in response to salinity stress and salicylic acid

Sources of variation	df	Germination percentage	Germination rate	Radicle length	Hypocotyl length	Radicle dry weight	hypocotyl dry weight	Seedling fresh weight	Seedling dryweight
Salt stress	3	**	**	*	*	**	**	**	**
SA	3	*	*	*	*	ns	*	*	*
S. stress × SA	9	*	*	ns	ns	ns	*	ns	ns

ns Non-significant, * Significantly at p < 0.05, ** Significantly at p < 0.01.

Table 2: Analysis of variance for the traits investigated in a wheat cultivar in response to salinity stress and gibberellic acid.

Sources of variation	df	Germination percentage	Germination rate	Radicle length	Hypocotyl length	Radicle dry weight	hypocotyl dry weight	Seedling fresh weight	Seedling dryweight
Salt stress	3	**	**	*	*	**	**	**	**
GA	2	*	*	*	**	*	*	*	**
S. stress ×GA	6	*	*	*	*	ns	ns	ns	ns

ns Non-significant, * Significantly at p < 0.05, ** Significantly at p < 0.01.

Table 3: Mean values of characters measured at germination and seedling growth stages of seed treated with SA and GA under NaCl stress in a wheat cultivar

Treatment	Germination		Radicle length (mm)	Hypocotyl length (mm)	Radicle dry weight (mg)	hypocotyl dry weight (mg)	Seedling fresh weight (mg)	Seedling dry weight (mg)
	percentage	Germination rate						
Salinity level mMol NaCl								
0	92.12 a	52.46 a	82.05 a	62.44 a	12.47 a	14.33 a	192.12 a	30.89 a
50	78.13 b	39.16 b	57.23 b	42.12 b	10.31 b	12.45 a	168.44 b	25.76 a
100	59.14 c	34.68 c	27.13 c	22.23 c	7.96 c	6.65 b	124.49 c	17.36 b
200	47.19 d	28.15 d	14.56 d	8.65 d	3.87 d	3.54 c	83.64 d	10.12 c
Salicylic acid m gr Lit								
0	90.24 b	42.12 b	90.12 b	89.90 b	7.16 a	8.23 c	165.92 c	24.29 c
1.5	95.16 a	39.24 c	112.45 a	110.11 a	8.12 a	11.44 a	184.66 a	28.36 a
3	95.04 a	45.72 a	88.23 b	90.11 b	6.52 a	10.02 b	174.39 b	26.12 b
4.5	88.28 c	47.29 a	70.24 c	80.05 c	6.54 a	10.11 b	143.23 d	19.18 d
Gibberellic acid mMol								
0	97.19 a	48.33 a	109.18 a	79.23 c	9.87 a	10.15 a	181.21 a	27.32 a
0.5	82.13 b	41.56 b	89.32 b	91.13 b	7.91 b	9.56 a	178.25 a	25.41 a
1	71.23 c	34.89 c	76.30 c	102.14 a	6.14 c	6.42 b	159.84 b	20.14 b

Means with similar letters in each column are not significantly different at 5 % level of probability (Duncan).

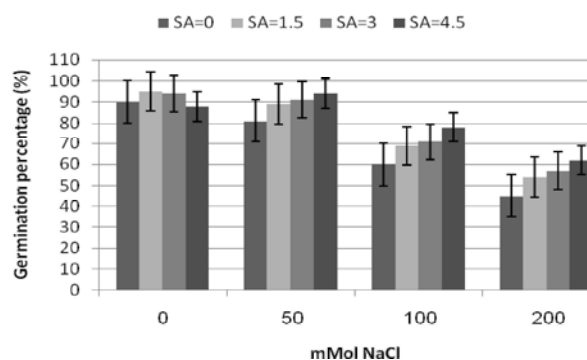


Fig. 1: Interactive effects of salinity and salicylic acid (SA) on germination percentage in wheat

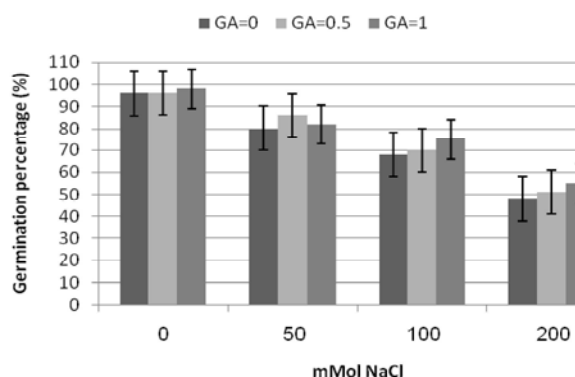


Fig. 2: Interactive effects of salinity and gibberellic acid (GA) on germination percentage in wheat

salinity condition. Salinity decreased germination percentage to about 19, 51 and 96% of controls (Table 3). SA increased germination percentage to 3 m gr Lit, but applying more decrease it. GA decreased germination percentage to about 38% in 1 m Mol than control (Table 3). Increasing of SA levels caused increases in germination under salinity condition. Germination percentage was significantly increased by SA under salinity conditions compared to non treatment of SA (Fig. 1). The seeds pretreated with GA also exhibited higher germination percentage in salinity treatments than untreated seeds (Fig. 2). Shakirova *et al.* [20], El-Tayeb [50] and Afzal [51] observed that SA treatment increases emergence percentage under salinity conditions. Growth regulators like GA3 are reported to alleviate the inhibitory effect of salinity on germination [37-40].

Germination Rate: The data (Table 1 and 2) indicated that there is significant difference in salinity levels, SA, GA and interaction effect of these two hormones and salinity. On average at 50, 100 and 200 mMol NaCl salinity germination rate was about 40, 60 and 93 %, respectively of control (Table 3). SA priming had effect on germination rate under both normal and saline conditions (Table 3 and Fig. 3). On the contrary, GA decreased germination rate to 41% in 1 m Mol treatment than zero treatment (Table 3). Under normal conditions, maximum germination rate (47.29) was achieved in those seeds which were primed with 4.5 mM salicylic acid as distilled water. When seeds were in 200 mMNaCl SA significantly improved germination (Fig. 3). However, Lowest germination rate was achieved in seeds primed with SA in100 or 200 mM NaCl (Fig. 3). Applying 3 and 4.5 m gr Lit SA, at all

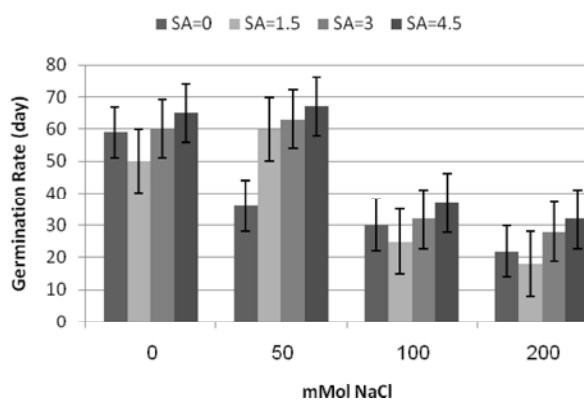


Fig. 3: Interactive effects of salinity and salicylic acid (SA) on germination rate in wheat

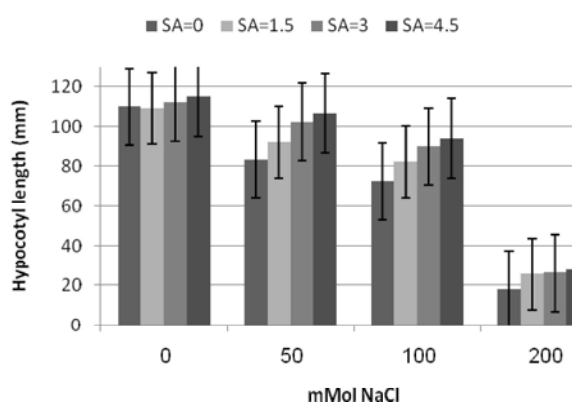


Fig. 5: Interactive effects of salinity and salicylic acid (SA) on hypocotyl length in wheat

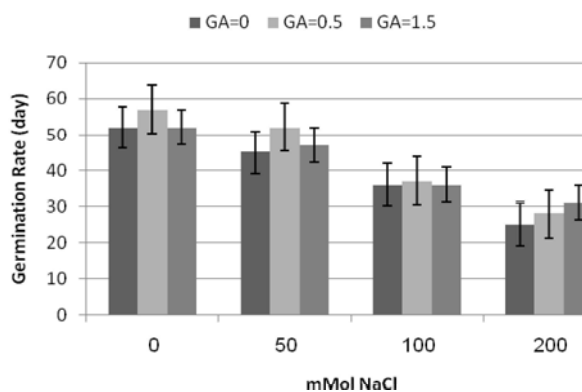


Fig. 4: Interactive effects of salinity and gibberellic acid (GA) on germination rate in wheat

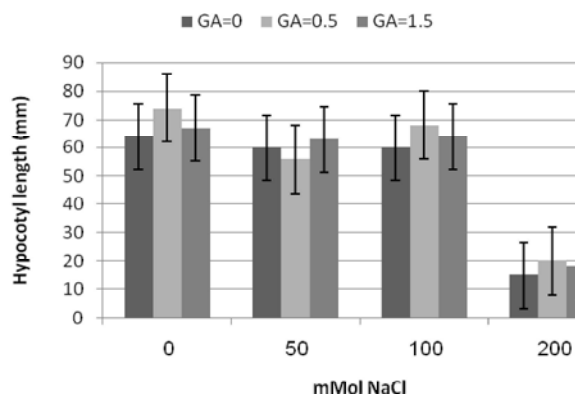


Fig. 6: Interactive effects of salinity and gibberellic acid (GA) on hypocotyl length in wheat

salinity levels increased germination rate to 40% than zero SA level. Regarding the effect of GA results from Fig. 4 indicated that the applications of GA (0.5 and 1 m Mol) were mitigated the negative effect of salinity levels on germination rate.

Rapid seed germination and stand establishment are critical factors to crop production under salt stress conditions particularly critical in semi-arid areas where favorable conditions in the seed zone may be brief. In many crop species, seed germination and early seedling growth are the most sensitive stages to salinity stress [34]. From present investigations, it is quite clear that seeds primed with various concentrations of salicylic acid proved to be effective in inducing salt tolerance at the seed germination stage in basil plants [52].

Radicle and Hypocotyl Length: These two traits were highly sensitive to salt with about 47% reduction even at the lowest concentration of 50 mMol NaCl (Table 4). With increasing salinity, radicle and hypocotyl length decreased progressively. The reason for reduced shoot

and root development may be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings. High salinity may inhibit root and shoot elongation due to slowing down the water uptake by the plant [53]. Priming with 1.5 m gr Lit SA showed maximum radicle and hypocotyl length and higher amount decreased both traits (Table 3). Priming with SA could not improve radicle length in all salinity levels (Table 1 and 3). However, treated seeds with SA produced the higher hypocotyl length in all salinity levels than untreated seeds (Fig. 5). The previous results were similar with that obtained by Zare *et al.* [54]. They stated that increasing in the application of GA3 led to an increase in shoot length of wheat plants under salinity stress, recently, Iqbal *et al.* [55] found that plant, height of *Cicer arietinum* was increased with GA3 treatment at 20 mg/l under NaCl at (0,8,12 and 16 ds/m). GA decreased radicle length, while increased hypocotyl length relatively (Table 3). The highest and the lowest significant values of hypocotyl length obtained by 1 m Mol GA and control treatments, respectively. Application of GA enhanced

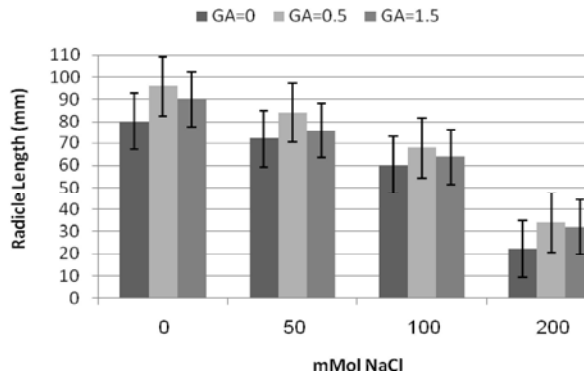


Fig. 7: Interactive effects of salinity and gibberellic acid (GA) on radicle length in wheat

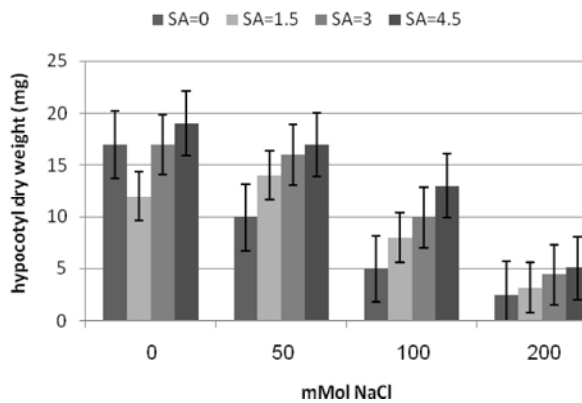


Fig. 8: Interactive effects of salinity and salicylic acid (SA) on hypocotyl dry weigh in wheat

radicle and hypocotyl length in all salinity levels compared to untreated seeds with treatment (Figs. 6 & 7). The treatment 0.5 m Mol GA gave the highest values in 50, 100 and 200 mMol NaCl. Patel and Pandey [56] on *Cassia montana* stated that root elongation was depressed by salinity levels (0.3,3.9,6.0,7.9,10.0,12.1 and 13.9 ds/m). Similarly Jaleel *et al.* [57] on *Catharanthus roseus* that root length was affected by salinity levels (15,30,45 and 60 mM).

Radicle and Hypocotyls Dry Weight: Variance analysis results of radicle and hypocotyls dry weight are shown in Table 1 and 2. According to Table 1 and 2, salinity ($P<0.01$) and gibberellic acid ($P<0.05$) influenced on radicle and hypocotyls dry weight significantly. Salicylic acid only affects on hypocotyls dry weight and did not affect on radicle dry weight (Table 1). Dry weight of radicle and hypocotyls decreased significantly under salinity condition compared with non salinity condition (Table 3). These results are similar to those reported by and Afzal [51] who found that dry weight was reduced by salt stress in wheat. Dry weight of hypocotyl was decreased due to

salinity stress but seedlings raised from seeds primed with SA improved dry weight of seedlings as compared to non treatment of SA under non salinity and salinity conditions (Fig. 8). Salt tolerance was increased in seeds subjected to the highest level of SA as indicated by hypocotyls dry weight. This may indicate that, treatment of seed with SA exhibited a significant increase in salt tolerance. These results was similar to the studies of El-Tayeb [50] reported that SA pretreatment increased dry weight in the stressed barley seedlings. In another work Gutierrez Coronado *et al.* [58], observed significant effect of SA on soybean increases in shoot growth and plant height. Khodary [59] reported that SA increased fresh and dry weight of shoot of salt stressed maize plants. Application of GA decreased both radicle and hypocotyls dry weight (Table 3).

Seedling Fresh and Dry Weight: Significant difference in seedling fresh and dry weight between salinity levels, salicylic acid and gibberellic acid was observed in this study (Table 1 and 2). On average at 50, 100 and 200 mMol NaCl seedling fresh weight were about 50, 100 and 135 %, respectively of the control (Table 3). Seedling dry weight at 50, 100 and 200 mMol NaC was reduced to about 70, 140 and 190 %, respectively of the control (Table 3). Priming with 1.5 m gr Lit SA showed maximum radicle and hypocotyl length and higher amount decreased both traits (Table 3). GA decreased seedling fresh and dry weight to about 20 and 35% in 1 m Mol than control (Table 3).

CONCLUSION

Increasing of SA and GA Levels Caused Increases in Germination under Salinity Condition: Germination percentage was significantly increased by SA and GA under salinity conditions compared to non treatment of them. SA priming had effect on germination rate under both normal and saline conditions. However, GA decreased germination rate in 1 m Mol treatment than zero treatment. When seeds were in the highest NaCl level SA significantly improved germination. Application of GA improved the negative effect of salinity levels on germination rate. Priming with SA could not improve radicle length in all salinity levels. However, treated seeds with SA produced the higher hypocotyl length in all salinity levels than untreated seeds. Application of GA enhanced radicle and hypocotyl length in all salinity levels compared to untreated seeds with treatment. Salt tolerance was increased in seeds subjected to the highest level of SA as indicated by hypocotyls dry weight. This may indicate that, treatment of seed with SA exhibited a significant increase in salt tolerance.

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