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Research Article
Germination and Physiological Traits to Ascertain the Ability of Hormonal Priming to Improve Salinity Tolerance in *Sorghum bicolor*

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Abstract

Background and Objective: Salinity is the highest environmental factor that affects seedling stages negatively, thus reduces plant development and production. The objective of this study was to explore the effects of gibberellic acid (GA3) pretreatment on sorghum (*Sorghum bicolor*) seed germination under salt stress. **Materials and Methods:** Seeds were soaked in a GA3 solution (50, 100 and 150 ppm in addition to a control), dried using a drypaper, transferred to Petri dishes and treated with sodium chloride (NaCl) solutions at different concentrations (0, 2000 and 4000 ppm). Germination traits, including the length of shoot and root, the ratio of shoot to root, the weight of shoot and root, germination percentage, germination energy, germination rate and germination time and physiological traits index's, including germination stress tolerance, seedling vigor, shoot and root length stress, fresh weight stress for shoot and root and germination speed were documented. Data was analyzed by two-way ANOVA. Treatments were arranged in a randomized complete block design, in three replications using SAS. **Results:** Salt stress induced by the NaCl solution significantly affected the germination and physiological traits ($p<0.01$). Priming seeds with GA3 (50, 100 and 150 ppm) showed a significant effect on these traits. Salt stress significantly decreased most traits, except for the mean germination time, in the absence of GA3. Increasing NaCl concentration decreased the germination and physiological traits; however, GA3 induced these traits only at the moderate salinity level (2000 ppm), whereas, no significant difference between primed and nonprimed seeds was detected at the high salinity level (4000 ppm). **Conclusion:** Priming sorghum seeds with GA3 play an important role in the plant response to mild salt stress.

Key words: Ameliorate, gibberellic acid, physiological traits, salinity, *Sorghum bicolor*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Water resources in Jordan is limited which causes this country to the third poorest country in the world in response to water resources, which have been estimated to a decline to <90 m^3 per capita annually by 2025[1]. The shortage in water resources will result in competition among different sectors in the use of water. Agriculture is a major sector that will be influenced by water scarcity due to shift in water use from agricultural to domestic uses associated with the increasing population. Water shortage will result in an increased use of low quality water, particularly saline water, to support the increasing demand for irrigation[2,3]. Improper irrigation management and high salinity of irrigation water are the major causes of salinity in soil[4].

Salinity is the main environmental factor creating an osmotic pressure[5] that reduces the production of plants due to poisonous effects of ions found in saline solutions, particularly in arid area, where annual precipitation is limited with high temperature which increase crop evapotranspiration and caused crop production mainly depend on irrigation[6]. Growth establishment after germination is the greatest sensitive stage to adverse environment condition[7]. Seed reserves and plant development decrease with increasing salinity[7,8]. Several approaches have been used to mitigate the antagonistic effects of saline water[9].

The first approach is to improve cultivars tolerant to salinity through breeding and genetic engineering or by budding onto accepting root stocks that tolerate saline water. The second approach is to apply antioxidants. The third approach is to develop the plant environment by removing salts from root areas, applying certain fertilizers to plants, using additives such as silicon, inoculating with bacteria and mycorrhizal fungi and priming seeds and young seedlings.

Priming methods assist rapid germination by enhancing some metabolic processes vital for germination and contribute to the establishment of a successful crop, particularly under adverse environmental conditions. Previous study has stated that seed priming decreases the adverse effects of NaCl on plants[10].

Different priming methods used to progress the performance, growth and production of various crops. Hormonal priming refers to seed pretreatment with different hormones, such as kinetin, salicylic acid (SA) and ascorbate, which enhance the development of seedlings of various crops. Several studies have reported a significant enhancement in crop production in salt-affected soils in reaction to hormonal priming[6,11,12]. Soaking the seeds with ascorbic acid and thiamin counter acts the negative effects of NaCl on wheat seedlings by suppressing salt stress[13]. Priming wheat seeds with SA reduces germination time by 50%, increases germination percentage and increases the biomass of shoots and roots under favorable and non-favorable climate condition[11]. The useful character of hormones in alleviating the effect of salinity on crops has been investigated[14].

Gibberellic acid (GA3) is a phytohormone that regulates many progressive processes in higher plants, includes germination, growth and development[15]. Gibberellic acid reduce the negative effects of salt during the growth stages after germination of Arabidopsis (*Arabidopsis thaliana*) complemented by a rise in salicylic acid, which is associated with plant responses to harmful environmental condition[16].

Sorghum (*Sorghum bicolor*) is essential crop in most countries. It is rich in protein, vitamins and minerals and has the capability to grown under rain-fed and irrigated areas. It has been characterized to be moderately tolerant to salinity[17]. Determining the improving effects of gibberellic acid by germinating sorghum bicolor seeds under saline water at various concentrations after priming with gibberellic acid was the main goal of this study.

MATERIAL AND METHODS

Priming procedure: A germination experiment was made in 2016 at Food Technology Laboratory, Department of Nutrition and Food Processing, Al-Balqa Applied University. Sorghum seeds were sterilized with sodium hypochlorite solution and then occupied in three concentrations of gibberellic acid (50, 100 and 150 ppm) in addition to a control. Soaked seeds were dried to retain to original weight[18].

Salinity induction: Three replicates of 20 primed seeds in their respective test hormonal solutions were grown in dishes that contained a filter paper. Ten milliliters of sodium chloride (NaCl) (analytical grade) solutions (0, 2000 and 4000 ppm) were added for each dishes. The filter paper was substituted every day to avoid salt buildup[19]. The seeds were endorsed to germinate at 26$\pm$1°C for 14 days. The seeds were considered as germinated seeds when radicle reaches 2 mm long.

Experimental layout: The NaCl concentration (0, 2000 and 4000 ppm) and GA concentration (0, 50, 100 and 150 ppm) were combined to make 12 treatments in this experiment which repeated three times. These treatments were arranged in a RCBD, in three replications with 20 seeds per replicate.

Germination parameter measurements

Morphological characters: The length of shoot (SL) and root (RL) were taken daily after germination, the ratio of shoot to root and the weight of shoot (SW) and weight of root (RW) were taken after 14 days. Abnormal seedlings (short, thick and spirally) shoots and roots were discarded from counting[20]. Germination percentage (GP) (documented every day for two weeks) and allometric data (shoot length:root length percentage) were respectively calculated using the equations below[21]:

$$\text{Germination percentage} = \frac{\text{No. of germinated seeds}}{\text{Total number of seeds}} \times 100 \quad (1)$$

$$\text{Allometry} = \frac{\text{Shoot length}}{\text{Root length}} \quad (2)$$

Germination rate was measured using mean germination time[22]:

$$\text{Mean germination time (MGT)} = \sum \text{Nidi}/\text{n} \quad (3)$$

where, n is the Number of seeds germinated after two weeks, Ni is the number of seed germinated on day di and di is the day through incubation period.

Germination energy (GE), germination speed index (GSI) and relative germination rate (RGR) were also calculated[23]:

$$\text{Germination energy} = \frac{a}{b} \quad (4)$$

$$\text{Germination speed index} = \sum \text{Gt}/\text{Dt} \quad (5)$$

$$\text{Relative germination rate} = c/d \quad (6)$$

where, a is the total germinated seed in NaCl concentration in fourth days, b is the total number of seeds to germinate, Gt is the germinated seed in t days, Dt is the number of germination days corresponding, c is the germinated percentage of treatment and d is the germination percentage of control.

Physiological traits: After 14 days the promptness index (PI) and various stress tolerance indexes includes germination (GSTI), shoot (PHSI) (%), root (RLSI) (%), shoot fresh weight (SFSI) (%), root fresh weight (RFSI) (%) and seedling vigor index (SVI) (%) were estimated using the following equations, as previously described[24].

$$\text{Promptness index (PI)} = \text{nd2(1)+nd4(0.75)+nd6(0.5)+nd8(0.25)} \quad (7)$$

where, nd2, nd4, nd6 and nd8 is the number of seeds germinated on the 2nd, 4th, 6th and 8th day, respectively.

$$\text{Germination stress tolerence index (\%)} = \frac{\text{PI of stressed seeds}}{\text{PI control seeds}} \times 100 \quad (8)$$

$$\text{Shoot length stress index (\%)} = \frac{\text{Shoot length of stressed plant}}{\text{Shoot length of control plants}} \times 100 \quad (9)$$

$$\text{Root length stress index(\%)} = \frac{\text{Root length of stressed plant}}{\text{Root length of control plants}} \times 100 \quad (10)$$

$$\text{Shoot fresh weight stress index (\%)} = \frac{\text{Shoot fresh weight of stressed plant}}{\text{Shoot fresh weight of control plants}} \times 100 \quad (11)$$

$$\text{Root fresh weight stress index (\%)} = \frac{\text{Root fresh weight of stressed plant}}{\text{Root fresh weight of control plants}} \times 100 \quad (12)$$

$$\text{Seedling vigour index} = \text{Germination percentage} \times \text{seedling length} \quad (13)$$

$$\text{Seedling length} = \text{Shoot length+root length} \quad (14)$$

Statistical measurements: All measurements were exposed to statistical analyses with two-way ANOVA for the factorial experiment in a randomized complete block design using SAS software version 9 and means were compared using the least significant difference test at a level of confidence of $p<0.01$[25].

RESULTS

A significant two-way interaction (hormonal priming with GA3 and NaCl stress) was detected ($p<0.01$) for all investigated traits except the shoot:root ratio (Table 1, 2).

Effect of priming techniques with GA3 on germination percentage and seedling development: The GA3 acts as important regulatory role in plant cells. Under control conditions, the primed seeds showed improvement in most of the studied traits. Seed soaking with 150 ppm of GA3 increased the length of shoot and roots, percentage of shoot to roots, shoot and root weight, germination percentage, germination energy and relative germination rate by 15, 12, 3, 11, 12, 18, 29 and 18%, respectively and decreased the mean germination time by 11%.

Table 1: Analyses of variance for morphological growth parameters of sorghum seeds primed with gibberellic acid at various concentrations and germinated using NaCl solutions

Source of variation	Shoot length (cm)	Root length (cm)	Shoot:root ratio	Shoot weight (g)	Root weight (g)	Germination (%)	Germination energy	Relative germination rate	Mean germination time
Salinity (B)	68.08 **	70.14**	0.047	0.89**	0.34**	0.23**	6786.11**	0.33**	18.77**
Gibberellic acid (A)	3.72 **	4.84**	0.0006	0.02**	0.02**	0.03**	711.8**	0.05**	1.45**
A*B	1.3 **	3.5**	0.0004	0.005**	0.006**	0.007**	291.6**	0.01**	0.92**
Error	0.38	0.54	0.006	0.0013	0.0008	0.002	34.7	0.003	0.23
C.V (%)	7.82	7.08	10.53	2.66	4.54	5.45	14.38	5.46	10.33

**Indicate significant difference at 1% probability level

Table 2: Analyses of variance for physiological traits of sorghum seeds primed with gibberellic acid at various concentrations and germinated using NaCl solutions

Source of variation	Germination stress tolerance index (%)	Seedling vigor index (%)	Plant height stress index (%)	Root length stress index (%)	Shoot fresh weight stress index (%)	Root fresh weight stress index (%)	Germination speed index (%)	Promptness Index (%)
Salinity (B)	6601**	464**	7553**	5071**	3580**	6319**	74.1**	26.44**
Gibberellic acid (A)	1894**	50**	413**	350**	79**	400**	7.44**	7.59**
A*B	442**	13**	145**	137**	20.6**	111**	1.76**	1.77**
Error	134.5	1.2	41.9	39	5.25	14.7	0.51	0.53
C.V (%)	13.86	7.18	7.82	7.08	2.66	4.54	13.76	13.86

**Indicate significant difference at 1% probability level

Further, this treatment increased the germination stress tolerance index, seedling vigor index, shoot length stress index, root length stress index, shoot fresh weight stress index, root fresh weight stress index and germination speed index by 20, 34, 15, 12, 11, 12 and 26%, respectively (Fig. 1-4).

Effect of NaCl on seed germination and seedling growth: With the exception of the mean germination time, the shoot length, root length, shoot:root ratio, shoot weight, root weight, germination percentage, germination energy and relative germination rate significantly decreased with increasing salinity levels ($p<0.01$) (Fig. 1, 2). At the highest salinity level, the reductions were noted to be 47, 38, 14, 31, 43, 30, 73 and 30%, respectively, whereas, the mean germination time increased by 73% at 4000 ppm of NaCl. These results propose that shoot and root growth are less tolerance to salinity than seed germination.

Physiological indices of seeds (primed and non-primed) were greatly affected by high NaCl concentration (4000 ppm). Physiological indices were also reduced at 2000 and 4000 ppm of NaCl. At 4000 ppm salinity level, the reductions were noted to be 43, 60, 47, 38, 31, 43 and 65% for the germination stress tolerance index, seedling vigor index, shoot length stress index, root length stress index, shoot fresh weight stress index, root fresh weight stress index and germination speed index, respectively (Fig. 3, 4).

Interaction between NaCl and GA3 on the percentage of germination and seedling development: The effects of GA3 on germination percentage and the growth of seedling after germination in the lack and presence of NaCl were explored to observe whether GA3 is responsible in preventing the inhibitory roles of NaCl.

The decrease in germination and physiological traits at 2000 ppm of NaCl was more pronounced in the seedlings developed from nonprime seeds than in the seedlings developed from the GA3-primed seeds. The application of different GA3 concentrations increased germination and physiological traits compared with seedlings developed from non-primed seeds.

At the moderate salinity level (2000 ppm), increased GA3 concentration decreased the adverse effects of salinity. Further, seed soaking with 150 ppm of GA3 increased the shoot length, root length, root weight, germination percentage and relative germination rate by 2, 11, 1, 4 and 4%, respectively, when germinated under the moderate salinity level (2000 ppm).

However, other germination traits slightly decreased for seeds grown under the moderate salinity level, even when primed with a high GA3 concentration. The reduction percentage ranged from 0% for the germination energy and mean germination time to 17% for the shoot weight, suggesting that GA3 (150 ppm) alleviates the negative effects of NaCl in sorghum.

To screen the tolerance of plants to salinity physiological indices were calculated. Seed priming with GA3 (150 ppm) and germinating under the moderate salinity level (2000 ppm) were increases by 5, 11, 2, 11, 1 and 3% for the germination stress tolerance index, seedling vigor index, shoot length stress index, root length stress index, root fresh weight stress index and germination speed index, respectively.

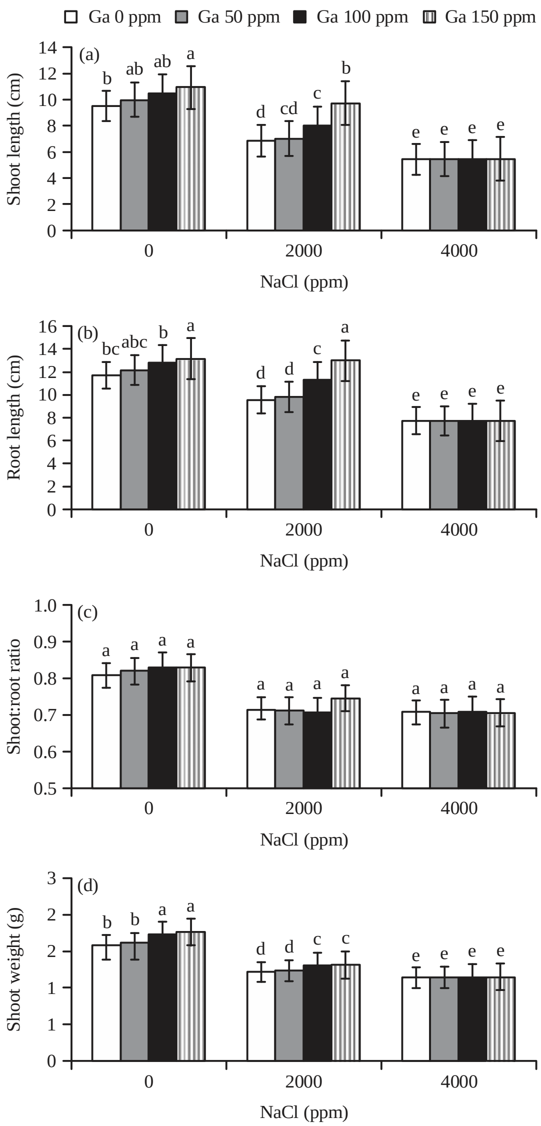

Fig. 1(a-d): Effect of seed priming with gibberellic acid on shoot and root length, shoot: root ratio and shoot weight of sorghum after germinated under salinity stress, bars (indicate for standard error) with the same letters are not significantly different at $p<0.05$

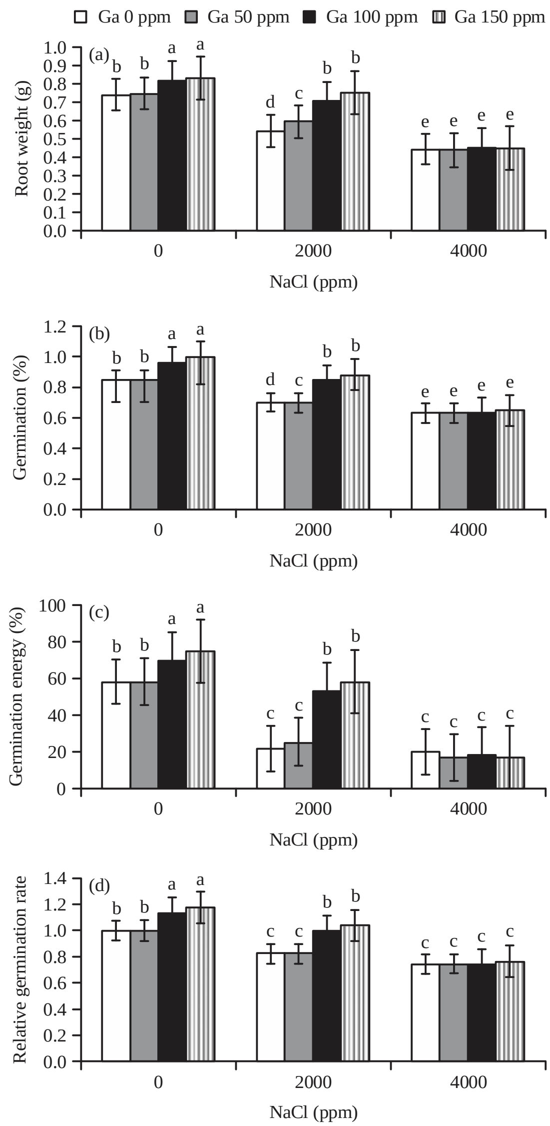

Fig. 2(a-d): Effect of seed priming with gibberellic acid on root weight, germination percentage, germination energy and relative germination rate in sorghum under salinity stress, bars (indicate for standard error) with the same letters are not significantly different at $p<0.05$

Germination percentage and other germination traits were not affected by priming with different concentrations of GA3 in reaction to the highest NaCl concentration (4000 ppm). The pattern of reaction to the higher NaCl concentration (4000 ppm) was the same for pretreated and no-treated seed with GA3, with high reductions in the germination percentage and other germination traits were recorded in reaction to this concentration, suggesting that the NaCl concentration (4000 ppm) induced reductions in the percentage of germination and the development of seedling after germination, which were insensitive to GA3.

Shoot and root length, percentage of shoot to root, shoot and root weight, germination percentage, germination energy and relative germination rate reduced in the non-primed seeds by 43, 34, 13, 28, 41, 25, 66 and 25%, respectively. Further, approximately the same percent reduction was observed after priming with 150 ppm of GA3: 42, 34, 13, 28, 39, 24, 71 and 24%, respectively (Fig. 1, 2). This result suggests that GA3 does not alleviate the adverse role of salt on sorghum growth under higher salinity.

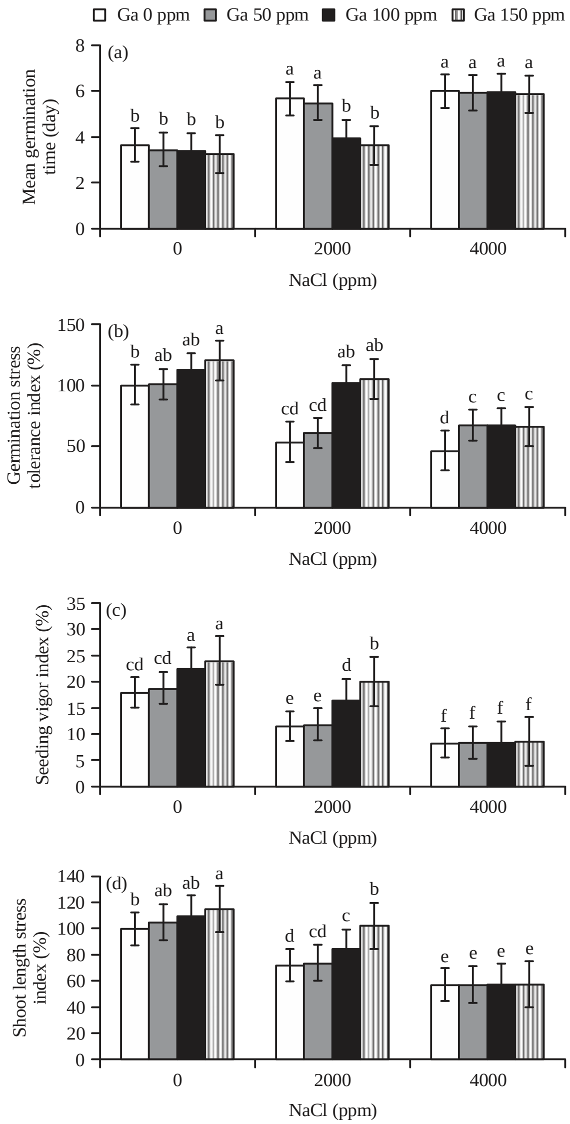

Fig. 3(a-d): Effect of seed priming with gibberellic acid on, mean germination time, germination stress tolerance index and shoot length stress index of sorghum after germinated under salinity stress, bars (indicate for standard error) with the same letters are not significantly different at $p<0.05$

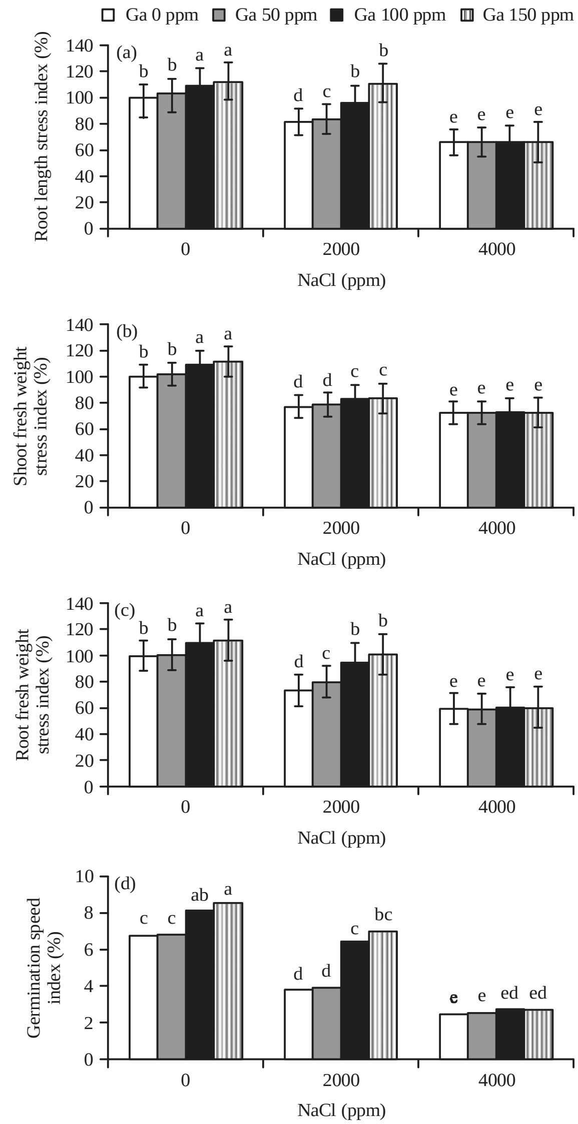

Fig. 4(a-d): Effect of seed priming with gibberellic acid on root length, shoot and root fresh weight and germination speed stress indexes of sorghum after germinated under salinity stress, bars (indicate for standard error) with the same letters are not significantly different at $p<0.05$

The physiological indices of sorghum seeds (primed and non-primed) were greatly affected by the high NaCl concentration (4000 ppm). The germination stress tolerance index, seedling vigor index, plant height stress index, root length stress index, shoot fresh weight stress index, root fresh weight stress index and germination speed index were reduced in the nonprimed seeds by 46, 36, 28,18, 23, 27 and 44%, respectively (Fig. 3, 4). The same reduction was originated in the primed seeds even at higher concentrations of GA3.

In this study, positive highly significant correlations ($p<0.001$) were observed between shoot length, root length, shoot weight, root weight, germination energy, relative germination rate, germination stress tolerance index, seedling vigor, shoot and root length stress index, shoot and root fresh weight stress index and germination speed index, whereas a negative correlation was observed the mean germination time and other traits investigated (Table 3), indicating functional connections between these traits.

Table 3: Correlation analysis between germination and physiological traits of sorghum bicolor seedlings exposed to salinity after priming the seeds with gibberellic acid

	SL	RL	SW	RW	GP	GE	RGR	MGT	GSTI	SVI	PHSI	RLSI	SFWSI	RFWSI	GSI
SL	1	0.91	0.89	0.95	0.91	0.92	0.91	-0.89	0.79	0.96	0.99	0.91	0.89	0.95	0.91
RL		1	0.82	0.94	0.89	0.90	0.89	-0.89	0.78	0.95	0.91	0.99	0.82	0.94	0.94
SW			1	0.88	0.85	0.88	0.85	-0.83	0.78	0.89	0.89	0.82	0.99	0.88	0.85
RW				1	0.92	0.93	0.92	-0.90	0.84	0.96	0.95	0.94	0.88	0.99	0.94
GP					1	0.93	0.99	-0.86	0.84	0.97	0.91	0.89	0.85	0.92	0.94
GE						1	0.93	-0.96	0.89	0.95	0.91	0.90	0.88	0.93	0.97
RGR							1	-0.86	0.84	0.98	0.91	0.89	0.85	0.92	0.94
MGT								1	-0.88	-0.90	-0.89	-0.89	-0.83	-0.90	-0.95
GSTI									1	0.84	0.79	0.77	0.78	0.84	0.85
SVI										1	0.96	0.95	0.88	0.96	0.96
PHSI											1	0.91	0.88	0.95	0.92
RLSI												1	0.82	0.94	0.94
SFWSI													1	0.88	0.86
RFWSI														1	0.94
GSI															1

SL: Length of shoot, RL: Length of root, SW: Weight of shoot, RW: Weight of root, GP: Germination percentage, GE: Germination energy, RGR: Relative germination rate, MGT: Mean germination time, GSTI: Germination stress tolerance index, SVI: Seedling vigor index, PHSI: Plant hormones shoot index, RLSI: Root length stress index, SFWSI: Shoot fresh weight stress index (%), RFWSI: Root fresh weight stress index, (GSI: Germination speed index

DISCUSSION

Germination stage is the most serious stage which determines the successes of plant establishment under adverse environment conditions[26]. The application of salt will result in the lowering of the water potential of growth media, thereby causing a reduction in water uptake and the buildup of poisonous ions. This causes an imbalance in the uptake of necessary elements[27] and a change in metabolic activities in seeds through the dehydration of proteins and disorder in enzymatic functions, which can negatively affect seed germination[28,29] . The poisonous effect of sodium and chloride ions comes from the disturbances in plant metabolism, which reduces seed germination indices[30] and limits the growth of rape seeds[31].

It was shown from this work that NaCl reduced the germination and physiological behaviors of sorghum seedlings. The decreased germination percentage, root and shoot lengths, germination speed, germination energy, germination index and seedling vigor index due to increasing salinity in this study were consistent with those reported previously[32-36].

The reduced seed germination traits at high salt concentrations might principally be because of osmotic stress[5], which affects the root zone of plants and therefore might alter physiological processes, subsequently reduced plant growth. This agrees with the other observations where salinity stress negatively affects the rate of starch remobilization by decreasing α-amylase activity[37] .

The supplementation of GA3 though seed priming can ameliorate salt toxicity and increase the seed germination, root and shoot lengths and seedling biomass and decrease the mean germination time of sorghum seeds.

Physiological traits, which help evaluate higher stress tolerance in plants[38] (germination tolerance, seedling vigor, shoot and root stress tolerance, fresh weight of shoot and root tolerance and germination speed), were improved after seed soaking with GA3 and germination using saline water.

This alleviation might be dependent on the GA3 concentration as well as the NaCl concentration in growth media. Furthermore, seed soaking with a high GA3 concentration (150 ppm) is more beneficial for plant growth. However, GA3 cannot reduce the adverse effects of high salinity (4000 ppm).

Seed priming is a technique that can be applied to counteract the adverse effects of environmental stress during germination and growth[39,40]. The process of germination and activities of antioxidant enzymes in primed seeds are significantly improved compared with nonprime seeds[41]. Hormone priming has been a frequently used method to progress germination in stressful environments[42-46]. Priming seeds influences the plant reaction to drought and salinity.

Acetyl salicylic acid and SA priming result in improvement in germination and growth of pepper seedling (*Capscum annum* L.) under high salinity[47].

Seed soaking with GA3 increases the germination of rye (*Secale montanum*) under drought[48]. In monocotyledons, GA3 enhance the synthesis of hydrolytic enzymes responsible for the hydrolysis of stored substrates necessary for seedling tissue synthesis[8]. This study exposed that when seeds are primed with GA3 and germinated in normal and stress conditions (2000 ppm NaCl), the seedlings exhibited better root and shoot lengths and higher seedling biomass accumulation, indicating the plant growth-promoting ability of GA3.

CONCLUSION

This study demonstrated that salinity stress has antagonistic effects on the germinated seeds and the development of seedling after germination in sorghum. Seed priming with GA3 enhanced the growth of sorghum under normal conditions and ameliorated the harmful effects of moderate salinity level (2000 ppm) by increasing the length of shoot and root, the weight of shoot and root, germination percentage, germination energy and relative germination rate and decreasing the mean germination time. Priming with 150 ppm GA3 was the most effective treatment.

Physiological traits index's also improved under saline conditions after seed priming with GA3 and germinating under the same salinity.

SIGNIFICANCE STATEMENTS

This study discovers the effects of GA3 priming after seed germination under saline water at various concentrations that can be beneficial for decreasing the toxic effects of sodium and chloride ions. Seed priming with GA3 improved the performance of sorghum under normal conditions and ameliorated the negative effects of moderate salinity level. This study will help the researcher to uncover the importance of GA3 as priming agent in decreasing the negative effects of salinity on plants that many researchers were not able to explore.

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