Response of some sorghum varieties to GA$_3$ concentrations under different salt compositions

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ABSTRACT

Germination is a very sensitive stage for the harmful effect of salinity stress. Plants respond differently to different types of salt. Gibberellic acid (GA$_3$) is the most important hormone to mitigate salt stress. A controlled germination study was conducted to evaluate the impact of different salts (0, 150 mM NaCl, and 150 mM Na$_2$SO$_4$) and different GA$_3$ concentrations (0, 50, 100, 250, and 300 mg L$^{-1}$) on the germination of the two most used Sudanese sorghum (Sorghum bicolor [L.] Moench) varieties Wadahmed and Tabat. The studied parameters were seed water uptake after 8 h imbibition, germination percentage, germination rate, shoot and root length, shoot and root fresh weight, shoot and root dry weight, and seed vigor index. Results showed that Na$_2$SO$_4$ reduced germination percentage, germination rate, shoot length, root length, root fresh weight, shoot dry weight, root dry weight, and seed vigor index by 17.0%, 50.0%, 86.99%, 89.03%, 76.8%, 54.5%, 66.6%, and 89.7%, respectively, when compared with the non-saline treatment. The maximum values for seed water uptake, germination percentage, germination rate, shoot length, and seed vigor index were achieved at 100 mg L$^{-1}$ GA$_3$. The Na$_2$SO$_4$ salt had a more harmful effect than NaCl. The 100 mg L$^{-1}$ GA$_3$ concentration can be recommended to improve seed water uptake, germination parameters, and seedling growth under salt stress in the two Sudanese sorghum varieties (Wadahmed and Tabat). ‘Wadahmed’ was more tolerant to salinity stress than ‘Tabat’ at the germination stage.

Key words: Germination, gibberellic acid, NaCl, Na$_2$SO$_4$, Sorghum bicolor.

INTRODUCTION

Sorghum (Sorghum bicolor [L.] Moench) is one of the five most important cereal crops grown worldwide (FAO/ITPS, 2015). Sorghum is currently grown in 116 countries (FAO, 2020). The potential uses of sorghum include food (grain), feed (grain and biomass), fuel (ethanol production), fiber (paper), fermentation (methane production), and fertilizer (use of organic byproducts) (Machado and Serralheiro, 2017). It is also considered a principal source of energy, protein, vitamins, and minerals for millions of people in semi-arid regions (Jacob et al., 2013).

World agriculture is facing many challenges such as producing 70% more food for the growing population; however, crop productivity is not increasing at the same rate as the demand for food. Lower productivity in most cases is attributed to various abiotic stresses. Salt stress is a major abiotic stress that reduces agricultural productivity (Hu et al., 2018). Salt stress (stress caused by excess sodium chloride and sodium sulphate) affects 20% of the total land area and more than 50% of the irrigated agricultural area. It is believed that 30% of agricultural land could be lost within the next 25 yr due to salt stress, a total loss that could reach 50% by 2050 (Hu et al., 2019). Mineral salts are found in all irrigation water, but the
composition and concentration of dissolved salts differs according to the water source and time of year. Since salts can damage plant growth, it is necessary for water managers to identify the concentration and composition of irrigation water at the different times of the year (Grattan, 2006).

Efforts have been made to improve the salinity tolerance of a variety of crops by selective breeding techniques, but the effective development of such salt-resistant plants is time-consuming and commercial success is limited. This requires the use of some other simple and cost-effective methods to screen and/or develop salinity tolerant crops. Different strategies are used to maximize plant growth under saline conditions. One strategy is to develop salt-tolerant genotypes. However, it is time-consuming, laborious, and highly dependent on existing genetic variability (Zhang et al., 2005). Priming with exogenous plant growth regulators is a strategy that might increase the performance of germination and emergence under stress conditions (Ghobani et al., 2011). Priming is a process in which seeds are treated with a test chemical under controlled conditions. This activates some pre-germination metabolic processes (physiological and chemical) followed by re-drying for a short time until seeds gain their initial weight before sowing. Several chemicals, including synthetic plant hormones, have been used for seed priming (Afzal et al., 2012). However, the effectiveness of these different priming agents varies under different stresses and between crop species. Plant hormones are active members of the signal cascade involved in the induction of plant stress responses. They have gained much attention worldwide. Gibberellic acid (GA3) is a plant hormone that can increase seed germination and seedling growth of crop plants under salt stress (Tsegay and Andargie, 2018).

Sorghum is the main food for most of the Sudanese population, especially in rural areas. These rural areas are subjected to salts under different concentrations and compositions. Most studies focus on the harmful effect of NaCl concentrations in sorghum. However, no research has been conducted to ensure which salt composition has the most impact on sorghum seed during the germination period; this stage has proven to be more sensitive to salt stress than other growth stages. The majority of sorghum areas in Sudan are cultivated with the Wadahmed and Tabat varieties. Therefore, we investigated how these two varieties can tolerate the same concentration of two different salts, NaCl and Na2SO4, and confirm which salt is more harmful for seed germination and early seedling growth of these varieties. The most abundant salts in the area cultivated with sorghum in Sudan are NaCl and Na2SO4. Early seedling growth is crucial for plant establishment and final crop yield. Mitigating methods should be developed to improve seedling growth under stress conditions. We also tried to reduce the effects of both salts by using a wide range of GA3 concentrations (0 to 300 mg L⁻¹).

**MATERIALS AND METHODS**

The sorghum (Sorghum bicolor [L.] Moench) ‘Wadahmed’ and ‘Tabat’ seeds used in this study were provided by the Sudanese Ministry of Agriculture. Selected seeds were uniform in size, shape, and color. Seeds were less than 12-month old and previously stored in paper bags under laboratory conditions (RH 40% to 60% at 15 to 20 °C) to maintain good germinability. Seeds were surface-sterilized with 1% HgCl₂ solution for 3 min. Seed germination was tested according to the International Seed Testing Association (ISTA) rules before starting the experiment.

**Salinity and seed treatments**

The study was conducted in the Joint International Research Laboratory of Agriculture and Agri-Product Safety, Yangzhou University, Yangzhou, China, in 2019. The experimental design was a factorial design with three factors: variety (‘Wadahmed’ and ‘Tabat’), salt (NaCl, Na2SO4, and distilled water as control), and gibberellic acid (GA3) concentration (four concentrations and a control without GA3), arranged in a completely randomized design with three replicates for each treatment. The salt factor included 0, 150 mM NaCl, and 150 mM Na2SO4. The hormone factor included 0, 50, 100, 250, and 300 mg GA3 L⁻¹. Each petri dish contained 20 seeds soaked in the target hormone solutions at room temperature for 12 h. When seed soaking was completed, solutions were discarded and seeds were re-dried to approximately original weight with forced air under shade for 48 h (Afzal et al., 2005). Twenty seeds of each replicate from each treatment were placed in a 9-cm petri dish with two sheets of filter paper (Hangzhou Special Paper Factory, Xinxing, Zhejiang Province, China) moistened with either distilled water or 5 mL NaCl or Na2SO4 solutions. Finally, seeds were incubated at 30 °C in complete darkness for 10 d. The relative humidity inside the incubation chamber was adjusted between 60% and 70% and evaporation rates were 7.6 mm d⁻¹.
Evaluations

Seed water uptake. The seeds in each petri dish were weighed before soaking and seed water uptake was measured 8 h after the start of water imbibition. To determine seed water uptake, seeds were carefully removed, drained, and quickly blotted with absorbent paper, weighed, and placed again in petri dishes. The environmental condition for seed water uptake was the same as for seed germination. Seed water uptake (%) was determined as \[ \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100 \]. Final weight is seed weight after water imbibition and initial weight is the initial seed weight before the start of water imbibition.

Germination parameters. The number of germinated seeds in each petri dish was counted every 24 h. Seeds were considered to be germinated when the radicles reached approximately 2 mm out of the seed coat (Patanè et al., 2009). After 2 d the filter paper was changed a further 2 mL saline solution or water was added to each dish. The germination percentage was recorded when no further germination occurred in the non-saline treatment. The germination rate was calculated by the formula \( \Sigma G/t \), where \( G \) is the percentage of germinated seeds and \( t \) is the total time of germination (d) (Timson, 1965).

Shoot and root growth. Five seedlings were randomly chosen from each petri dish after the germination test. Roots and shoots were excised from the seedling and root and shoot length was measured with a vernier caliper. Shoots and roots were weighed to determine fresh weight. Shoot and root dry weights were measured after drying the samples in a forced-air dryer at 80 ºC for 48 h.

Seed vigor index. After determining the germination percentage, the seed vigor index (VI) was calculated by the formula \( \text{VI} = (\text{shoot length} + \text{root length}) \times \text{germination percentage} \) (Tavsanoglu et al., 2015).

Data analyses

The experimental design was factorial with three factors (variety, GA\(_3\) concentration, salt type) arranged in a completely randomized design with three replicates. Data for each variable were subjected to ANOVA with the statistical package DPS 7.05 for Windows (Tang and Feng, 1997); according to the design, means were separated by the LSD test (\( P \leq 0.05 \)) when \( F \) values were significant.

RESULTS

The variety factor significantly affected all the study parameters, except root fresh weight and root and shoot dry weights. All the measurements were significantly affected by salinity. The GA\(_3\) concentrations, taken separately, had nonsignificant effects on shoot and root fresh weights and shoot and root dry weights. The interaction between two factors was nonsignificant for most of the parameters. However, interaction among the three experimental factors was significant only for seed water uptake after 8 h imbibition (Tables 1 and 2).

| Source of variation | Water uptake | Germination percentage | Germination rate | Shoot length | Root length |
|---------------------|--------------|------------------------|------------------|--------------|-------------|
|                     | MS | F value | MS | F value | MS | F value | MS | F value | MS | F value |
| Variety (V)         | 5374.0 | 12.12* | 1033.6 | 5.97* | 50.80 | 14.48* | 2.4 | 8.30* | 13.20 | 14.20* |
| Salinity (S)        | 6130.9 | 13.83* | 2151.9 | 12.40* | 463.40 | 132.20* | 503.8 | 1754.80* | 299.96 | 324.70* |
| Hormone (H)         | 1640.4* | 3.70* | 459.3 | 2.65* | 65.80 | 18.70* | 8.6 | 30.00* | 2.70 | 2.95* |
| V × S               | 329.8 | 0.74ns | 11.9 | 0.06ns | 5.40 | 1.50ns | 4.7 | 16.20* | 5.30 | 5.80* |
| V × H               | 1246.3 | 2.81* | 99.5 | 0.57ns | 2.08 | 0.50ns | 0.6 | 2.19ns | 0.90 | 1.02ns |
| S × H               | 1388.3 | 3.13* | 99.6 | 1.74ns | 24.90 | 7.09* | 6.2 | 21.80* | 0.22 | 0.24ns |
| H × S × V           | 1926.6 | 4.34* | 317.5 | 1.83ns | 5.60 | 1.60ns | 0.5 | 1.80ns | 0.80 | 0.87ns |

*Significant at \( P \leq 0.05 \); MS: mean square; ns: nonsignificant.
Effect of salinity on germination, salt tolerance index, seed vigor index, and seedling growth

The germination percentage decreased by 17% at 150 mM Na₂SO₄ when compared with the control. However, there was non-significant difference between 150 mM NaCl and the control for the germination percentage. Both salts significantly decreased the germination rate. The germination rate decreased by 26.08% and 50% for NaCl and Na₂SO₄, respectively, as compared with the non-salinity treatment. Root length decreased with salt type, especially Na₂SO₄ that decreased by 89.03% compared with the control. Root fresh and dry weights were negatively affected by both salts. The Na₂SO₄ salt reduced root fresh and dry weights, shoot dry weight, and seed vigor index by 76.8%, 54.5%, 66.6%, and 89.7% respectively. However, both salts had the same effect on the salt tolerance index and shoot and root dry weights (Table 3).

Effect of variety on germination, seed vigor index, and seedling growth

There was a significant difference between the two varieties for germination, seed vigor index, and shoot and root growth. ‘Wadahmed’ was better than ‘Tabat’ for all the mentioned measurements. The germination percentage, germination rate, shoot length, root length, seed vigor index, and shoot fresh weight were higher in ‘Wadahmed’ by 8.8%, 12.0%, 8.0%, 20.7%, 17.3%, and 12.8%, respectively (Table 4).
Effect of gibberellic acid (GA₃) concentration on germination parameters and shoot and root growth

The highest germination percentage was achieved at 100 mg L⁻¹ GA₃, followed by the 50 mg L⁻¹ concentration. However, the lowest germination percentage was recorded at 300 mg L⁻¹. All hormone concentrations increased the germination rate compared with the control. The highest germination rate was reported at 100 mg L⁻¹. However, there were nonsignificant differences between 50, 250, and 300 mg L⁻¹. Shoot length improved with all the hormone concentrations, and shoot length increased 61.80%, 59.92%, 56.17%, and 52.10% compared with the control at 50, 100, 250, and 300 mg L⁻¹, respectively. Root length was negatively affected by high GA₃ concentrations. However, the maximum root length was recorded at the lowest concentration (50 mg L⁻¹ GA₃). The seed vigor index was enhanced by all the hormone concentrations and the highest seed vigor index was reached at 100 mg L⁻¹ (Table 5).

Table 5. Effect of gibberellic acid (GA₃) concentrations on germination and shoot and root growth of sorghum ‘Wadahmed’ and ‘Tabat’.

| GA₃ (mg L⁻¹) | Germination (%) | Germination rate | Shoot length (cm) | Root length (cm) | Seed vigor index |
|-------------|----------------|-----------------|-------------------|-----------------|----------------|
| 0           | 83.10bc        | 8.4c            | 2.67c             | 3.39b           | 0.23c          |
| 50          | 86.70b         | 12.5b           | 4.32a             | 3.83a           | 0.37ab         |
| 100         | 92.20a         | 13.3a           | 4.27ab            | 3.44ab          | 0.39a          |
| 250         | 81.94bc        | 12.4b           | 4.17ab            | 2.98c           | 0.37ab         |
| 300         | 80.80c         | 12.0b           | 4.06b             | 2.86c           | 0.35b          |

Means followed by the same letters are not different according to LSD test (P = 0.05).

Figure 1. Effects of salinity and gibberellic acid (GA₃) on salt tolerance index of sorghum ‘Wadahmed’ and ‘Tabat’.

Columns with different letters differ according to LSD test (P < 0.05).

Table 6. Effect of salinity and gibberellic acid (GA₃) concentrations on germination rate and shoot length of sorghum ‘Wadahmed’ and ‘Tabat’.

| GA₃ (mg L⁻¹) | 0 mM NaCl | 150 mM NaCl | 0 mM Na₂SO₄ | 150 mM Na₂SO₄ |
|-------------|-----------|-------------|-------------|--------------|
| 0           | 9.083h    | 8.833h      | 7.333i      | 5.347d       |
| 50          | 15.920c   | 13.060d     | 8.639h      | 9.487b       |
| 100         | 18.250a   | 11.720f     | 9.972g      | 9.727a       |
| 250         | 17.750ab  | 12.470de    | 6.875ij     | 9.633ab      |
| 300         | 17.580b   | 12.000ef    | 6.472j      | 8.830c       |

Means followed by the same letters are not different according to LSD test (P = 0.05).
Effect of interaction between salinity and gibberellic acid (GA₃) concentration on germination rate, shoot length, and salt tolerance index

At 150 mM NaCl, the highest germination rate was reported at 50 mg L⁻¹ GA₃. However, at 150 mM Na₂SO₄, the highest germination rate was recorded at 100 mg L⁻¹ GA₃. All the GA₃ concentrations failed to improve the salt tolerance index when compared with untreated seed (Figure 1). The Na₂SO₄ salt decreased shoot length by 68.2% compared with NaCl. The maximum shoot length was observed at 50 mg L⁻¹ GA₃ in both salt types (Table 6).

Effect of interaction between variety, salinity, and hormone on seed water uptake after 8 h imbibition

The highest water uptake at 150 mM NaCl was reported for ‘Wadahmed’ with 50 mg L⁻¹ GA₃. However, the other GA₃ concentrations decreased water uptake compared with the untreated seeds for both salts in both varieties (Table 7). In the Variety × Salinity interaction, both salts decreased root length, but there was nonsignificant difference between varieties (Figure 2).

In the Salinity × GA₃ concentration interaction, only the 50 mg L⁻¹ GA₃ rate succeeded in enhancing the seed vigor index at 150 mM Na₂SO₄. With NaCl, the seed vigor index was increased by all GA₃ concentrations, except 250 mg L⁻¹ GA₃ (Figure 3). At all the GA₃ concentrations, shoot dry weight substantially decreased with both salts. However, shoot dry weight improved by 90.4% at 300 mg L⁻¹ GA₃ for 150 mM Na₂SO₄ (Figure 4).

Table 7. Effect of variety, salinity, and gibberellic acid (GA₃) on water uptake after 8 h imbibition.

| Variety  | Salinity   | Control  | 50 mg L⁻¹ GA₃ | 100 mg L⁻¹ GA₃ | 250 mg L⁻¹ GA₃ | 300 mg L⁻¹ GA₃ |
|----------|------------|----------|---------------|---------------|---------------|---------------|
| Wadahmed | 0          | 22.82m   | 14.70n        | 60.24gh       | 89.08a        | 17.79mn       |
|          | 150 mM NaCl| 76.67cd  | 85.50ab       | 46.36jkl      | 67.75ef       | 82.47abc      |
|          | 150 mM Na₂SO₄| 56.88hi | 56.89hi        | 65.17fg       | 60.16fg       | 63.05fgh      |
| Tabat    | 0          | 19.99mn  | 51.33ijk      | 45.80jkl      | 16.22mn       | 23.65m        |
|          | 150 mM NaCl| 73.31de  | 52.85ijj      | 44.11kl       | 78.51bcd      | 39.31l        |
|          | 150 mM Na₂SO₄| 71.75de | 74.84de        | 69.32ef       | 43.35l        | 23.60m        |

Means followed by the same letters are not different according to LSD test (P = 0.05).

Figure 2. Effect of variety and salinity on root length of sorghum ‘Wadahmed’ and ‘Tabat’.
DISCUSSION

Saline soils contain multiple types of soluble salt components and each one has a different effect on the initial growth of plants; in addition, the composition of soluble salts in saline soils greatly differs among locations. Our study tested two types of soluble salts, Na₂SO₄ and NaCl, on seed germination and early seedling growth of two Sudanese varieties (Wadahmed and Tabat). The NaCl and Na₂SO₄ salts are the most abundant in the area cultivated with sorghum in Sudan. Our results showed that seed water uptake at 8 h was enhanced by the lowest GA₃ concentration (50 mg L⁻¹) in ‘Wadahmed’ (Table 7). Nimir et al. (2014) mentioned that under NaCl stress, the exogenous application of GA₃ at 100 mg L⁻¹ significantly increased seed water uptake at 4 and 24 h compared with the control. Our results showed that the germination percentage and germination rate of both varieties decreased more with Na₂SO₄ than NaCl (Table 3). These results concur with Kaymakanova (2009), who indicated that the germination of seeds treated with Na₂SO₄ was more strongly inhibited than those treated with NaCl in three bean (Phaseolus vulgaris L.) cultivars. Hussain et al. (2018) indicated that seedling emergence or early seedling growth of two different rice cultivars were inhibited by increased NaCl concentrations. The decreased germination percentage and germination rate could be due to the fact that Na₂SO₄ increased osmotic pressure, which slowed the seed imbibition process of sorghum; thus, seed metabolism activation and seed germination was delayed. Ibrahim (2016) pointed out that the absorption of toxic ions has been shown to affect embryo viability and cause biochemical toxic influences, including disruption of the structure of enzymes and other macromolecules, damage to cell organelles and plasma membrane, disruption of respiration, photosynthesis, and protein synthesis. Oxidative stress has also proven to be an important factor for the inhibition of seed germination, the effect is caused by the impact on the balance of reactive oxygen species production and scavenging or detoxification.

In our study, different GA₃ concentrations had different effects on the germination parameters, but the highest germination percentage, germination rate, and seed vigor index were achieved at 100 mg L⁻¹ (Table 5). Our results
concur with findings by Nimir et al. (2014), who indicated that germination parameters improved at 100 mg L⁻¹ GA₃ under salt stress. Zhu et al. (2019) indicated that the appropriate concentration of GA₃ markedly reduced salt stress and improved seed germination of sorghum seeds; the optimum concentration for seed germination of sweet sorghum was 288 μM GA₃. In the second step of the germination stage (enzyme activation) after water absorption, gibberellins activated the formation of hydrolytic enzymes. The α-amylase was activated in the aleurone cells, which are responsible for the hydrolysis of storage macromolecules such as starch and proteins; these are converted into available forms to the embryo, used to increase size, and raise the osmotic content of the seed to increase water potential (Bradford and Nonogaki, 2008).

The shoot and root lengths in seedling growth are the most important parameters under salinity stress because roots are in contact with the soil, absorb soil water, and provide it to other plant parts (Ibrahim et al., 2016). Root and shoot lengths provide important evidence for plant response to salinity stress (Jamil and Rha, 2004). We found that both shoot and root lengths decreased more with Na₂SO₄ than NaCl (Tables 3 and 6). Plants grown in the presence of Na₂SO₄ showed an immediate and sustained reduction of their growth parameters, accompanied by senescence symptoms such as chlorosis, necrosis, and leaf abscission (Reginato et al., 2014). The inhibiting effect of Na₂SO₄ in the initial growth of seedlings is 20% stronger than for NaCl (Kaymakanova, 2009). *Prosopis strombulifera* plants treated with Na₂SO₄ underwent structural cell and tissue alterations, resulting in changed growth patterns at different levels of organization. Anatomical and histological differences in leaf stem and roots were observed in plants treated with Na₂SO₄ when compared with non-salinized plants or plants grown with high NaCl concentrations (Reinoso et al., 2004).

Our results show that shoot growth was enhanced by all the GA₃ concentrations; however, root growth only improved at 50 mg L⁻¹ (Table 5). Shoot dry weight only improved at high GA₃ concentrations with Na₂SO₄ (Figure 3). Jiao et al. (2019) mentioned that castor bean seed soaked in 86 mg L⁻¹ GA₃ significantly increased shoot length, stem diameter, dry weight, and promoted leaf growth under salt treatments, while seed treated with other GA₃ concentrations had little effect on seedling growth. Sedghi et al. (2010) suggested that seed priming with GA₃ in medicinal plants of pot marigold (*Calendula officinalis* L.) and sweet fennel (*Foeniculum vulgare* Mill.) accelerated metabolic activity and subsequently increased radicle and plumule weight, especially under salinity stress conditions. Gibberellin concentrations in plants under salt stress are lower than those in unstressed plants. Therefore, limited growth caused by salinity is at least partly due to a decreased gibberellin concentration (Llanes et al., 2014). Gibberellin enhances maize seedling growth and establishment under saline soil conditions by improving nutrient levels and membrane permeability (Tuna et al., 2008).

Seed priming with GA₃ at appropriate concentrations successfully reduced different salt types on the germination and growth of shoots and roots of early sorghum seedlings. This knowledge is useful for farmers in order to adopt exogenous amendments to improve seed germination and seedling growth under salinity conditions. This application is inexpensive and can be easily managed, especially when seeds are sown by hand. For a commercial farm, where seeds are usually planted with a machine, seeds could probably be damaged by mechanical management if they are soaked with hormone solutions. This problem could likely be solved by processing the seeds with coating agents containing exogenous hormones at suitable concentrations.

**CONCLUSIONS**

An appropriate amount of gibberellic acid (GA₃) has been reported in many crops to mitigate the harmful effect of abiotic stress. Our study examined several GA₃ concentrations to improve two types of salt stress on two sorghum varieties. The maximum values for seed water uptake, germination percentage, germination rate, shoot length, and seed vigor index were achieved at 100 mg L⁻¹ GA₃. Root length was only improved by the lowest GA₃ concentration (50 mg L⁻¹). The Na₂SO₄ salt caused a higher reduction than NaCl on germination, germination rate, root fresh weight, root dry weight, shoot dry weight, and seed vigor index. ‘Wadahmed’ was more tolerant to salt stress than ‘Tabat’. The Na₂SO₄ salt had a more harmful effect than NaCl at the germination stage for both varieties.

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