

# Effects of gibberellic acid on water uptake and germination of sweet sorghum seeds under salinity stress

Guanglong Zhu<sup>1,2,3</sup>, Linlin An<sup>2</sup>, Xiurong Jiao<sup>1</sup>, Xubing Chen<sup>2</sup>, Guisheng Zhou<sup>1,2,3,4\*</sup>, and Neil McLaughlin<sup>5</sup>

<sup>1</sup>Yangzhou University, Joint International Research Laboratory of Agriculture and Agri-Product Safety, Yangzhou, 225009, Jiangsu, China.

<sup>2</sup>Yangzhou University, Key Lab of Crop Genetics & Physiology of Jiangsu Province, Agricultural College, Yangzhou 225001, Jiangsu, China.

<sup>3</sup>Yangzhou University, Jiangsu Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou 225009, China.

<sup>4</sup>Yangzhou University, Joint Laboratory in Agricultural Sciences between AAFC, 225009, Jiangsu, China.

\*Corresponding author (gszhou@yzu.edu.cn).

<sup>5</sup>Agriculture and Agri-Food Canada, Ottawa Research and Development Center, 960 Carling Ave., Ottawa, Ontario, Canada.

Received: 5 November 2018; Accepted: 11 February 2019; doi:10.4067/S0718-58392019000300415

## ABSTRACT

Sweet sorghum (*Sorghum bicolor* [L.] Moench) is a potential feedstock crop in biomass energy development, and is much more resistant to saline soils than crops. Healthy seed germination is critical for the growth cycle of plants, and determines the establishment of seedlings and subsequent crop production. High salinity conditions can result in difficulty for seed germination and delays the germination period. So, screening salt-tolerant genotypes and method for healthy seed germination under salinity stress are vital to crop production and food security. Therefore, a controlled study was conducted to explore the interactive amendment effects of exogenous gibberellic acid (GA<sub>3</sub>) and salinity on seed germination process of sweet sorghum. Seeds were presoaked in different levels of GA<sub>3</sub> water solutions (0, 144, 288, and 576 μM) and then cultivated in gradient NaCl solutions (0, 50 and 100 mM). The effects of salinity and external GA<sub>3</sub> on seed water uptake and germination characteristics were investigated. Compared with the effects of 0 μM GA<sub>3</sub> at 0 mM NaCl, slight salt stress of 50 mM NaCl improved the cumulative water uptake, germination and germination index, but high salinity level of 100 mM NaCl significantly inhibited these germination traits. However, either 100 mM NaCl or 576 μM GA<sub>3</sub> had significantly negative effects on seed cumulative water uptake, cumulative germination, germination index, and length of germ and radicle. The appropriate concentration of GA<sub>3</sub> prominently relieved salt stress and improved the seed germination of sorghum seeds, and the optimum concentration for seed germination of sweet sorghum was 288 μM GA<sub>3</sub> at each salinity level.

**Key words:** GA<sub>3</sub>, germination, saline stress, sweet sorghum, water uptake.

## INTRODUCTION

Compared to other conventional crops, sweet sorghum (*Sorghum bicolor* [L.] Moench) can survive in lower quantity of water and fertilizer. It is a potential feedstock crop in biomass energy development. Sweet sorghum is much more resistant to saline soils than corn (*Zea mays* L.) or sugarcane (*Saccharum officinarum* L.), which are currently the main bioenergy resources in the world (Almodares et al., 2011). Sweet sorghum crops have a great potential for manufacturing syrups for sweetening food and beverages, carbohydrates, and most importantly, as a raw material for fuel alcohol production worldwide (Ratanavathi et al., 2004). In China, people can take advantage of broad saline lands by planting salt-tolerant

sweet sorghum varieties. Although it has been found that a number of sweet sorghum varieties can survive under salinity soil condition, however, their seed germination and seedling establishment are still difficult on marginal saline lands.

Currently, more than 900 million hectares or about 20% of the total agricultural land are affected by salinity around the world. Salinity is becoming an increasingly serious problem limiting crop production worldwide (Munns and Tester, 2008). Salinity can affect crop growth from germination stage, the very beginning of the life cycle of a crop plant. Healthy seed germination plays an important role in the growth cycle of plants, and determines the establishment of seedlings and subsequent crop production (Bahrani and Pourreza, 2012). High salinity conditions can result in difficult seed germination and delays in the germination time (Nyagah and Musyimi, 2009). The inhibition of salinity on seed germination was mainly due to water deficit and/or the toxic influence of ions, such as absorbing excessive  $\text{Na}^+$  and  $\text{Cl}^-$  ions (Murillo et al., 2002).

The water uptake is the first stage preparing for seed germination. Under adequate supply of oxygen and optimal temperature conditions, the most important factor in seed germination is water status. Viable seeds have the ability to break dormancy and begin germination after absorbing enough water. However, water quality varies greatly caused by degrading environments and salinization has an important effect on inhibiting germination and subsequent root elongation (Saberli and Moradi, 2017). It was reported that water uptake of tomato plants declined with increasing salinity, causing significant reductions in morphological and/or physiological parameters, stomatal density and water conductance (Romero et al., 2001).

Plant hormones are active members of plant regulation, and are involved in the induction of plant stress responses (Pedranzani et al., 2003). They make plants adapt to serious abiotic stress conditions, and help crops to improve their tolerance, capacity to adverse environments (Srivastava and Srivastava, 2007). Gibberellic acid ( $\text{GA}_3$ ) is an important plant hormone, which plays a vital role in regulating the signal pathways, seed germination and plant growth (Cavusoglu and Sulusoglu, 2015). It has been shown that  $\text{GA}_3$  is related to salinity tolerance of *Arabidopsis thaliana* L. (Sun, 2008).

There is abundant evidence showed that  $\text{GA}_3$  has a positive effect on water uptake and germination of crops under normal conditions, but little research is in the literature on sweet sorghum grown in saline soils. Furthermore, the related mechanism of  $\text{GA}_3$  in regulating germination is still not well documented. Therefore, the objectives of this research were to explore the effects of  $\text{GA}_3$  amendment on water uptake and germination of sweet sorghum at different salinity levels; and screen the optimal concentrations of  $\text{GA}_3$  to sweet sorghum germination under saline conditions.

## MATERIALS AND METHODS

A controlled study was conducted in Joint International Research Laboratory of Agriculture and Agri-Product Safety, Ministry of Education of China, Yangzhou University (32.30° N, 119.25° E), Jiangsu Province, China. The seeds of sweet sorghum 'Chuntian 1', kindly provided by Beijing Sangliang Technological Development Center, were used. The variety is relatively salt-tolerant and being popularly grown in China.

### Material and cultivation

The study was arranged in two-factorial randomized complete block design with three replicates. The two factors were NaCl (0, 50, and 100 mM water solutions) and gibberellic acid ( $\text{GA}_3$ ; 0, 144, 288, and 576  $\mu\text{M}$  water solutions). For each replicate of each treatment, 50 uniform and healthy seeds were selected and surface-sterilized using 1% sodium hypochlorite solution for 10 min, and then rinsed thoroughly six times with deionized water and dried by air, and fresh weight was considered as initial weight. After that, all the seeds were cultivated in Petri dishes with treatment solutions. The diameter of the Petri dish was 9 cm. A double layer of filter paper was placed in each Petri dish, and 7 mL of different treatment solutions of NaCl and  $\text{GA}_3$  were infused. Then the Petri dishes were covered with lids and placed in a germinator (Model ZLC-100, Hangzhou Shuolian Instrument Co., Ltd., Hangzhou, Zhejiang, China) with a natural light 12:12 h diurnal cycle, constant temperature 25 °C and humidity 60%. In order to maintain the treatment levels, each Petri dish was carefully injected with 5.0 mL treatment solutions every 8 h to replenish evaporation and solution absorbed by seeds. The filter paper was changed every 48 h during the testing period.

### Observations and measurements

The seeds in each Petri dish were weighed before soaking and during seed water uptake at 8, 16, 24, 32, 40, 48, 56 and 64 h after the beginning of water imbibition. In order to measure seed cumulative water uptake and water uptake rate, seeds were carefully removed, drained, blotted with absorbent paper, weighed, and, returned to Petri dishes quickly. Germination parameters, including cumulative water uptake, cumulative germination, and germination index at each measurement time were calculated as the following:

$$\text{Cumulative water uptake } Y_t (\%) = (X_t - X_0) / (X_0)^t \times 100 \quad [1]$$

where  $X_0$  is the initial weight of the 50 sweet sorghum seeds (g) and  $X_t$  is the weight at t (h).

$$\text{Cumulative germination } (\%) = S_t \times S_0^{-t} \times 100 \quad [2]$$

where  $S_t$  is the number of seeds germinated at t (h) and  $S_0$  is the initial number of seeds (50) subjected to the germination test at time t = 0 h.

$$\text{Germination index } G_t = G_{t-1} + (\text{count}_t - \text{count}_{t-1}) \times S_0^{-t} \times (t/24) \quad [3]$$

where  $G_t$  is germination index at time  $t_i$  (h),  $\text{count}_t$  is the number of seeds germinated at time  $t_i$ ,  $\text{count}_{t-1}$  is the number of seeds germinated at time  $t_{i-1}$ ,  $S_0$  is the number of seeds subjected to the germination test, and  $t_i$  is observation time.

Seeds were considered to be germinated when radicle length reached approximately 0.2 mm. Both radicle and germ lengths were precisely measured using a vernier caliper after 32 h. The test was terminated after 64 h in the germinator.

### Statistical analysis

The experiment was designed as a factorial design with two experimental factors (three salinity levels, and four hormones gradients) arranged in a completely randomized design with three replicates. The data of each variable were then subjected to ANOVA with the statistical package of DPS 7.05 for Windows (Tang and Feng, 1997) according to this design. When “F” values were significant, means were separated by the least significant difference test (LSD,  $P \leq 0.05$ ).

## RESULTS

### Cumulative water uptake

There was nonsignificant effects of salt, GA<sub>3</sub> or Salt × GA<sub>3</sub> interaction on cumulative water uptake at the first 32 h except Salt × GA<sub>3</sub> interaction at 16 h (Table 1). The effect of salt on cumulative water uptake was nonsignificant at any time point, but the cumulative water uptake was significantly affected by GA<sub>3</sub> and Salt × GA<sub>3</sub> at 40 and 48 h, 16 and 48 h, respectively (Table 1). Some of the pairwise comparisons were significantly different from each other as affected by Salt × GA<sub>3</sub> at 16-48 h. Cumulative water uptake was inhibited at whole germination period at high GA<sub>3</sub> or high salt. Compared with control, the cumulative water uptake was increased by about 8%-12% at 50 mM salinity level during 8-56 h. However, when salinity was increased to 100 mM NaCl, the cumulative water uptake was decreased by about 14%-16% during the period of 8-48 h. Statistical analysis indicated that the inhibition of seeds cumulative water uptake caused by salinity could be alleviated by application of GA<sub>3</sub>. With increased concentration of GA<sub>3</sub>, the cumulative water uptake was gradually increased at low and middle level but decreased at high level of GA<sub>3</sub> at 100 mM NaCl. In general, water uptake was promoted by 288 μM GA<sub>3</sub> but then decreased at 576 μM GA<sub>3</sub> under each salinity level during whole germination period. Salinity and GA<sub>3</sub> treatments showed the most prominent effect during the time period of 32-48 h, the highest cumulative water uptake was recorded at 50 mM NaCl and 288 μM GA<sub>3</sub> (Table 2).

### Cumulative germination

The ANOVA showed that cumulative germination of sweet sorghum seeds were significantly influenced by salinity during the germination period, but by GA<sub>3</sub> only during 32-48 h ( $P \leq 0.05$  and  $P \leq 0.01$ ). However, Salt × GA<sub>3</sub> had nonsignificant effects on cumulative germination in any growth period (Table 1). Compared with control, 50 mM NaCl significantly increased the cumulative germination by 23% at 8 h and 17% at 16 h, but then declined with 54% and 42.2%, respectively, when salinity concentration was increased to 100 mM NaCl. Compared to non-GA<sub>3</sub>, cumulative germination was significantly increased at the levels of 144 and 288 μM GA<sub>3</sub> during 32-48 h, but decreased at 576 μM GA<sub>3</sub> at all salinity levels. For example, the cumulative germination declined by 7.5% from 288 to 576 μM GA<sub>3</sub> at 50 mM NaCl at 48 h (Table 3). As a whole, during the germination period of 32-56 h, the highest cumulative germination was presented by applying 288 μM GA<sub>3</sub> regardless of the salinity levels.

**Table 1. Significance levels of salt, gibberellic acid (GA<sub>3</sub>) and Salt × GA<sub>3</sub> interaction on different germination parameters for sweet sorghum seeds at different measurement times.**

Parameters	Factor	Time (h)							
		8	16	24	32	40	48	56	64
Cumulative water uptake	Salt	ns	ns	ns	ns	ns	ns	ns	-
	GA <sub>3</sub>	ns	ns	ns	ns	*	*	ns	-
	Salt × GA <sub>3</sub>	ns	*	ns	ns	ns	*	ns	-
Cumulative germination	Salt	-	***	**	**	**	*	*	-
	GA <sub>3</sub>	-	ns	ns	**	*	**	ns	-
	Salt × GA <sub>3</sub>	-	ns	ns	ns	ns	ns	ns	-
Germination index	Salt	-	***	***	***	***	***	***	-
	GA <sub>3</sub>	-	ns	ns	ns	ns	*	ns	-
	Salt × GA <sub>3</sub>	-	ns	ns	ns	ns	ns	ns	-
Radical length	Salt	-	-	-	***	ns	***	***	***
	GA <sub>3</sub>	-	-	-	***	***	***	***	***
	Salt × GA <sub>3</sub>	-	-	-	ns	***	***	***	***
Germ length	Salt	-	-	-	***	*	***	***	**
	GA <sub>3</sub>	-	-	-	ns	***	***	***	***
	Salt × GA <sub>3</sub>	-	-	-	ns	ns	***	***	***
Ratio of radicle and germ lengths	Salt	-	-	-	*	***	**	***	***
	GA <sub>3</sub>	-	-	-	ns	**	***	***	***
	Salt × GA <sub>3</sub>	-	-	-	ns	**	***	***	***

\*, \*\*, \*\*\*Significant at the 0.05, 0.01, 0.001 probability levels, respectively.  
ns: Nonsignificant; -: no measurements at that time.

**Table 2. Cumulative water uptake of sweet sorghum seeds as influenced by gibberellic acid (GA<sub>3</sub>) amendment at different salinity levels.**

Salt	GA <sub>3</sub>	Cumulative water uptake						
		8 h	16 h	24 h	32 h	40 h	48 h	56 h
mM	μM	%						
0	0	32.1abc	40.0ab	43.6ab	47.4ab	55.1bcde	65.7bc	71.6ab
	144	33.2ab	39.3abc	42.2ab	47.9ab	57.0abcd	68.5abc	75.3ab
	288	33.9a	40.8a	45.9a	52.0a	62.1ab	69.5abc	77.7a
	576	31.4abc	39.8ab	42.9ab	46.9ab	53.3de	62.9cd	73.5ab
50	0	36.4a	43.0a	47.3a	52.4a	61.4ab	69.8abc	77.7a
	144	34.4a	43.4a	47.8a	51.6a	62.5a	68.9abc	77.7a
	288	34.4a	42.4a	47.2a	52.6a	63.6a	70.1abc	76.0a
	576	26.3c	34.0c	38.8b	43.8b	54.2cde	58.0d	66.6b
100	0	27.0bc	34.5bc	38.8b	42.2b	48.6e	56.6d	70.7b
	144	31.1abc	39.6ab	43.6ab	46.9ab	60.5abc	70.7ab	78.6a
	288	34.1a	43.0a	46.8a	51.2a	61.7ab	73.2a	80.5a
	576	33.2ab	42.3a	44.7ab	51.4a	63.0a	71.8ab	77.0a

Means in the same column and followed by the same letter indicate nonsignificant difference (P ≥ 0.05).

### Germination index

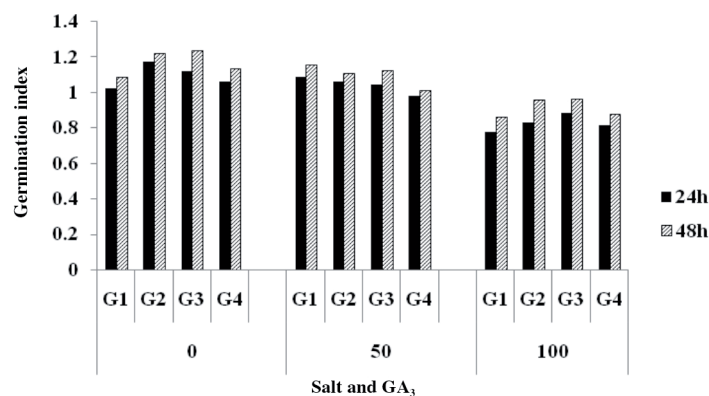
According to the ANOVA, salt stress had a significant effect on germination index, but GA<sub>3</sub> had not except at 48 h. The Salt × GA<sub>3</sub> interaction did not significantly affect germination index at any sampling time (Table 1). Compared with control, germination index was slightly decreased at 50 mM NaCl but it was significantly inhibited at 100 mM NaCl (Figure 1). At the level of 0 and 100 mM NaCl, germination index was improved by 144 and 288 μM GA<sub>3</sub>, but it was suppressed at 576 μM GA<sub>3</sub> and 50 mM NaCl. At each salinity level, the highest germination index was recorded at 0 mM NaCl by 144 μM GA<sub>3</sub>, 50 mM NaCl by 0 μM GA<sub>3</sub>, and 100 mM NaCl by 288 μM GA<sub>3</sub> (Figure 1).

**Table 3. Cumulative germination of sweet sorghum seeds as influenced by gibberellic acid (GA<sub>3</sub>) amendments at different salinity levels.**

Salt	GA <sub>3</sub>	Cumulative germination (%)						
		8 h	16 h	24 h	32 h	40 h	48 h	56 h
mM	μM	%						
0	0	8.7abc	39.3cd	70.0ab	72.7bcd	77.3abcd	79.3abc	82.7abc
	144	10.7ab	56.7a	73.3a	74.0abc	80.0ab	80.7ab	83.3ab
	288	12.0a	53.3ab	67.3abc	80.0a	82.7a	84.0a	85.3a
	576	10.7ab	50.0abc	65.3abcd	71.3bcd	75.3abcde	76.0bcd	80.0abc
50	0	11.3a	47.3abc	68.0abc	72.0bcd	74.0bcde	80.0ab	81.3abc
	144	8.0abc	45.3abcd	71.3ab	70.7cd	76.7abcd	80.0ab	80.7abc
	288	9.3abc	42.0bcd	69.3ab	77.3ab	78.7abc	81.3ab	83.3ab
	576	6.0abcd	40.7bcd	68.7abc	68.7cd	73.3bcde	74.0cd	79.3bc
100	0	4.0cd	22.7e	60.7cd	67.3d	70.0de	72.7d	77.3c
	144	4.7bcd	37.3cd	57.3d	70.0cd	72.0cde	76.0bcd	78.0bc
	288	3.3cd	32.0de	67.3abc	72.0bcd	76.0abcd	80.7ab	80.7abc
	576	1.3d	32.7de	63.3bcd	68.0cd	68.0e	72.7d	77.3c

Means in the same column and followed by the same letter indicate nonsignificant difference ( $P \geq 0.05$ ).

**Figure 1. Germination index of sweet sorghum seeds calculated at 24 and 48 h for various combinations of salt and gibberellic acid (GA<sub>3</sub>).**



G1, G2, G3, and G4 indicate GA<sub>3</sub> concentrations of 0, 144, 288 and 576 μM, respectively. Salinity levels are 0, 50 and 100 mM NaCl.

### Radicle length

Salinity, GA<sub>3</sub> and Salt × GA<sub>3</sub> significantly affected radicle length mainly after 48 h water absorption ( $P \leq 0.001$ ) (Table 1). Radicle length was improved at the low salinity level but greatly inhibited under high salt stress. Compared with 0 mM NaCl, radicle length was increased by 5%-6% at 50 mM NaCl during 48-64 h. With rising salinity to 100 mM NaCl, radicle length was significantly decreased by 51%-18% during 8-64 h. The reduction in radicle length caused by salinity was significantly alleviated by external application of 144 and 288 μM GA<sub>3</sub>. Under 100 mM NaCl, radicle length was increased by 26%-22% when applied 288 μM GA<sub>3</sub> during 40-64 h, but it was prominently inhibited by 576 μM GA<sub>3</sub> under all salt treatments. Overall, the radical length was prominently enhanced by application of 288 μM GA<sub>3</sub> during all growth period from 32 to 64 h under each salinity treatment except at 32 h with 0 mM NaCl (Table 4).

### Germ length

The effects of salt, GA<sub>3</sub> and Salt × GA<sub>3</sub> were significant on the germ length at 32, 40, and 48 h after water uptake initiation ( $P \leq 0.001$ ) (Table 1). On the average, there was nonsignificant difference in germ length between 0 and 50 mM NaCl treatments. However, compared to 0 mM NaCl, germ length was decreased by 37%-12% at 100 mM NaCl during 32-56 h.

**Table 4. Radicle length of sweet sorghum seeds at different times as influenced by gibberellic acid (GA<sub>3</sub>) amendments at different salinity levels.**

Salt	GA <sub>3</sub>	Radicle length				
		32 h	40 h	48 h	56 h	64 h
mM	μM	mm				
0	0	3.7bc	5.0b	8.4bc	9.4f	19.0d
	144	4.9a	4.3cd	7.9d	10.2e	19.8cd
	288	4.4ab	5.5a	8.8b	13.8b	22.1b
	576	3.3cd	3.2e	5.2g	8.5g	15.8fg
50	0	2.3f	5.1b	8.8b	10.7d	20.2c
	144	3.1cde	5.0b	8.1cd	13.8b	21.7b
	288	3.4c	5.7a	10.4a	15.3a	23.9a
	576	2.5def	3.2e	5.3fg	7.5h	16.1ef
100	0	1.8f	4.2cd	7.1e	9.0f	15.5fg
	144	2.3ef	4.5c	8.1cd	11.9c	17.1e
	288	2.3ef	5.7a	8.6bc	14.1b	20.0cd
	576	1.9f	4.0d	5.8f	8.2g	14.9g

Means in the same column and followed by the same letter indicate nonsignificant difference ( $P \geq 0.05$ ).

The longest germ length was achieved at the concentration of 288 μM GA<sub>3</sub> under 0 and 50 mM NaCl, but higher GA<sub>3</sub> of 576 μM inhibited germ length at each salinity level during all tested periods. However, at the salinity of 100 mM NaCl, the longest germ length was recorded by 144 μM GA<sub>3</sub> during 40-48 h and 288 μM GA<sub>3</sub> during 56-64 h (Table 5).

#### The ratio of radicle length to germ length

The effects of salt, GA<sub>3</sub> and Salt × GA<sub>3</sub> on the ratio of radicle length to germ length mainly came up after 40 h of germination ( $P \leq 0.001$ ) (Table 1). The concentration of 144 μM GA<sub>3</sub> significantly improved radicle length/germ length at 0 mM NaCl at 32, 48 and 64 h, and at 50 mM NaCl during all germination period except at 48 h. However, under 100 mM NaCl, radicle length/germ length was promoted by 288 μM GA<sub>3</sub> at 32 and 40 h, and by 576 μM GA<sub>3</sub> at 56 and 64 h (Table 6).

**Table 5. Germ length of sweet sorghum seeds at different times as influenced by gibberellic acid (GA<sub>3</sub>) amendments at different salinity levels.**

Salt	GA <sub>3</sub>	Germ length				
		32 h	40 h	48 h	56 h	64 h
mM	μM	mm				
0	0	1.9abcd	2.7bc	4.2cd	5.7e	13.5cd
	144	2.1ab	2.8ab	3.8d	7.1d	11.8ef
	288	2.5a	3.2a	5.2a	11.0a	16.4a
	576	1.7bcde	2.2de	3.2e	5.6e	11.9ef
50	0	1.4cde	2.5bcd	4.1cd	6.0e	13.8c
	144	1.9abc	2.3cde	4.6b	6.0e	11.4f
	288	1.7bcde	2.9ab	5.4a	8.5c	14.2bc
	576	1.4cde	1.9e	3.1e	4.4fg	12.4e
100	0	1.2e	2.1de	3.2e	5.0f	12.7de
	144	1.3de	2.9ab	4.2bc	6.2e	12.7de
	288	1.3de	2.8ab	4.0cd	9.4b	15.1b
	576	1.4cde	2.0e	3.3e	4.3g	9.0g

Means in the same column and followed by the same letter indicate nonsignificant difference ( $P \geq 0.05$ ).

**Table 6. The ratio of radicle and germ length of sweet sorghum seeds as influenced by gibberellic acid (GA<sub>3</sub>) amendment at different salinity levels.**

Salt	GA <sub>3</sub>	Radicle length/Germ length				
		32 h	40 h	48 h	56 h	64 h
mM	μM					
0	0	2.12ab	1.92abc	2.03bc	1.67cd	1.41cd
	144	2.61a	1.57d	2.11ab	1.46e	1.67b
	288	1.82bc	1.74bcd	1.69e	1.26f	1.35de
	576	2.04abc	1.51d	1.63e	1.52de	1.33de
50	0	1.73bc	2.10a	2.18ab	1.81bc	1.48c
	144	2.09ab	2.21a	1.77de	2.30a	1.91a
	288	2.09ab	2.01ab	1.96c	1.81bc	1.69b
	576	1.83bc	1.68ad	1.75e	1.71c	1.31de
100	0	1.77bc	2.01ab	2.25a	1.80bc	1.23e
	144	1.95bc	1.58d	1.92cd	1.92b	1.35de
	288	2.12ab	2.08a	2.15ab	1.49e	1.32de
	576	1.47c	2.04a	1.77de	1.94	1.67b

Means in the same column and followed by the same letter indicate nonsignificant difference ( $P \geq 0.05$ ).

## DISCUSSION

Salinity inhibits water uptake, delays seed germination, slows down growth rate, changes metabolic and reduces biomass production (Munns, 2002). In most cases, salinity does harm plant survival by disturbing different plant mechanisms (Tavakkoli et al., 2010). Salinity at higher levels usually causes both hypertonic and hyperosmotic stresses and can lead to plant death. These effects may cause membrane damage, nutrient imbalance, altered levels of growth regulators, enzymatic inhibition and metabolic dysfunction (Sudhir and Murthy, 2004), changes in C and N metabolism (Kim et al., 2004), and decreased photosynthesis rate, which ultimately leads to plant death (Mahajan and Tuteja, 2005; Hasanuzzaman et al., 2012). Conventional crops, such as wheat and rice, are sensitive to high salinity conditions and their biomass production and yield gain are impeded sharply at high salinity levels (Baharani and Haghjoo, 2011).

However, as for sweet sorghum, it has been proved to be more tolerant to salt stress as compared with conventional crops aforementioned (Ratanavathi et al., 2004), but the poor germination under severe salt stress is still a crucial problem to limit sweet sorghum production. An increasing evidence showed that the growth of sorghum is seriously restrained at 250 mM rather than at 125 mM NaCl (Ibrahim, 2004). In the present study, cumulative water uptake, cumulative germination and germination index were all exhibited with similar tendencies at 100 mM NaCl. These results are consistent with Yang and Li (2014). These damage to seed germination and seedling growth is closely related to Na<sup>+</sup> accumulation. Excessive accumulation of Na<sup>+</sup> can cause a range of osmotic and metabolic problems for plants (Hoai et al., 2003). Most toxic effects of NaCl can be attributed to Na<sup>+</sup> toxicity that can result in the dormancy of seeds and delay germination. It is known that toxic effects of Na<sup>+</sup> are largely due to its ability to compete with K<sup>+</sup> for binding sites, essential for cellular function (Yildirim et al., 2009). As a major plant macronutrient, K plays important roles on stomatal behavior, osmoregulation, enzyme activity, cell expansion, neutralization of non-diffusible negatively charged ions, and membrane polarization (Qin et al., 2010). On the other hand, Na and Cl ions can enter into the cells and have direct toxic effects on cell membranes, as well as on metabolic activities in the cytosol (Cha-Um and Kirdmanee, 2010).

On the contrary, slight salinity improved germination parameters at the level of 50 mM NaCl. Similar results were found by other scientists (Nimir et al., 2017). There is evidence that low salinity sometimes stimulates photosynthesis of *Bruguiera parviflora*. Parida et al. (2004) observed that the rate of photosynthesis increased at low salinity while decreased at high salinity. But the related mechanism is still not clear.

The capacity of crops tolerant to stress can be improved by a number of ways, including selection and breeding, genetic modifications, and use of osmoprotectants and growth regulating substances (Parida et al., 2004). In this regard, attention has come to be focused on the use of plant growth regulators, such as GA<sub>3</sub>, kinetin, and salicylic acid, which are known

to regulate plant responses to adverse external environments and to regulate the expression of a number of stress-induced genes. At high salinity levels, the germination of sweet sorghum is deteriorated. The osmotic regulation, together with the toxic effects of Na<sup>+</sup> and Cl<sup>-</sup> ions, reduces water uptake and causes an imbalance of essential nutrients during seed germination (Willenborg et al., 2004). In the present study, we observed that the cumulative water uptake, cumulative germination, germination index and the length of radicle and germ of sweet sorghum seeds were significantly improved by applying 144 and 288 µM GA<sub>3</sub>. Similar results were found in cotton (*Gossypium barbadense* L.) and castor (*Ricinus communis* L.) seeds when GA<sub>3</sub> was amended at appropriate concentrations (Zhou et al., 2014). Also with wheat seeds, the greatest improvement in seed germination was achieved when seeds were presoaked in 50 mg GA<sub>3</sub> L<sup>-1</sup> (Parashar and Varma, 1988). As for sweet sorghum, the optimum GA<sub>3</sub> concentration to promote seed germination is 288 µM GA<sub>3</sub> in this study. At this level, most of the parameters were improved under each salinity levels during different germination periods (Tables 2-6).

The probable mechanism is that GA<sub>3</sub> can break seed dormancy, stimulate seed embryos, thereby promote plant metabolic reactions, repair the integrity of damaged cell and improve seed viability. Nimir et al. (2017) reported that GA<sub>3</sub> caused a reduction in Na<sup>+</sup> content and partly decreased the content of other ions. These results agreed with those of Kaya et al. (2010), who reported that stressed maize plants significantly accumulated less Na<sup>+</sup> upon application of GA<sub>3</sub>. In another study, application of GA<sub>3</sub> counteracted the adverse effects of NaCl salinity on relative water content, electrolyte leakage, and chlorophyll content (Ahmad et al., 2011).

According to previous studies, external seed treatment with GA<sub>3</sub> could be a possible method of reversing the effects of salt stress (Tuna et al., 2008). However, when GA<sub>3</sub> level was increased to 576 µM, most germination parameters were suppressed as shown in the present study (Tables 2-6). This results indicated that application of low levels of GA<sub>3</sub> can regulate plant growth and have positive effects, but high levels of GA<sub>3</sub> may have opposite effects. This result is similar to Baskin et al. (1998), who reported that high level of GA<sub>3</sub> reduced the ratio of radicle length to germ length, but the suitable GA<sub>3</sub> concentration improved the growth rate of the germ relative to the radicle. The application of 288 µM GA<sub>3</sub> can relieve the harmful effects of salinity on water uptake and germination of sweet sorghum. Nevertheless, these results need to be further confirmed in field environment due to the difference in soil environment and NaCl water solution.

## CONCLUSION

Sweet sorghum can be tolerant to mild salinity stress, it can be recognized as one of the excellent candidate crops used to exploit the area of coastal shoaly land. Low concentrations of salinity and gibberellic acid (GA<sub>3</sub>) can enhance water absorption and germination of sweet sorghum, but high levels of these treatments can bring the opposite effects. The 288 µM GA<sub>3</sub> is the optimum concentration which can be applied to promote seed germination of sweet sorghum under salinity stress conditions.

## ACKNOWLEDGEMENTS

This study was financially supported in part by Jiangsu Provincial Key R&D Program (BE2016345), China National Key R&D Program (SQ2017YFNC050027), Jiangsu Provincial Independent Agriculture Innovation Program (CX(16)1005-5), the Natural Science Foundation of Jiangsu Province of China (BK20180923), the Natural Science Foundation of Jiangsu Higher Education Institutions of China (17KJB210008), and Open Research Project of Joint International Research Laboratory of Agriculture and Agri-Product Safety, the Ministry of Education of China; Yangzhou University (JRK2018003).

Guanglong Zhu and Linlin An contributed equally to this paper and are considered first coauthors.

## REFERENCES

- Ahmad, P., Nabi, G., and Ashraf, M. 2011. Cadmium-induced oxidative damage in mustard plants [*Brassica juncea* (L.) Czern. & Coss.] can be alleviated by salicylic acid. *South African Journal of Botany* 77:36-44.
- Almodares, A., Hadi, M.R., and Kharazian, Z.A. 2011. Sweet sorghum: salt tolerance and high biomass sugar crop. p. 441-460. In Matovic, M. (ed.) *Biomass-detection, production and usage*. InTech, Rijeka, Croatia. doi:10.5772/19044.



- Bahrani, A., and Haghjoo, M. 2011. Response of some wheat (*Triticum aestivum* L.) genotypes to salinity at germination and early seedling growth stages. *World Applied Sciences Journal* 13:887-897.
- Bahrani, A., and Pourreza, J. 2012. Gibberellic acid and salicylic acid effects on seed germination and seedlings growth of wheat (*Triticum aestivum* L.) under salt stress condition. *World Applied Sciences Journal* 18:633-641.
- Baskin, J.M., Nan, X.Y., and Baskin, C.C. 1998. A comparative study of seed dormancy and germination in an annual and a perennial species of Senna (*Fabaceae*). *Seed Science Research* 8:501-512.
- Cha-Um, S., and Kirdmanee, C. 2010. Effect of glycine betaine on proline, water use, and photosynthetic efficiencies, and growth of rice seedlings under salt stress. *Turkish Journal of Agriculture and Forestry* 34:517-527.
- Cavusoglu, A., and Sulusoglu, M. 2015. Effects of gibberellic acid (GA<sub>3</sub>), indole-3-acetic acid (IAA) and water treatments on seed germination of *Melia azedarach* L. *Scientific Papers. Series B, Horticulture* 59:319-326.
- Hasanuzzaman, M., Hossain, M.A., da Silva, J.A.T., and Fujita, M. 2012. Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor//Crop stress and its management: Perspectives and strategies. p. 261-315. Springer, Dordrecht, The Netherlands.
- Hoai, N.T.T., Shim, I.S., Kobayashi, K., and Kenji, U. 2003. Accumulation of some nitrogen compounds in response to salt stress and their relationships with salt tolerance in rice (*Oryza sativa* L.) seedlings. *Plant Growth Regulation* 41:159-164.
- Ibrahim, A.H. 2004. Efficacy of exogenous glycine betaine application on sorghum plants grown under salinity stress. *Acta Botanica Hungarica* 43:307-318.
- Kaya, C., Tuna, A.L., and Okant, A.M. 2010. Effect of foliar applied kinetin and indole acetic acid on maize plants grown under saline conditions. *Turkish Journal of Agriculture and Forestry* 34:529-538.
- Kim, Y., Arihara, J., Nakayama, T., Nakayama, N., Shimada, S., and Usui, K. 2004. Antioxidative responses and their relation to salt tolerance in *Echinochloa oryzicola* Vasing and *Setaria viridis* (L.) Beauv. *Plant Growth Regulation* 44:87-92.
- Mahajan, S., and Tuteja, N. 2005. Cold, salinity and drought stresses: an overview. *Archives of Biochemistry and Biophysics* 444:139-158.
- Munns, R. 2002. Comparative physiology of salt and water stress. *Plant Cell and Environment* 25:239-250.
- Munns, R., and Tester, M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology* 59:651-681.
- Murillo, A.B., Lopez, A.R., Kaja, C., Larrinaga, M.J., and Flores-Hernandez, H.A. 2002. Comparative effects of NaCl and polyethylene glycol on germination, emergence and seedling growth of cowpea. *Journal of Agronomy and Crop Science* 188:235-247.
- Nimir, N.E.A., Zhou, G., Guo, W., Ma, B., Lu, S., and Wang, Y. 2017. Effect of foliar application of GA<sub>3</sub>, kinetin, and salicylic acid on ions content, membrane permeability, and photosynthesis under salt stress of sweet sorghum [*Sorghum bicolor* (L.) Moench]. *Canadian Journal of Plant Science* 97(3):525-535. doi.org/10.1139/cjps-2016-0110.
- Nyagah, A.H., and Musyimi, D.M. 2009. Effects of sodium chloride solution stress on germination and growth of passion fruits seedlings. *Agricultural and Biological Science Journal* 4:49-53.
- Parashar, A., and Varma, S.K. 1988. Effect of pre-sowing seed soaking in gibberellic acid, duration of soaking, different temperatures and their interaction on seed germination and early seedling growth of wheat under saline conditions. *Plant Physiology and Biochemistry* 15:189-197.
- Parida, A.K., Das, A.B., and Mohanty, P. 2004. Investigations on the antioxidative defense responses to NaCl stress in amangrove, *Bruguiera parviflora*: differential regulations of iso-forms of some antioxidative enzymes. *Plant Growth Regulation* 42:213-226.
- Pedranzani, H., Racagni, G., and Alemano, S. 2003. Salt tolerant tomato plants show increased levels of jasmonic acid. *Plant Growth Regulation* 41:149-158.
- Qin, J., Dong, W.Y., He, K.N., Yu, Y., Tan, G.D., Han, L., et al. 2010. NaCl salinity-induced changes in water status, ion contents and photosynthetic properties of *Shepherdia argentea* (Pursh) Nutt. seedlings. *Plant, Soil and Environment* 56:325-332.
- Ratanavathi, C.V., Dayakar, R.B., and Seet, H.N. 2004. Sweet sorghum: A new raw material for fuel alcohol, in study report on technological aspects in manufacturing ethyl alcohol from cereal grains in Maharashtra. Part II. Department of Scientific & Industrial Research, Ministry of Science & Technology, Government of India, New Delhi and Mitcon Consultancy Services Limited, Pune, India.
- Romero, A.R., Soria, T., and Cuartero, J. 2001. Tomato plant water uptake and plant water relationships under saline growth conditions. *Plant Science* 160:265-272.
- Saberali, S.F., and Moradi, M. 2017. Effect of salinity on germination and seedling growth of *Trigonella foenum-graecum*, *Dracocephalum moldavica*, *Satureja hortensis* and *Anethum graveolens*. *Journal of the Saudi Society of Agricultural Sciences* (In Press).
- Srivastava, N.K., and Srivastava, A.K. 2007. Influence of gibberellic acid on <sup>14</sup>CO<sub>2</sub> metabolism, growth and production of alkaloids in *Catharanthus roseus*. *Photosynthetica* 45:156-160.
- Sudhir, P., and Murthy, S.D.S. 2004. Effects of salt stress on basic processes of photosynthesis. *Photosynthetica* 42:481-486.
- Sun, T.P. 2008. Gibberellin metabolism, perception and signaling pathways in Arabidopsis. *The Arabidopsis Book* 6:e0103.
- Tang, Q.Y., and Feng, M.G. 1997. Practical statistics and DPS data processing system. China Agricultural Press, Beijing, China [in Chinese].

- Tavakkoli, E., Rengasamy, P., and Mc Donald, G.K. 2010. High concentrations of Na<sup>+</sup> and Cl<sup>-</sup> ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. *Journal of Experimental Botany* 61:4449-4459.
- Tuna, A.L., Cengiz, K., Murat, D., and David, H. 2008. The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. *Environmental and Experimental Botany* 62:1-9.
- Willenborg, C.J., Gulden, R.H., Johnson, E.N., and Shirliffe, S.J. 2004. Germination characteristics of polymer-coated canola (*Brassica napus* L.) seeds subjected to moisture stress at different temperatures. *Agronomy Journal* 96:786-791.
- Yang, C.J., and Li, G.Y. 2014. Effect of NaCl stress on germination of birch seeds. *Chemical and Pharmaceutical Research* 6:1980-1986.
- Yildirim, E., Karlidag, H., and Turan, M. 2009. Mitigation of salt stress in strawberry by foliar K, Ca and Mg nutrient supply. *Plant, Soil and Environment* 55:213-221.
- Zhou, G., Nimir, N., Lu, S., Zhai, F., and Wang, Y. 2014. Gibberellic acid and salinity affected growth and antioxidant enzyme activities in castor bean plants at early growth stage. *Agronomy Journal* 106(4):1340. doi:10.2134/agronj14.0044.