Bangladesh Agron. J. 2022, 25(1): 115-128

GERMINATION AND GROWTH PERFORMANCE OF SEEDLINGS OF ASCORBIC ACID, SILICON AND GIBBERELLIC ACID TREATED SECONDARY SEED OF WHEAT UNDER SALT STRESS

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(**Received:** 16 June 2022, **Accepted:** 23 June 2022)

Keywords: wheat, salt stress, ascorbic acid, silicon, gibberellic acid

Abstract

Considering the effect of salt stress on morph-physiological and biochemical changes of wheat (*Triticum aestivum* L. var. BARI Gom-26) as well as mitigation of the adverse effect through exogenous application of Ascorbic Acid (AsA), Silicon (Si) and Gibberellic Acid (GA3), the experiment was conducted at Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. In the field experiment, four levels of salt stress (0, 50, 80, 120 mM NaCl) were applied at 20 days after sowing and grown up to harvest. AsA (2 mM ascorbic acid), Si (200 µM SiO_2), GA3 (100 µM gibberellic acid) were applied as foliar spraying at 20 days interval. Seeds were collected from the field experiment which used as secondary seeds as planting materials for second experiment to evaluate the influence of AsA, Si and GA3 on growth performance and physiological attributes of seedlings under salt stress. Experiment revealed that AsA, Si and GA3 enhanced the germination and growth performance of seedling under salinity stress. Overall, GA3 significantly increased the seed germination (%) and seedling growth parameters, while silicon mostly improved the fresh weight and chlorophyll (*a*, *b* and *a*+*b*) and AsA showed better relative water contents with other parameters. Considering the results of experiments, GA3 performed better than the AsA and Si in mitigating salt stress.

Introduction

Among abiotic stresses, salt stress is one of the critical determinants limiting crop production (Wassmann *et al*., 2009). About 20% of the world total agricultural areas are affected by salinity (FAO, 2015). It is expected that, by the middle of the twenty-first century, 50% of the world's arable area will be influenced by salinity (Machado and Serralheiro, 2017).

Saline conditions crucially influence various plant development phases; germination and early seedling are acknowledged as more delicate growth stages in most plant species (Ibrahim *et al*., 2016). Higher salinity induced both ionic and osmotic stresses in the plant that causes growth inhibition, yield reduction and even death of the plant (Hasanuzzaman *et al*., 2013; Nahar *et al*., 2016). The immediate response of plants to higher salinity is osmotic stress that reduces cell expansion, cell division, and stomatal closure consequently inhibits leaf area, photosynthesis and growth of the plant. In the later stage, plants experience ionic stress due to the accumulation of Na^+ and Cl^- at the toxic level. Higher accumulation of Na^+ causes premature senescence of adult leaves by disrupting protein synthesis and interfering with enzyme activity (Wu and Wang, 2012; Rahman *et al*., 2016a). Salt-induced osmotic stress, ionic toxicity and a lower rate of photosynthesis increase the production of reactive oxygen species (ROS). A higher composition of ROS interrupts the antioxidant defense system and hence causes oxidative stress (Hasanuzzaman *et al.*, 2013).

Mitigation of abiotic stress including salinity by using antioxidants is one of the common approaches for plant biologists in recent times. The antioxidants including ascorbic acid (AsA), play a significant role in the mitigation of additional cellular reactive oxygen species function caused by salinity stresses (Venkatesh and Park, 2014). Reduced Ascorbic acid (AsA) is the non-enzymatic antioxidant component that plays a vital role in the AsA-GSH cycle. As an efficient electron donor, AsA directly reacts with ROS (Hasanuzzaman *et al*., 2011). Enhanced synthesis of AsA plays a crucial role in conferring stress tolerance by detoxifying ROS and maintaining other antioxidants GSH, α-tocopherol etc (De Tullio *et al*., 2004).

The effect of plant elements in mitigating the effect of abiotic stress in plants including salinity has become a matter of interest in recent decades. Among them, silicon (Si) is the second most abundant element in the soil. Although it is a non-essential element, it has some beneficial role for plant growth and development. The beneficial role of Si plays a role against various abiotic stresses (Srivastava *et al*., 2015). Silicon reduces Na^+ uptake acting as a mechanical barrier. Accumulation of Si by plant reduces transpiration through deposition in the cell wall of leaves which decreases Na^+ uptake and transpiration. Also, Si application improves salt stress tolerance by improving the water status of the plant (Wang *et al*., 2015). Moreover, Si mitigates salt-induced oxidative stress by detoxifying ROS by regulating the antioxidant defense system (Zhu and Gong, 2014).

Of the plant hormones, gibberellic acid (GA3) helps enhance plant growth and development under abiotic stress conditions (Ryu and Yong-Gu, 2015). The exogenous application of GA3 developed sufferance under abiotic stress by induction and developing of the endogenous levels of salicylic acid (Alonso- Ramírez *et al*., 2009). Moreover, seed priming with GA3 alleviate the drastic effect of salinity and increase grain weight and grain quality by improving photosynthetic pigments, leaf area and plant growth (Shaddad *et al*., 2013). Foliar applications of GA3 also confer salt stress tolerance by increasing germination percentage, plant growth and up regulating antioxidant enzyme (Tabatabei, 2013). A different study showed that GA3 has a significant outcome on various of the biological processes that happen in plants, before-mentioned as better germination percentage in various plants under natural circumstance (Al Sahil, 2016).

Although many studies revealed the mechanisms, by which AsA, Si and GA3 alleviate salt stress through ionic and osmotic homeostasis, regulating antioxidant defence and another biochemical process, it was necessary to further study to understand the mechanisms how they alleviate salt-induced damages in wheat.

Materials and Methods

Plant materials and growth conditions

The experiment was conducted at Plant Physiology Laboratory, Sher-e-Bangla Agricultural University, Dhaka (90°77´ E longitude and 23°77´ N latitude) during October to December, 2018. Wheat (*Triticum aestivum* L. var. BARI Gom-26) seeds were collected from the previous experiment where ascorbic acid (2 mM), silicon (200 µM SiO_2) and gibberellic acid (100 µm)were exogenously applied under different level of salt stress (0, 50, 80 and 120 mM NaCl). The collected seed of the previous experiment was named as secondary seed. The secondary seed of BARI Gom-26 were surface sterilized with 70% ethanol for 8–10 min for sterilization. Seeds were rinsed several times with distilled running water. Fifty seeds were sown in hydroponic plates with 250 ml of distilled water. The growth performance and physiological attributes data were collected from 10 days old seedling.

Germination (%)

A germination test was carried out in the Plant Physiology Laboratory. Fifty seeds were soaked in water for one hour then spread on a layer of moistens filter paper in the Petri dish. The lid was put on the Petri dish and placed in the dark room and recorded the number of seeds that germinate daily. Germination % was determined by using the following formula:

$$\text{Germination \%} = (\text{Seeds germinated} / \text{Total seed}) \times 100$$

Measurement of fresh and dry biomass, relative water content and chlorophyll (chl) content

For fresh weight (FW) and dry weight (DW) measurement, 10 seedlings from each treatment were selected and uprooted carefully, weighed and deliberated as FW. DW was determined after drying the seedlings at 80 °C for 48 h. Leaf relative water content was measured in accordance with Barrs and Weatherly (1962). Leaf laminas were weighed (FW) then placed immediately between two layers of filter paper and submerged in distilled water in a Petri dish for 24 h in a dark place. Turgid weight (TW) was measured after gently removing excess water with a paper towel. Dry weight was measured after 48 h oven drying at 70 °C. Leaf relative water content (RWC) was determined by using the following formula:

$$\text{RWC (\%)} = (\text{FW}-\text{DW}) / (\text{TW}-\text{DW}) \times 100$$

Chlorophyll (chl) content was measured as a fresh leaf sample of 0.25g was taken from randomly selected seedlings to measure the chlorophyll content. The samples were homogenized with 10 ml of acetone (80% v/v) using pre-cooled pestle and mortar and the homogenate was centrifuged at 10,000 × g for 10 min. The absorbance of the supernatants was measured with a UV visible spectrophotometer at 663 and 645 nm for chl *a* and chl *b* respectively. The amount of chlorophyll contents was calculated using the equations suggested by Arnon (1949).

Chlorophyll *a* (µg/ml) = 12.7 (A663) - 2.69 (A645)
Chlorophyll *b* (µg/ml) = 22.9 (A645) - 4.68 (A663)
Total chlorophyll (µg/ml) = 20.2 (A645) + 8.02 (A663)

Shoot and Root length

Seedlings were taken randomly and shoot-root length was measured in centimeters with the scale.

Root shoot ratio

Length basis root-shoot ratio was calculated by following the method of Khandakar (1980) to estimate root efficiency to support production.

$$\text{Root-shoot ratio (\%)} = \frac{\text{Root length}}{\text{Shoot length}} \times 100$$

Vigor index

Seedling vigor index was calculated by using by the formula of Abdul-Baki and Anderson (1973).

$$\text{Vigor index (VI)} = \text{Germination (\%)} \times \text{Seedling length (cm)}$$

Survivability (%)

$$\text{Survivability (\%)} = \frac{\text{Number of servable seedling}}{\text{Total number of seed}} \times 100$$

Statistical analysis

The data obtained for different parameters, mean values of each parameter were calculated and subjected to based CoStat v.6.400 (CoStat, 2008) software. The average of three replications (n = 3) was used to determine mean (± SD) for each treatment and mean differences were compared using LSD (0.05) test.

Results and Discussion

Germination percentage

In this study, germination percentage significantly increased in secondary seed with previously exogenous application of GA3 under minimal or no salt stresses (0 mM and 50 mM) (Figure 1). Neither higher salt stress 80 mM or 120 mM showed significant variations with the application of GA3. Germination percentage significantly increased 1.33% and 1.66% in control and 50 mM NaCl, respectively in the secondary seeds of previously exogenous application of GA3 in salinity stress. However, results showed that the percentage of germination of secondary seed decrease collaterally with the increase of salt stresses throughout the treatments. In the present study, germination percentage increased in secondary seeds. These results revealed that secondary seeds from saline conditions with exogenous application of AsA, Si, and GA3 increased seed performances. Usually, salt stresses reduce photosynthetic parameters, chlorophyll content and stomatal opening, and hinder the normal growth of plant, while affecting the proper seed development (Neill *et al*., 2002; Netondo *et al*., 2004).

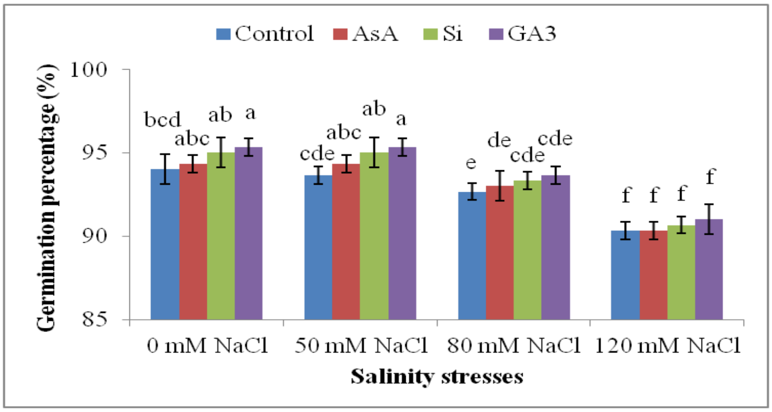

Fig. 1. Germination percentage of salt-stress induced secondary seeds from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM SiO_2 and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Relative water content

Relative water content of seedlings of secondary seed showed insignificant variations. RWC of seedlings of secondary seeds previously treated in 100 mM NaCl with different mitigating agents (AsA, 2 mM), Si, 200 µM) and GA3, 100 µM), while GA3 showed significant results in control and AsA in 50 mM NaCl, respectively (Figure 2). The highest increase of RWC of secondary seedling was observed in previously treated 50 mM NaCl by AsA application under salt stressed. RWC is considered as an important parameter for evaluating plants for tolerance to salinity stress (Boyer *et al*., 2008). In this study, salinity stress led to a significant decrease of RWC in wheat with increasing NaCl concentration (Figure 2). In the previous studies, the RWC of the plant decreased under salinity stress conditions in a diverse group of plants (Polash *et al*., 2018; Keyvan, 2010). Decrease in RWC was due to loss of turgor that results in physiological drought for cell extension processes (Shamsi and Kobraee, 2013). However, when salt treated wheat plants were supplemented with AsA, Si and GA3, plant showed increased RWC which was due to the retention in water in their tissue (Figure 2). The enhanced water content in plants due to exogenous application of protectants was also observed by other researchers (Hasanuzzaman *et al*., 2013).

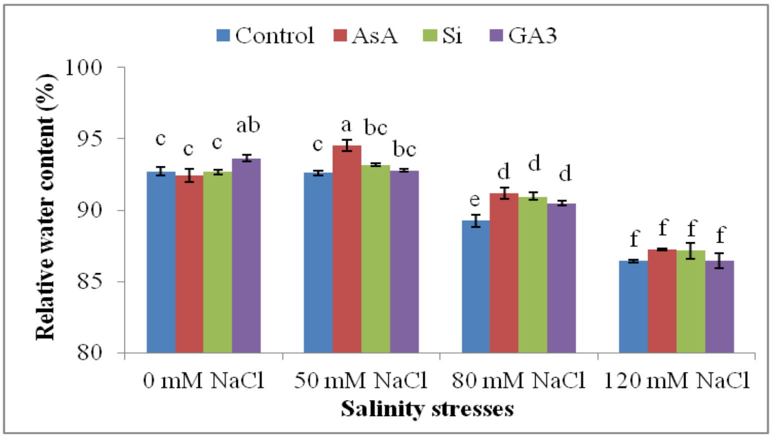

Fig. 2. Relative water content of salt stress induced secondary seeds from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM SiO_2 and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Growth Parameter

Shoot and root length

Shoot length of seedlings from secondary seed significantly increased (4.0%, 7.4%, 9.5% and 10.6%, respectively) with previously exogenous application of GA3 under different salinity level (Figure 3). Shoot lengths were higher in lower or minimal salt stressed. The exogenous application of AsA (2 mM), Si (200 µM) and GA3 (100 µM) in previous experiment under different salt stress improved the shoot length of seedling derived from secondary seeds. In addition, shoot lengths were decreased respectively in previously increased doses of salt treated seeds.

Root length of seedlings from secondary seeds were significantly increased with previously exogenous application of Si (200 µM) and GA3 (100 µM) under different salt stresses. The highest results (9.6%, 19%, 13% and 8.5% increase under 0, 50, 80 and 120 mM NaCl) were found from the GA3 (100 µM) application under all the salt stresses (Figure 4). Results showed the similar type of reduction in root length as shoot length under salt stresses.

Salinity stress restricts plant growth due to osmotic stress and ionic toxicity (Munns *et al.*, 2006). Under salt stress, root cells may lose water instead of absorbing it due to the hyperosmotic pressure of the soil solution. As a result physiological drought induced by salinity stress, resulting in decreased cell elongation, cell expansion, wilting and ultimately, decreased plant shoot and root growth (Munns and Tester, 2008). However, supplementation of osmoprotectants and synthetic plant growth regulator like AsA, Si and GA3 increased adaptation of wheat cultivars to salinity to some extent. This may be achieved through osmoregulation which in turn increased osmotic adjustment by increasing water flow and water status by using the organic solutes (Islam and Mehraj, 2014).

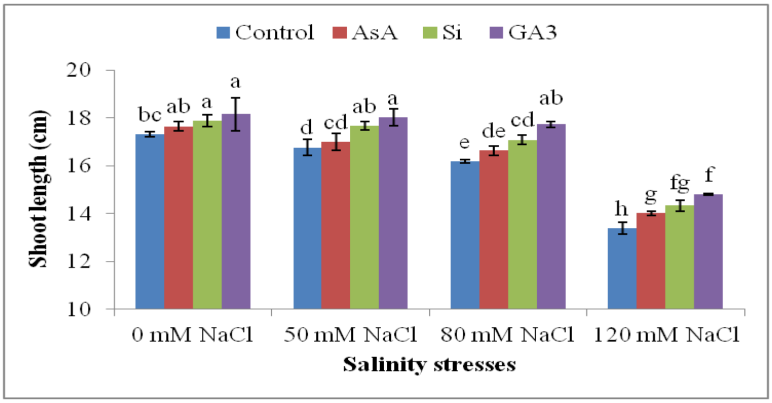

Fig. 3. Shoot length of salt stress induced secondary seeds from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM SiO_2 and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

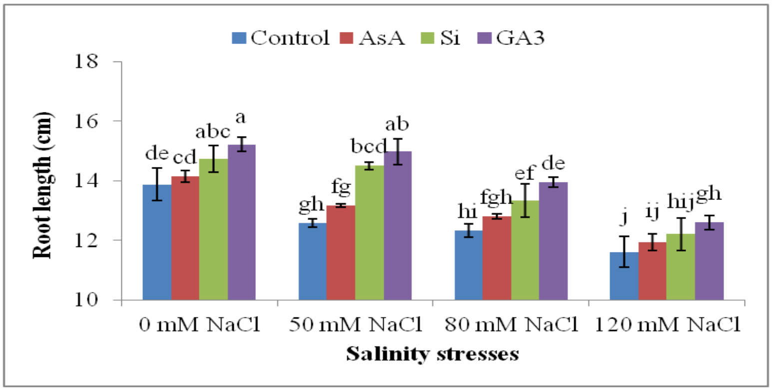

Fig. 4. Root length of seedlings of secondary seeds induced different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM SiO_2 and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Root-shoot ratio

The seedlings of secondary seeds showed significant increase (10.8%) in root-shoot ratio. The highest root-shoot ratio was found in previously applied 120 mM NaCl conditions. Previously applied GA3 (100 µM) under 120 mM NaCl treated secondary seeds showed effective result, while other doses of mitigating agents AsA (2 mM), Si (200 µM) and GA3 (100 µM)) were found ineffective under different salt stressed seeds (Figure 5). The root-shoot ratio increased simultaneously with increased salt stresses in every previously treated seeds.

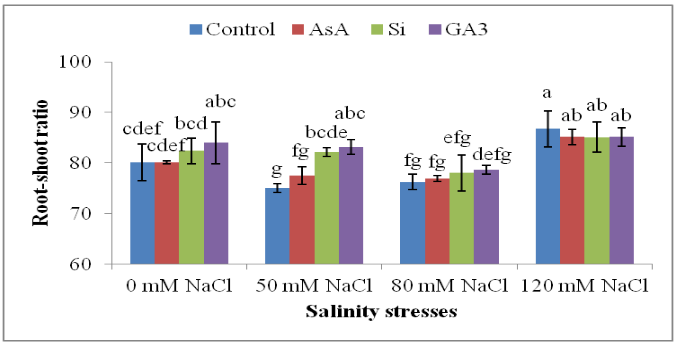

Fig. 5. Root-shoot ratio of seedlings of secondary seeds derived from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM SiO_2 and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Fresh and dry weight seedling^{-1}

Fresh weight of seedlings of secondary seed derived from different salt treated plant showed significant variations with different mitigating agents AsA (2 mM), Si (200 µM) and GA3 (100 µM). The highest increases in FW were observed by Si (3.84%) in 80 mM NaCl and GA3 (12.5%) under 120 mM NaCl salt stressed secondary seedlings (Figure 6). Fresh weight in previously treated plants seeds were decreased with the increased salt stresses.

Dry weight of previously affected salt stressed seedlings of secondary seed showed significant variation and increased by Si (200 µM) and GA3 (100 µM). Exogenous application of salt stress mitigating agent Si (200 µM) increased the seedling DW by 9.52% under 50 mM NaCl and 80 mM NaCl salt stressed conditions, while GA3 (100 µM) increased DW of salt stressed secondary seedlings by 12.5% under 120 mM salt stress (Figure 7). Dry weights in different treatments were also decreased with the increased salt stresses as like as FW of seedlings.

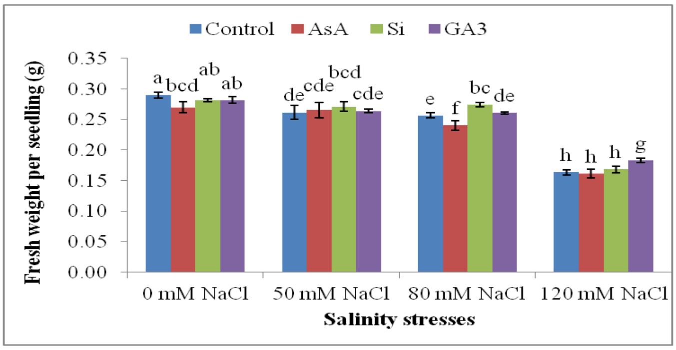

Fig. 6. Fresh weight per seedling of salt stressed secondary seedlings from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM SiO_2 and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Reduction of biomass, a significant disturbance in photosynthetic parameters along with reduced chlorophyll contents, reduction of stomatal conductance, photosynthesis rates, and inhibition of metabolic phenomena, and increased ROS generation which can increase oxygen-induced cellular damage (Neill *et al*., 2002). However, previously application of GA3, AsA and Si increased biomass of secondary seedlings. GA3 is plant hormones that are associated with various plant growth and development processes. GA3 plays a central role in tolerance to salinity stress by improving the activity of antioxidant enzymes and preventing lipid peroxidation, increased the accumulation of proline and potassium and increased the different physiological parameters, ultimately produced biomass (Miceli *et al*., 2019; Alsudays *et al*., 2020). On the other hand, application of AsA helps to uptake nutrients (N, P and K), protects metabolic processes against H_2O_2 and other toxic derivatives of oxygen affected many enzyme activities, minimize the damage caused by oxidative processes through synergistic function with other antioxidants and stabilize membranes (Farahat *et al*., 2013). Furthermore, it was showed that Si supplementation ameliorated the adverse effects of NaCl on plants growth, biomass, and oxidative stress by increasing the content of proline and cytokinin (Zhu *et al*., 2020). Si supplement could induce salt tolerance in plants by improving photosynthesis, membrane integrity, and detoxification of toxic radicals and elevating its antioxidant capacity under salinity stress (Robatjazi *et al*., 2020).

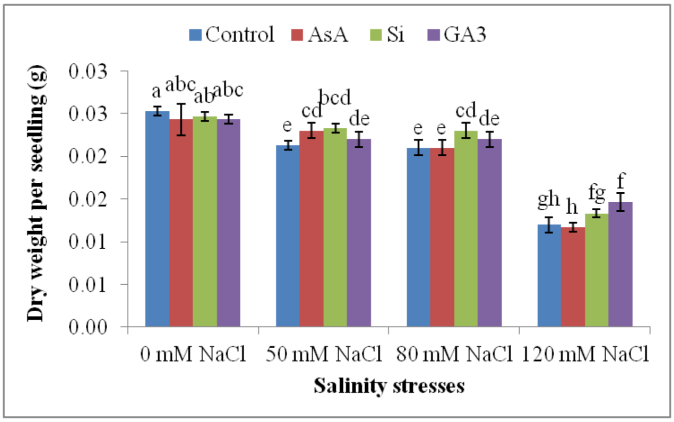

Fig. 7. Dry weight per seedling of salt stressed secondary seedlings from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM SiO_2 and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Vigor index

Salt stress mitigating agents (AsA, 2 mM; Si, 200 µM and GA3, 100 µM) previously treated salt stressed secondary seed showed significant increase in seedling vigor index. AsA treated plants seeds increased 2.27, 3.54, 3.61, and 3.83%, respectively; Si treated plants seeds increased 5.63, 11.17, 7.41 and 6.55%, respectively and GA3 treated plants seeds increased 8.49, 14.45, 12.26 and 10.43%, respectively in 0, 50, 80 and 120 mM NaCl stresses (Figure 8). The highest vigor indexes were found from the GA3 (100 µM) treated plants seeds in all the salt stresses.

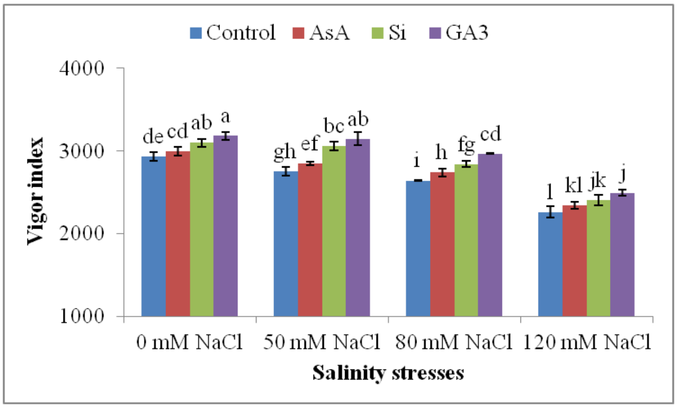

Fig. 8. Vigor index of previously salt treated seedlings of secondary seeds from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM SiO_2 and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Survivability percentage

There were no significant variations in survivability percentages with mitigating agents (AsA, 2 mM; Si, 200 µM and GA3, 100 µM) in different slat stressed secondary seeds except for 120 mM NaCl treatment. Survivability percentages increased 3.74% and 4.68% by AsA (2 mM) and GA3 (100 µM), respectively (Figure 9). However, survivability percentages decreased with increased salt stresses in salt stressed secondary seeds.

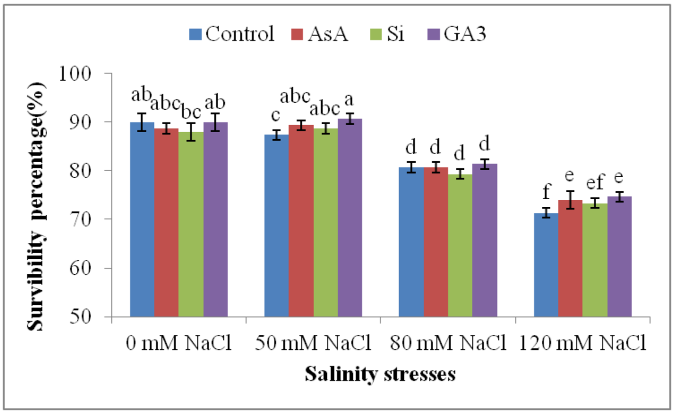

Fig. 9. Survivability percentage of previously salt treated seeding of secondary seeds from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM SiO_2 and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Chlorophyll content

In this experiment, chlorophyll (*a*, *b* and *a*+*b*) of salt stressed secondary seedlings did not show any significant variation with the application of salt stress mitigating agents (AsA, 2 mM), Si, 200 µM) and GA3,100 µM)) under previously four different salt stress conditions including control treatment (0, 50, 80 and 120 mM) (Table 1).

Table 1. Chlorophyll (*a*, *b* and *a*+*b*) of salt stressed secondary seeds of different salinity level comprising of salt stress mitigating agents.

Treatments	chl *a* (nmol/g DW)	chl *b* (nmol/g DW)	chl (a+b) (nmol/g DW)

Control	0.67 ± 0.02 ab	0.38 ± 0.02 ab	1.06 ± 0.01 ab
AsA	0.66 ± 0.01 abc	0.39 ± 0.03 ab	1.05 ± 0.02 abc
Si	0.69 ± 0.02 a	0.41 ± 0.03 a	1.10 ± 0.02 ab
GA3	0.67 ± 0.01 ab	0.38 ± 0.04 ab	1.05 ± 0.03 ab
50 mM NaCl	0.68 ± 0.01 a	0.40 ± 0.02 ab	1.08 ± 0.01 ab
50 mM NaCl + AsA	0.67 ± 0.01 ab	0.40 ± 0.25 ab	1.07 ± 0.03 ab
50 mM NaCl + Si	0.70 ±0.02 a	0.41 ± 0.04 a	1.11 ± 0.02 a
50 mM NaCl + GA3	0.68 ± 0.01 a	0.39 ± 0.04 ab	1.07 ± 0.04 ab
80 mM NaCl	0.67 ± 0.05 ab	0.37 ± 0.01 bc	1.04 ± 0.05 abc
80 mM NaCl + AsA	0.66 ± 0.05 abc	0.37 ± 0.01 bc	1.03 ± 0.06 bc
80 mM NaCl + Si	0.69 ±0.05 a	0.40 ± 0.02 ab	1.08 ± 0.05 ab
80 mM NaCl+ GA3	0.67 ± 0.05 ab	0.36 ± 0.03 bcd	1.03 ± 0.06 bc
120 mM NaCl	0.60 ± 0.04 cd	0.33 ± 0.01 cd	0.93 ± 0.05 d
120 mM NaCl + AsA	0.58 ± 0.05 d	0.33 ± 0.01 cd	0.92 ± 0.07 d
120 mM NaCl + Si	0.61 ± 0.04 bcd	0.36 ± 0.01 bcd	0.97 ± 0.05 cd
120 mM NaCl + GA3	0.60 ± 0.04 cd	0.32 ± 0.02 d	0.92 ± 0.07 d
LSD$_{(0.05)}$	**0.029**	**0.020**	**0.037**
CV (%)	**5.57**	**6.70**	**4.38**

AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM SiO_2 and 100 µM gibberellic acid, respectively. Means (±SD) were calculated from three replications (n = 3) for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

However, the application of salt stress mitigation agent Si (200 µM) in showed simultaneous increase in chlorophyll (*a*, *b* and *a*+*b*) under different salt treatments in secondary seedlings. The highest increase in chlorophyll *a* (2.23, 2.20, 2.08 and 2.5% in 0, 50, 80 and 120 mM, respectively), chlorophyll *b* (7.74, 4.68, 7.68 and 9.02% in 0, 50, 80 and 120 mM, respectively) and chlorophyll *a+b* (4.18, 3.11, 4.10 and 4.76% in 0, 50, 80 and 120 mM, respectively) were found in salt stressed secondary seedlings from previously treated plants by Si (Table 1). In addition, the lowest Chlorophyll *a*, *b* and *a+b* were found from the highest salt stress 120 mM). Salinity stress often causes alteration in photosynthetic pigment biosynthesis (Cuin *et al*., 2010). Kiani-Pouya and Rasouli (2014) reported that the chlorophyll content decreased significantly under severe salinity stress in wheat. However, exogenous application of AsA, Si and GA3 in salt treated plant increased chl content (Tahir *et al*., 2012).

Conclusion

The application of salt stress mitigating agents like AsA, Si and GA3 all together improve the plant growth and development as well as enhance the secondary seed performances. In this study seeds and seedlings performances from the previously exogenous applied salt stress mitigating agents in wheat plant's seeds showed significant variations in germination percentage, shoot length, root length, fresh and dry weight seedling^{-1} and vigor indexes. However, the performances of secondary seed and seedlings in this study elucidates that exogenous application of salt stress mitigating agents may significantly reduce the stress impacts in wheat plants under different salt stresses as well as in secondary seeds during their germination and seedling growth. In this study, secondary seeds from GA3 application in salt stressed plants significantly have increased the seed germination and seedling growth parameters, while silicon mostly improved the fresh weight and chlorophyll (*a*, *b* and *a*+*b*) and AsA showed better relative water contents with other parameters in secondary seeds. Preceding research hardly explain the secondary seeds performances, thus further research is necessary on this topic. The results of this study have evaluated the secondary seeds performances which plants treated by salt stress mitigating agents during salt stress conditions.

Acknowledgement

The authors are thankful to Dr. Mirza Hasanuzzaman, Professor, Department of Agronomy, Sher-e-Bangla Agricultural University for providing necessary facilities and successful execution of laboratory work to conduct the study.

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