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Effects of Gibberellic Acid and Kinetin on Germination and Ion Accumulation in a Bangladesh Wheat Variety Under Salt Stress Conditions

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Abstract: Salinity is the major environmental stress that restricts on agricultural productivity in arid and semiarid regions by a reduction in the germination rate. Experiments were carried out to assess the role of gibberellic acid (GA_3) and kinetin on germination and ion accumulation in a Bangladesh wheat (*Triticum aestivum* L.) variety, namely Akbar under salt stress conditions. Increasing salt (NaCl) stress conditions consistently decreased the rate of germination of wheat. Gibberellic acid alone or in combination with kinetin alleviated the inhibitory effects of salinity on germination. However, kinetin alone further decreased the rate of germination under salt stress. Salt (NaCl) stress increased the accumulation of Na^+ and Cl^- while it decreased K^+ accumulation in germinating seeds. Gibberellic acid caused an increase in K^+ accumulation and a decrease in Na^+ and Cl^- accumulation in the germinating seeds. Kinetin increased Cl^- accumulation and decreased K^+ accumulation in salinity stressed wheat seedlings. Therefore, GA_3 prominently relieved salt stress and improved the seed germination of wheat.

Keywords: Gibberellic Acid, Kinetin, Ion Accumulation, Salinity, Wheat

1. Introduction

Salinity and inadequate precipitation are the main factors limiting agricultural production in arid and semi-arid regions around the world [1]. The detrimental effects of salt stress on cultivated land have influenced ancient and modern civilizations. Nearly 20% of the irrigated land is affected by salinity globally [2]. More than 30% of cultivable land in Bangladesh is located in the coastal belts, where about of 1.2 million hectares is affected by different degrees of salinity and the area is increasing with the impact of climate change [3]. Due to increasing salinity and a rapid increase in population, Bangladesh will face a large deficit in food grain in near future [4]. To increase agricultural production, more land must be brought under cultivation and/or productivity per unit area must be increased [5]. However, the possibility for increasing cultivation area is minimal under Bangladesh conditions.

Chloride (Cl^-) and sodium (Na^+) are the most common ions associated with saline conditions. Salinity damage is commonly associated with excessive Na^+ and Cl^- uptake by the plant [6]. Both ions are generally absorbed in the same way by plants and contribute to salt toxicity synergistically. Thus, maintaining low levels of sodium in leaves is linked to salt tolerance in wheat and other species. [7]. However, it is also well known that NaCl toxicity is largely attributable to the effect of Na^+, and only rarely those of Cl^- [8]. Sodium (Na^+) toxicity is also strongly linked to the plant's ability to sustain the acquisition and distribution of K^+ in plants [9].

According to Debez *et al.* [10] the inhibitory effect of salinity on germination could be related to a decline in endogenous levels of hormones. Inclusion of plant growth regulators at the start of pre-soaking and other pre-plant treatments of many crops has shown better seed performance. The phytohormones send signal from roots to shoot when the roots perceived abiotic stresses in its surrounding [11].

Wheat is the 2nd most important cereal after rice in Bangladesh. Demand for wheat is increasing at an increasing rate in Bangladesh for its large use in diverse food items. Though the wheat is largely grown in the northern part of Bangladesh, it is now being expanding in the southern part where salinity is a threat for potential crop harvest. Therefore, research on various issues for reducing harmful effects of salinity on wheat productivity is a time demanding in Bangladesh context. Therefore, the present study was aimed to investigate the role of GA_3 and kinetin either alone or in combination on germination and ion accumulation in wheat (cv. Akbar) under saline condition. Akbar is a popular wheat variety in Bangladesh which was found relatively salt tolerant in a study of Akhtar [12].

2. Materials and Methods

2.1. Plant Material

Triticum aestivum L. cv. Akbar was used as plant material. The seeds were obtained from Bangladesh Agricultural Research Institute, Gazipur, Bangladesh. The variety is popularly cultivated in the farmers' field of Bangladesh and also found relatively salt tolerant in the laboratory experiment [12].

2.2. Treatments

Five different levels of NaCl solutions i.e. 0, 50, 100, 200 and 300 mM were used in this experiment. Each salinity treatment was also contained 0.1 mM $CaSO_4$ to remove free space ions [13]. Gibberellic acid and kinetin (both) were applied at 10^{-6} M and 10^{-5} M. The concentration of GA and kinetin was determined by conducting preliminary experiment at the Jahangirnagar University.

2.3. Method of Germination of Seeds

The seeds were surface sterilized to avoid fungal infection by soaking the seeds with 0.1% sodium hypochlorite for three minutes followed by washing 7 to 8 times with tap water and three times with distilled water. Fifty surface sterilized seeds were placed on Whatman filter paper No.1 in a petridish (11 cm). Three replications were used for each treatment. Filter papers were soaked with 7 ml of respective solutions i.e. 0, 50, 100, 200 and 300 mM NaCl. In case of control (0 mM NaCl), the filter paper was soaked with 7 ml of 0.1 mM $CaSO_4$. The seeds were allowed to be germinated in dark at 25°C ± 1°C /16°C ± 1°C day/night temperature respectively, in a growth cabinet (Model EA-7BH, Environ AIR Sydney, Australia). Seeds were considered to be germinated when radicle and plumule could be clearly distinguished. The germination of seed was recorded at 24, 48, 72 and 96 hours after the seeds place for germination.

2.4. Application of GA_3 and Kinetin Either Alone or Combination

In order to study the influence of GA_3 and kinetin on germination under saline conditions, seeds were allowed to germinate in 100, 200, 300 mM NaCl solution with or without 10^{-6} M and 10^{-5} M GA_3 or kinetin. Germination technique was the same as described in above.

2.5. Methods of Extraction and Analysis of Na^+, K^+ and Cl^- in Plumule and Radicle

Seeds (those germinated after 48 hours of soaking with 100, 200 mM NaCl with or without GA_3 and/or kinetin) were used for analysis of Na^+, K^+ and Cl^- ions in plumule and radicle. Plumules and radicles were separated from cotyledons. The radicle was washed with 0.1 mM $CaSO_4$. Plumules and radicles were then dried in an oven at 80°C till a constant weight reached. Ions were extracted from plumule and radicles according to the method of Karmoker and Van Steveninck [13]. Na^+ and K^+ ions were measured by flame photometer (Gallenkamp-model FGA 330-C) at a wavelength of 589 nm (for Na^+) and 767 nm (for K^+). $C1^-$ was measured by titrametric method using potassium chromate as an indicator.

3. Results and Discussion

3.1. Effects of Salinity on Germination

Salt (NaCl) stress (50-300 mM) decreased the rate of germination of wheat. The inhibition of rate of germination was directly proportional to the concentration of NaCl used and sudden decreased over 250 mM (Figure 1). This result is in agreement with the work of Rahman *et al.* [14]. Earlier reports showed that salinity decreased the rate of germination in wheat [15], Triticale [16] and in maize [17]. Accumulation of Na^+ and Cl^- in plumule and radicle increased with the increase in NaCl salinity concentration with a consistent inhibition of germination (Table 1). It is apparent from this result that accumulation of excessive amount of Na^+ and Cl^- in germinating seed is responsible for inhibition of germination under saline conditions. On the contrary, salt stress decreased the amount of endogenous K^+ of plumule and radicle (Table 1) which had an adverse effect on the growth of the embryo and thus further enhanced the inhibition of germination rate.

Salinity-induced inhibition of germination may be due to an increase in salinity- induced abscisic acid (ABA) level in germinating seeds [18]. ABA-induced inhibition of germination under saline condition occurs by preventing mobilization of reserve food, which may indicate germination depends on it [19]. Thus, salinity induced increase in accumulation of ABA may lead to observe the inhibition of germination rate. K^+ content in the plumule and radicle was inhibited by salt stress (Table 1) which may be due to an increase in K^+ efflux as a result of an increase in ABA level in salt stressed plant [20]. This result is consistent with the findings of Begum *et al.* [21] who stated that ABA affected K^+/Na^+ selectivity in beet root disc.

Table 1. *Effects of NaCl-salinity stress on rate of germination and accumulation of Na^+, K^+ and Cl^- in wheat (var. Akbar).*

	Ion accumulation (mequiv. g^{-1} dry tissue)			
	Na^+	K^+	Cl^-	Germination percentage
0.1 mM $CaSO_4$	0.185 (0.05)	0.645 (0.05)	1.250 (0.20)	98 (0.10)
0.1 mM $CaSO_4$ + 100 mM NaCl	0.557 (0.02)	0.604 (0.03)	3.605 (0.05)	92 (0.10)
0.1 mM $CaSO_4$ + 200 mM NaCl	0.825 (0.50)	0.564 (0.04)	4.002 (0.06)	82 (0.50)

Each value is the mean of five replicates, and the standard deviation is shown in parenthesis.

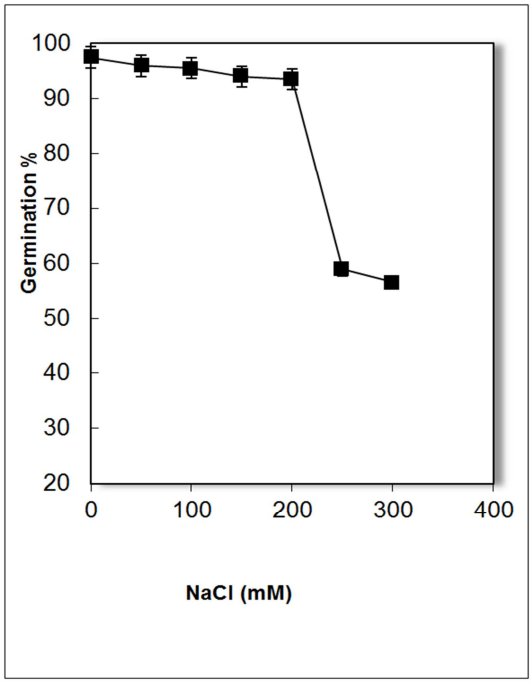
Moreover, inhibition of germination due to salinity as suggested in previous reports [22] was attributed to a decrease water content, that affect the synthesis of hydrolytic enzymes such as amylase limiting the hydrolysis of food reserves from storage tissues as well as to impaired translocation of food reserves from storage tissue to developing embryo axis [23].

3.2. Influence of GA_3 and Kinetin Either Alone or in Combination on Germination

Application of GA_3 (10^{-6} M, 10^{-5} M) along with NaCl (100 mM) nullified the inhibitory effect of salinity on germination in Akbar (Figure 2). Application of kinetin (10^{-6} M and 10^{-5} M) along with NaCl (100 mM) caused further inhibition of germination of Akbar as compared to that of 100 mM NaCl. Application of GA_3 and kinetin (10^{-6} M and 10^{-5} M) along with NaCl (100 mM) eliminated the inhibitory effect of salinity on germination in Akbar (Figure 2). When applied together with 200 mM NaCl and GA_3 (10^{-6} M and 10^{-5} M) caused 17% increase in germination of seeds of Akbar as compared to that of control (200 mM NaCl) (Figure 3). Application of 200 mM NaCl along with kinetin (10^{-5} M and 10^{-6} M) further decreased the rate of germination as compared to inhibition caused by 200 mM NaCl alone (Figure 3). The rate of germination was increased by 23% (10^{-5} M) and 20% (10^{-6} M) in Akbar when seeds were treated with 200 mM NaCl + GA_3 + kinetin as compared to control (200 mM NaCl) (Figure 3). A combination of 300 mM NaCl and GA_3 increased the rate of germination by 35% (10^{-5} M) and 37% (10^{-6} M) in Akbar (Figure 4) as compared to control (300 mM NaCl) (Figure 4). Interaction of 300 mM NaCl and 10^{-5} M GA_3 + 10^{-5} M kinetin caused 13% more inhibition of germination in Akbar as compared to that in 300 mM NaCl (control) (Figure 4).

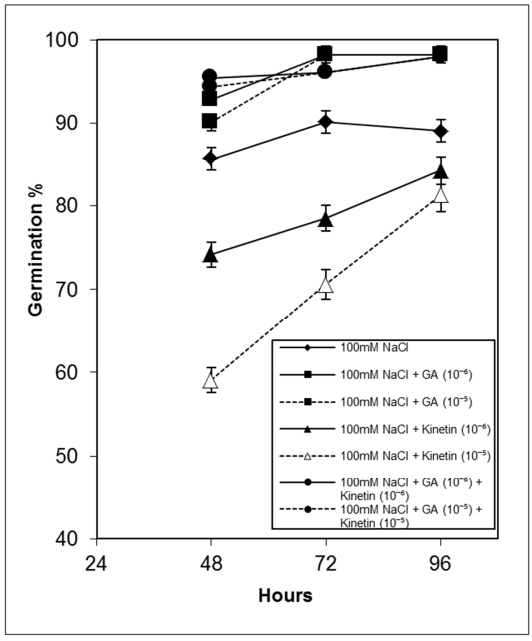
The alleviation of germination by GA_3 in saline condition (Figures 2 to 4) may be due to GA_3-induced decrease in Na^+ and Cl^- accumulation in germinating seeds (Tables 2 and 3). This result is supported by Aldesquy and Ibrahim [24] who observed that GA_3 inhibited Na^+ and Cl^- accumulation in root and shoot of wheat. A combination of 100 to 300 mM NaCl and kinetin had an inhibitory effect on germination (Figures 2 to 4). Babu and Kumars' [25] observation that kinetin reduced seed germination by 6.8% at 3.5 percent soil salt level is consistent with our data.

Interaction of GA_3, kinetin and NaCl salinity increased the rate of germination in wheat (Figures 2 and 3). Similarly, Kabar [26] discovered that combining GA3 with kinetin enhanced the percentage of germination in 1.75% NaCl stressed barley seed.



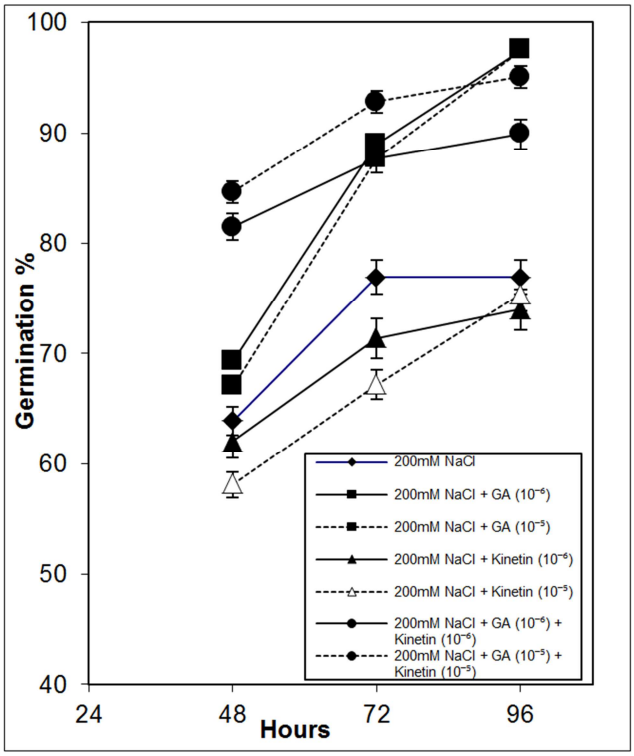
Bars represent ± standard error.

Figure 1. *Effect of NaCl salinity on germination of wheat seed var. Akbar.*



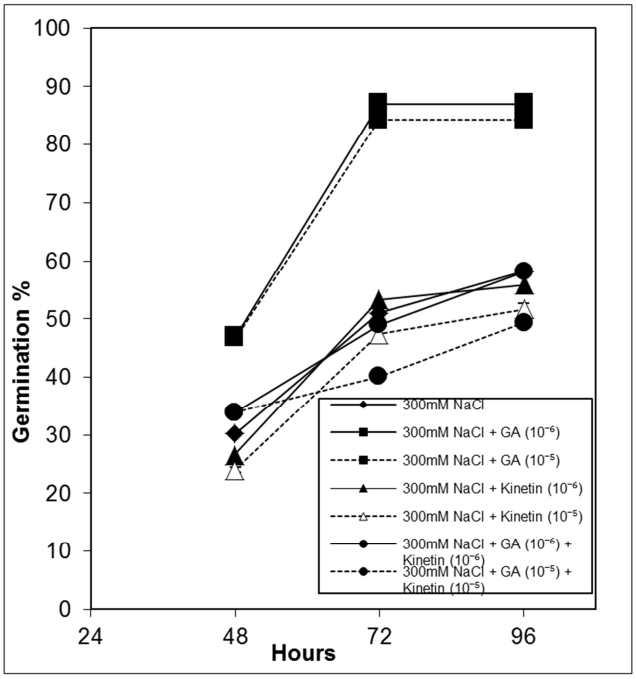
Bars represent ± standard error.

Figure 2. *Interaction of 100 mM NaCl gibberellic acid and kinetin on rate of germination of wheat seed (var. Akbar).*



Bars represent ± standard error.

Figure 3. *Interaction of 200 mM NaCl gibberellic acid and kinetin on rate of germination of wheat seed (var. Akbar).*



Bars represent ± standard error.

Figure 4. *Interaction of 300 mM NaCl gibberellic acid and kinetin on rate of germination of wheat seed (var. Akbar).*

3.3. Influence of GA_3 and Kinetin Either Alone or Combination on Na^+, K^+ and Cl^- Accumulation

Application of GA_3 (10^{-6} M, 10^{-5} M) along with 100 mM NaCl decreased Cl^- accumulation by 52% (10^{-6} M) and 54% (10^{-5} M) in plumules and 22% (10^{-6} M) and 27% (10^{-5} M) in radicles respectively, as compared to 100 mM NaCl (control) of germinating seeds of Akbar (Table 2). (10^{-5} M) in plumules and 22% (10^{-6} M) and 27% (10^{-5} M) in radicles respectively, as compared to 100 mM NaCl (control) of germinating seeds of Akbar (Table 2). However, GA_3 (10^{-6} M, 10^{-5} M) increased K^+ accumulation by 25% (10^{-6} M) and 16% (10^{-5} M) in plumules and 32% (10^{-6} M) and 26% (10^{-5} M) in radicles respectively, as compared to 100 mM NaCl (control) in germinating seeds of Akbar (Table 2). Similar effects were also observed when GA_3 (10^{-6} M, 10^{-5} M) along with 200 mM NaCl were used (Table 3).

When kinetin (10^{-6} M and 10^{-5} M) were used along with 100 mM NaCl, there was an increase in the accumulation of Cl^- by 22% (10^{-6} M) and 25% (10^{-5} M) in plumules and 19% (10^{-6} M) and 28% (10^{-5} M) in radicles respectively, as compared to control (100 mM NaCl) in germinating seeds of Akbar (Table 2).

There was decrease in the accumulation of K^+ by 24% (10^{-6} M) and 21% (10^{-5} M) in plumules and 5% (10^{-6} M) and 3% (10^{-5} M) in radicles respectively, in germinating seeds of Akbar when kinetin at a concentrations of 10^{-6} M and 10^{-5} M were used along with 100 mM NaCl as compared to control (100 mM NaCl) (Table 2). Similar results were obtained when 200 mM NaCl and kinetin (10^{-5} M and 10^{-6} M) were used (Table 3).

Accumulation of total Cl^- was also decreased by 34% (10^{-6} M) and 31% (10^{-5} M) when 100 mM NaCl in combination with GA_3 and kinetin were used and total K^+ increased by 17%. K^+ decreased by 17% (10^{-6} M) and 7% (10^{-5} M) when GA_3, kinetin and 200 mM NaCl were used together (Table 3).

NaCl (100 and 200 mM) along with GA_3 and kinetin either alone or in combination did not have much effect on Na^+ accumulation in Akbar (Tables 2 and 3). A 7% increase in Na^+ accumulation was observed in Akbar following application of 100 mM NaCl and kinetin (Table 2). In Akbar, a decrease of total Na^+ by 19% was observed when GA_3, kinetin and 200 mM NaCl were used as compared to control (Table 3).

Interaction of salinity and GA_3 increased the amount of K^+ in plumule and radicle (Tables 2 and 3). GA_3 may cause activation of the uptake of extruded K^+ in place of Na^+ influx. This view is confirmed by a prior investigation in which GA3 was found to elevate K^+ levels in wheats' developing grains. [24].

Kinetin increased the uptake of Cl^- in salt treated germinating seeds (Tables 2 and 3). This result is in agreement with the findings of Naeem *et al.* [27]. It is suggested that salinity induced-inhibition of germination may be due to the accumulation of excess amount of Cl^- in the developing young embryo of the seed. Interaction of GA_3 and kinetin with NaCl stress decreased the accumulation of Cl^- in radicle and plumule upto a certain level (Tables 2 and 3). Similar results were observed by Naeem *et al.* [27].

Table 2. *Effects of gibberellic acid and kinetin on accumulation of Na^+, K^+ and Cl^- in germinating wheat seeds (var. Akbar) in 100 mM NaCl.*

TREATMENTS	Ion accumulation (mequiv. g^{-1} dry tissue)								
	PLUMULE			RADICLE			TOTAL		
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
100 mM NaCl + 0.1 mM $CaSO_4$	0.445 (0.04)	0.567 (0.05)	5.344 (0.10)	0.592 (0.01)	0.599 (0.04)	3.930 (0.70)	0.552 (0.10)	0.603 (0.02)	3.803 (0.40)
100 mM NaCl + 0.1mM $CaSO_4$ + 10^{-6} M GA	0.372 (0.03)	0.752 (0.02)	2.582 (0.90)	0.547 (0.02)	0.730 (0.06)	3.048 (0.08)	0.488 (0.01)	0.715 (0.30)	2.298 (0.25)
100 mM NaCl + 0.1mM $CaSO_4$ + 10^{-5} M GA	0.367 (0.03)	0.672 (0.08)	2.478 (0.07)	0.637 (0.02)	0.671 (0.02)	2.920 (0.05)	0.527 (0.02)	0.691 (0.02)	2.224 (0.10)
100 mM NaCl + 0.1mM $CaSO_4$ + 10^{-6} M Kinetin	0.571 (0.04)	0.446 (0.04)	6.885 (0.70)	0.668 (0.08)	0.471 (0.03)	4.828 (0.30)	0.594 (0.02)	0.652 (0.03)	5.304 (0.30)
100 mM NaCl + 0.1 mM $CaSO_4$ + 10^{-5} M Kinetin	0.492 (0.05)	0.429 (0.01)	7.168 (0.30)	0.572 (0.02)	0.480 (0.01)	5.481 (0.07)	0.562 (0.02)	0.585 (0.05)	5.708 (0.40)
100 mM NaCl + 0.1 mM $CaSO_4$ + 10^{-6} M (GA+K)	0.460 (0.08)	0.295 (0.02)	3.022 (0.10)	0.612 (0.09)	0.345 (0.03)	4.382 (0.30)	0.586 (0.02)	0.643 (0.01)	2.524 (0.04)
100 mM NaCl +0.1 mM $CaSO_4$ + 10^{-5} M (GA+K)	0.392 (0.03)	0.299 (0.01)	1.496 (0.40)	0.616 (0.02)	0.304 (0.02)	3.417 (0.40)	0.516 (0.01)	0.726 (0.03)	2.624 (0.20)

Table 3. *Effects of gibberellic acid and kinetin on accumulation of Na^+, K^+ and Cl^- in germinating wheat seeds (var. Akbar) in 200 mM NaCl.*

TREATMENTS	Ion accumulation (mequiv. g^{-1} dry tissue)								
	PLUMULE			RADICLE			TOTAL		
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
200 mM NaCl + 0.1 mM $CaSO_4$	0.609 (0.04)	0.496 (0.03)	4.931 (0.10)	0.900 (0.04)	0.558 (0.10)	2.859 (0.30)	0.795 (0.02)	0.565 (0.04)	4.245 (1.0)
200 mM NaCl + 0.1 mM $CaSO_4$ + 10^{-6} M GA	0.496 (0.04)	0.771 (0.02)	4.371 (0.36)	0.990 (0.04)	0.651 (0.03)	2.447 (0.25)	0.783 (0.03)	0.672 (0.04)	3.006 (6.0)
200 mM NaCl + 0.1 mM $CaSO_4$ + 10^{-5} M GA	0.456 (0.02)	0.736 (0.02)	2.636 (0.30)	0.880 (0.10)	0.612 (0.02)	2.365 (0.30)	0.832 (0.02)	0.887 (0.20)	2.666 (1.0)
200 mM NaCl + 0.1 mM $CaSO_4$ + 10^{-6} M Kinetin	0.758 (0.05)	0.346 (0.02)	9.508 (0.35)	0.766 (0.04)	0.616 (0.05)	4.093 (1.60)	0.799 (0.02)	0.524 (0.04)	6.212 (1.0)
200 mM NaCl + 0.1 mM $CaSO_4$ + 10^{-5} M Kinetin	0.927 (0.20)	0.349 (0.04)	15.930 (0 2.0)	0.835 (0.07)	0.605 (0.02)	7.908 (0.20)	0.812 (0.06)	0.414 (0.02)	9.222 (2.0)
200 mM NaCl + 0.1 mM $CaSO_4$ + 10^{-6} M (GA+K)	0.714 (0.03)	0.462 (0.01)	4.998 (0.80)	0.736 (0.02)	0.461 (0.02)	2.263 (0.30)	0.707 (0.02)	0.417 (0.03)	3.573 (0.80)
200 mM NaCl + 0.1 mM $CaSO_4$ + 10^{-5} M (GA+K)	0.559 (0.03)	0.483 (0.01)	4.970 (0.60)	0.705 (0.04)	0.517 (0.03)	2.381 (0.70)	0.646 (0.02)	0.442 (0.10)	3.700 (0.09)

Each value is the mean of five replicates, and the standard deviation is shown in parenthesis.

On the whole the results suggested that salinity-induced inhibition of germination may be due to accumulation of excess amount of Na^+ and Cl^- in the developing embryo of wheat and concomitant reduction of accumulation of K^+ (Table 1). The partial nullification of inhibitory effect of salinity following GA_3 treatment (Figures 2 to 4) may be due to the less accumulation of Na^+ and increased accumulation of K^+ in germinating seeds (Tables 2 and 3).

4. Conclusions

It is concluded that salinity-induced inhibition of germination was related to the accumulation of excess amount of Na^+ and Cl^- in the developing embryos of wheat and reduction in accumulation of K^+. The partial reduction of inhibitory effect of salinity following GA_3 treatment was probably due to the reduction of accumulation of Na^+ in germinating seed and proportionate increase in K^+ accumulation.

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