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EFFECTS OF GIBBERELLIC ACID AND KN ON SEED GERMINATION AND ACCUMULATION OF Na^+ AND K^+ IN THE SEEDLINGS OF TRITICALE-I UNDER SALINITY STRESS

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Abstract

Effects of GA and Kn on seed germination and accumulation of Na^+ and K^+ in the seedlings of Triticale cultivar was investigated under salinity stress. Salinity decreased the rate of seed germination and ion accumulation in the seedlings but both GA and Kn stimulated these processes under stress. Salinity increased the accumulation of Na^+ in radicles and plumules with concomitant inhibition of that of K^+, GA and Kn, on the other hand, reduced the rate of Na^+ accumulation and increased that of K^+ in the seedlings. The interrelationship between the effect of salinity and growth regulators on seed germination and the accumulation of Na^+ and K^+ in the seedlings is discussed.

Introduction

Seedlings are the most vulnerable stage in the life cycle of plants and germination determines when and where seedling growth begins (Lianes *et al.* 2005). Salinity, in general, has inhibitory effects on germination of seeds (Kaymakanova 2009, Zhang *et al.* 2010, Abari *et al.* 2011, Akbarimoghaddam *et al.* 2011, Kaveh *et al.* 2011). Earlier, it was also reported that salinity decreased germination of seeds of barley, wheat and rice (Kumar *et al.* 1988, Begum *et al.* 1992, Babu and Kumar 1975 and Queiroz and Nakagawa 1992).

Phytohormones like gibberellic acid and Kn were reported to overcome the inhibitory effect of salinity on germination. A combination of 3% NaCl and 50 ppm Kn increased the germination by 100 per cent as compared to that of control whereas 3.5% soil salt concentration along with 50 ppm Kn reduced the germination in wheat (Babu and Kumar 1975). Gibberellic acid and Kn increased the rate of germination of barley and lettuce seeds under salt-stress treatment (Kabar 1989, Kabar and Baltepe 1989). Phytohormone, in general, was found to regulate the transport of ions in plants (Karmoker 1984). Hence, phytohormone-induced alleviation of the inhibitory effect of salinity may be due to the phytohormone-induced changes in ionic balance in seedlings. The delay in germination is mainly due to higher Na^+ accumulation in the seeds of wheat (Begum *et al.* 1992, Akbarimoghaddam *et al.* 2011). Triticale cultivar was used as plant material because the report on the effect of salinity on germination of seeds of Triticale is rare. Triticale (X *Triticosecale* Wittmack, hexaploid chromosome) is the first successful man-made crop which is a close relative of wheat.

The effect of GA and Kn on seed germination and accumulation of Na^+ and K^+ in the developing seedlings under salinity stress is reported.

Materials and Methods

The seeds of Triticale (*Triticosecale* Wittmack) were obtained through the courtesy of Bangladesh Agriculture Research Institute, Gazipur.

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Methods of germination of seeds: The seeds were surface sterilized in 5.25% sodium hypochlorite solution for two minutes. Seeds were then rinsed seven times in tap water followed by washing four times in distilled water. The sterilized seeds dipped in distilled water were aerated for 30 minutes with an air compressor. Thirty such seeds were placed on Whatman filter paper No.1 contained in a Petri dish (11 cm diam.). Three replicates were used for each treatment. Filter paper was soaked with 6 ml of NaCl solution at concentrations of 25, 50, 100, 200, 300 and 400 mM each prepared in 0.1 mM $CaSO_4$ ± selected concentration of GA and Kn and the Petri dish was covered.

In case of control, the filter paper was soaked with 6 ml of 0.1 mM $CaSO_4$. The seeds were allowed to germinate in the dark at 28°C ±1°C/22°C ±1°C day/night temperature. However, 3 ml of test solution was added after 72 h from the time of sowing. Seeds were considered to be germinated when radicles and plumules could be clearly distinguished. Seed germination activity was recorded at 48, 72 and 96 hrs from the time of sowing.

Extraction and analysis of Na^+ and K^+ in radicles and plumules: Radicles and plumules were separated from cotyledons at 48, 72 and 96 hrs from the date of sowing. Each set of radicles or plumules was washed in two changes of 0.1mM $CaSO_4$ contained in a small beaker (50 ml each) for two minutes to remove free space ions and were dried in an oven (Memmert, M-3132, Germany) at 75°C for 72 hrs to a constant weight. Na^+ and K^+ were extracted by hot water following Karmoker and Van Steveninck (1978). After necessary dilution Na^+ and K^+ in the extract was measured using a flame photometer (Jenway PEP-7, UK) at a wavelength of 767 and 589 nm, respectively.

Results and Discussion

Effects of GA and Kn on seed germination under salinity stress: Different concentrations of NaCl (100 - 400 mM) caused an inhibition of the rate of germination in Triticale. There was a progressive rate of inhibition of seed germination with the rise of salinization of seeds (Fig. 1). Similar salinity-induced decrease in germination was found in wheat (Begum *et al.* 1992), *Acacia* sp. (Abari *et al.* 2011) and in tomato lines (Kaveh *et al.* 2011).

Application of GA (10^{-6} M) under salinity stress due to 100 mM NaCl alleviated the inhibitory effect of salinity leading to an increase in the rate of germination (Fig. 2). GA (10^{-6} M) increased the rate of germination by 24 - 13% from 48 - 96 hrs of treatment even under 200 mM NaCl stress (Fig. 2). Similarly, interaction of NaCl-salinity (100 and 200 mM) and Kn (10^{-5} M) nullified the inhibitory effect of salinity on germination at 48 and 96 hrs of treatment (Fig. 3). Igbinnosa and Okonkwo (1992) found that interaction of sodium hypochlorite and Kn stimulated germination of cowpea witchweed *(Striga gesnerioides)*.

The combined effect of gibberellic acid (10^{-6} M) and Kn (10^{-5} M) was stimulatory at both 100 and 200 mM NaCl stress on seed germination. At 200 mM salinity stress, GA and Kn significantly increased the rate of germination by 61% at 48 hrs of treatment and the stimulatory effect was sustained up to 96 hrs of treatment (Fig. 4). Kabar (1989) observed that under NaCl-salinity stress, GA and Kn applied together increased the percentage of seed germination of lettuce.

Effects of GA/Kn on Na^+ and K^+ accumulation in seedlings under salinity stress: NaCl-salinity increased the accumulation of Na^+ in radicles and plumules while it decreased that of K^+ (Table 1). This result is supported by Akbarimoghaddam *et al.* (2011) who found that salinity increased the accumulation of Na^+ and decreased that of K^+ in wheat. Similar increase in Na^+ and decrease in K^+ content following salinity treatment was found in germinating cotton seeds (Chachar *et al.* 2008).

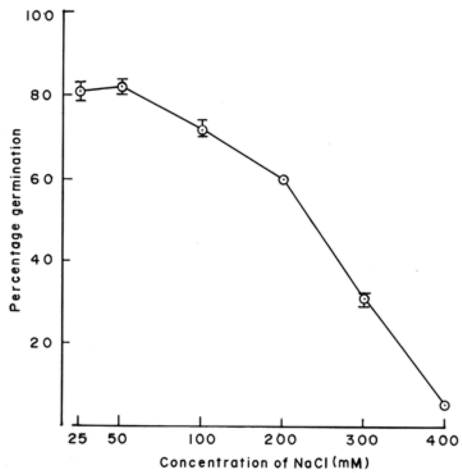

Fig. 1. Effects of NaCl salinity stress on germination of seeds of Triticale. Each value is the mean of three replicates. Bars represent ± standard error of the mean value.

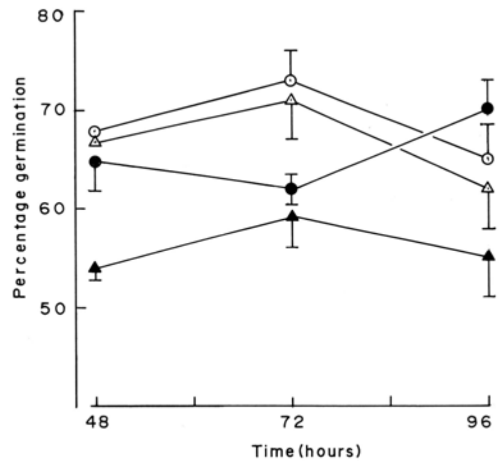

Fig. 2. Interaction of salinity and 10^{-6} M GA on germination of seeds of Triticale. ● represents 100 mM NaCl (control), ○ represents 100 mM NaCl + 10^{-6} M GA, ▲ represents 200 mM NaCl (control), Δ represents 200 mM NaCl + 10^{-6} M GA. Otherwise as Fig. 1.

Interaction of 100 mM NaCl and GA nullified the stimulatory effect of salinity on Na^+ accumulation in radicles and plumules whereas it increased that of K^+ by 37% at 48 hrs of treatment (Table 2). On the other hand, 200 mM NaCl-salinity and GA decreased the accumulation of Na^+ but increased that of K^+ in the radicles and plumules at 72 and 96 hrs of treatment (Table 2). Stimulatory effect of GA (10^{-4} M) on K^+ transport in excised cotyledons of cucumber was reported (Ezekiel *et al.* 1978).

Application of NaCl along with Kn negated the stimulatory effect of salinity on Na^+ accumulation in radicles and plumules and the inhibitory effect of that on K^+ accumulation (Table 3). Much earlier, it was reported that Kn prevented net accumulation of Na^+ in beet root tissue (Van Steveninck 1972).

When salinity (100 and 200 mM), GA and Kn were applied together, the stimulatory effect of NaCl-stress on Na^+ accumulation was leveled leading to a decrease while inhibitory effect of salinity on K^+ accumulation was nullified leading to an increase of that in radicles and plumules of seedlings of Triticale (Table 4). Similarly, Kabar (1989) observed that NaCl-salinity stress, GA and Kn together increased the percentage of germination in lettuce seeds.

Accumulation of excessive amount of Na^+ in radicles and plumules (Table 1) in germinating seeds may be responsible for the inhibition of germination (Fig. 1). Furthermore, decrease in the endogenous K^+ content in the radicles and plumules (Table 1) would have adverse effect on the growth of embryo leading to a further inhibition of the rate of germination (Fig. 1).

Table 1. Effects of NaCl-salinity on accumulation of Na^+ and K^+ in radicles and plumules of germinating Triticale seeds.

Treatment	Na^+ and K^+ content (μequiv./g dry tissue)											
	Duration of treatment (hr)											
	48				72				96			
	Radicle		Plumule		Radicle		Plumule		Radicle		Plumule	
	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+
0.1 mM $CaSO_4$	0.170 ± 0.009	0.838 ± 0.297	0.108 ± 0.022	0.796 ± 0.241	2.77 ± 0.045	6.73 ± 0.120	1.00 ± 0.053	7.39 ± 0.070	2.29 ± 0.028	5.01 ± 0.519	0.719 ± 0.010	6.19 ± 0.250
100 mM NaCl + 0.1 mM $CaSO_4$	0.319 ± 0.039	0.739 ± 0.071	0.306 ± 0.048	0.558 ± 0.017	Harvested							
200 mM NaCl + 0.1 mM $CaSO_4$	No germination				1.85 ± 0.183	0.480 ± 0.047	0.677 ± 0.007	0.419 ± 0.012	Harvested			
300 mM NaCl + 0.1 mM $CaSO_4$	No germination								2.09 ± 0.016	0.411 ± 0.053	0.936 ± 0.018	0.368 ± 0.005

Each value is the mean of three replicates ± standard error.

Table 2. Interaction of salinity and 10^{-6}M GA on accumulation of Na^+ and K^+ in radicles and plumules of germinating Triticale seeds. Otherwise as Table 1.

Treatment	Na^+ and K^+ content (μequiv./g dry tissue)											
	Duration of treatment (hr)											
	48				72				96			
	Radicle		Plumule		Radicle		Plumule		Radicle		Plumule	
	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+
100 mM NaCl + 0.1 mM $CaSO_4$	13.08 ± 0.245	9.18 ± 3.986	7.74 ± 1.218	11.11 ± 0.095	14.46 ± 0.335	5.42 ± 0.609	4.23 ± 0.035	7.37 ± 0.361	10.27 ± 0.802	4.31 ± 0.139	3.39 ± 0.378	5.15 ± 0.069
100 mM NaCl + 0.1 mM $CaSO_4$ + 10^{-6}M GA	12.64 ± 0.316	12.59 ± 1.683	3.28 ± 0.860	9.89 ± 0.453	12.34 ± 0.421	4.25 ± 0.574	4.82 ± 0.182	6.36 ± 0.413	7.31 ± 1.007	4.90 ± 0.831	4.25 ± 0.006	4.74 ± 0.361
200 mM NaCl + 0.1 mM $CaSO_4$	23.29 ± 3.126	8.54 ± 0.409	12.14 ± 0.401	7.88 ± 1.56	18.11 ± 0.141	7.59 ± 0.179	8.69 ± 0.980	6.37 ± 0.006	7.16 ± 0.136	4.89 ± 1.355	8.69 ± 0.834	3.43 ± 0.248
200 mM NaCl + 0.1 mM $CaSO_4$ + 10^{-6}M GA	17.24 ± 2.424	7.58 ± 1.389	7.21 ± 1.337	5.49 ± 0.774	15.50 ± 0.098	7.05 ± 0.277	8.49 ± 1.509	6.81 ± 1.079	6.16 ± 0.153	4.04 ± 0.185	6.00 ± 0.468	4.14 ± 0.014

Table 3. Interaction of salinity and 10^{-5}M Kn on accumulation of Na^+ and K^+ in radicles and plumules of germinating Triticale seeds. Otherwise as Table 1.

Treatment	Na^+ and K^+ content (µequiv./g dry tissue) Duration of treatment (hr)											
	48				72				96			
	Radicle		Plumule		Radicle		Plumule		Radicle		Plumule	
	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+
100 mM NaCl + 0.1 mM $CaSO_4$	21.17 ±0.632	14.64 ±0.779	9.86 ±0.788	11.42 ±0.336	15.25 ±0.314	7.90 ±0.104	7.43 ±0.552	6.16 ±0.025	9.84 ±0.161	5.57 ±0.447	3.79 ±0.302	4.48 ±0.353
100 mM NaCl + 0.1 mM $CaSO_4$ + 10^{-5} M Kn	15.69 ±0.977	8.16 ±1.027	10.16 ±0.529	12.42 ±0.147	15.61 ±0.418	8.93 ±0.378	7.61 ±0.766	9.17 ±3.240	11.33 ±0.396	6.54 ±0.018	4.29 ±0.187	3.79 ±0.112
200 mM NaCl + 0.1 mM $CaSO_4$	22.04 ±1.54	9.13 ±0.684	17.69 ±1.51	8.04 ±0.822	19.54 ±0.337	7.62 ±0.265	9.53 ±0.219	4.11 ±0.379	13.79 ±0.423	5.46 ±0.287	6.13 ±0.166	4.65 ±0.237
200 mM NaCl + 0.1 mM $CaSO_4$ + 10^{-5} M Kn	22.21 ±1.55	8.06 ±0.644	11.65 ±1.04	9.17 ±0.687	20.01 ±4.581	8.07 ±0.101	11.08 ±0.148	6.72 ±0.258	14.72 ±0.328	7.02 ±0.313	4.84 ±0.183	4.61 ±0.294

Table 4. Interaction of salinity and 10^{-6}M GA and 10^{-5}M kinetin on accumulation of Na^+ and K^+ in radicles and plumules of germinating Triticale seeds. Otherwise as Table 1.

Treatment	Na^+ and K^+ content (µequiv./g dry tissue) Duration of treatment (hr)											
	48				72				96			
	Radicle		Plumule		Radicle		Plumule		Radicle		Plumule	
	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+
100 mM NaCl + 0.1 mM $CaSO_4$	22.12 ±0.870	16.35 ±1.32	9.32 ±0.276	10.62 ±0.127	13.02 ±2.60	8.03 ±0.789	3.62 ±0.993	5.18 ±0.263	14.31 ±2.27	4.80 ±0.382	4.52 ±0.032	3.87 ±0.481
100 mM NaCl + 0.1 mM $CaSO_4$ + 10^{-6} M GA + 10^{-5} M Kn	18.30 ±0.960	13.68 ±0.091	5.13 ±0.280	9.21 ±0.050	10.35 ±0.291	8.07 ±0.578	4.07 ±0.238	4.97 ±0.347	11.08 ±1.37	6.04 ±0.101	5.06 ±0.346	5.24 ±0.704
200 mM NaCl + 0.1 mM $CaSO_4$	24.51 ±0.183	7.81 ±0.576	14.25 ±0.210	6.11 ±0.117	20.23 ±0.797	7.22 ±0.104	6.71 ±0.355	5.59 ±0.063	25.04 ±0.594	6.79 ±0.704	7.37 ±0.187	4.58 ±0.077
200 mM NaCl + 0.1 mM $CaSO_4$ + 10^{-6} M GA + 10^{-5} M Kn	22.07 ±1.78	8.59 ±0.540	6.78 ±0.186	8.81 ±0.468	15.88 ±0.182	6.07 ±0.133	4.48 ±0.274	6.86 ±0.332	20.72 ±1.47	5.59 ±0.037	5.46 ±0.139	4.61 ±0.109

Stimulation of germination by GA (Fig. 2) might be due to GA-induced inhibition of Na^+ accumulation with a concomitant increase in K^+ accumulation in radicles and plumules of Triticale (Table 2).

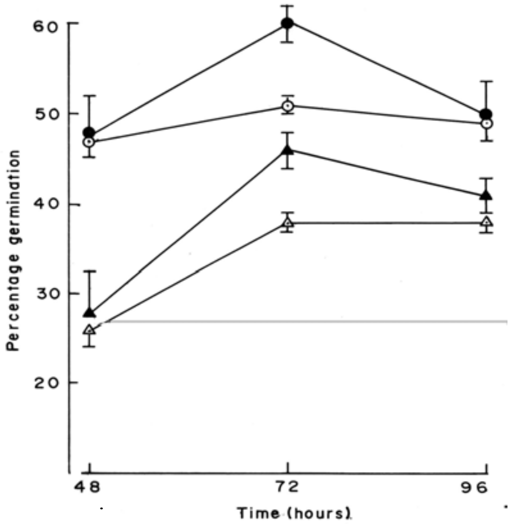

Fig. 3. Interaction of salinity and 10^{-5} M Kn on germination of seeds of Triticale. ● represents 100 mM NaCl (control), ○ represents 100 mM NaCl + 10^{-5} M Kn, ▲ represents 200 mM NaCl (control), Δ represents 200 mM NaCl + 10^{-5} M Kn. Otherwise as Fig. 1.

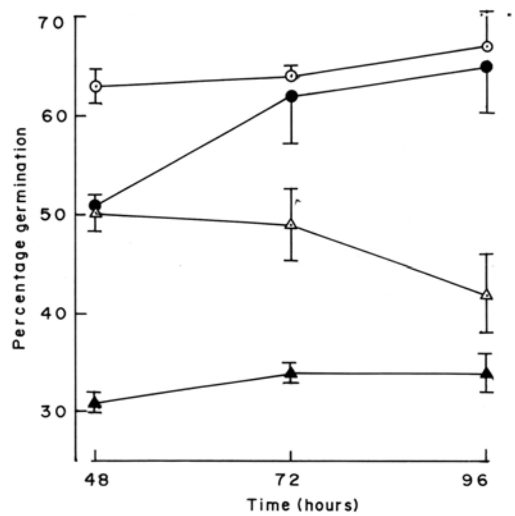

Fig. 4. Combined effect of 10^{-6} M GA and 10^{-5} M Kn on germination of seeds of Triticale under salinity stress. ● represents 100 mM NaCl (control), ○ represents 100 mM NaCl + 10^{-6} M GA + 10^{-5} M Kn, ▲ represents 200 mM NaCl (control), Δ represents 200 mM NaCl + 10^{-6} M GA + 10^{-5} M Kn. Otherwise as Fig.1

In conclusion, inhibition of germination by salinity might be due to the salinity-induced increase in accumulation of Na^+ with concomitant inhibition of that of K^+ in the radicles and plumules of germinating embryo of Triticale. The nullification of an inhibitory effect of salinity on germination leading to a stimulation of that following interaction of GA and Kn might be due to GA- and Kn-induced decrease in Na^+ accumulation with concomitant increase in the uptake of extruded K^+ in germinating embryo of Triticale.

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