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EFFECTS OF NaCl STRESS ON ACCUMULATION OF K^+, Na^+, Cl^-, NO_3^-, SUGAR AND PROLINE CONTENTS IN THE SEEDLINGS OF TRITICALE-I

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Abstract

NaCl stress at 50 - 150 mM NaCl caused a 12 - 16-fold increase in Na^+ accumulation in the root and 3 - 10-fold in the shoot of Triticale-I with a concomitant decrease in that of K^+ and Cl^-. NaCl stress increased the accumulation of reducing, total sugars and proline both in the root and shoot of the seedlings. The mechanism of salt tolerance of Triticale-I plants with respect to ionic relation and accumulation of sugars and proline were discussed.

Introduction

Salinity stress caused an imbalance of nutrients in plants (Song *et al.* 2006) due to the competition of Na^+ and Cl^- with nutrients such as K^+ and NO_3^- (Jouyban 2012). Salt stress undesirably affects plant growth and productivity during all developmental stages (Abari *et al.* 2011).

Na^+ and Cl^- content in both shoots and roots increased with the increase in salinity in a wide range of plant species (Wang *et al*. 2012 and Majid *et al.* 2012). Salinity stress had an inhibitory effect on accumulation of K^+ in barley and rice (Tavakkoli *et al.* 2011 and Alamgir *et al.* 2007).

Salt stress decreased NO_3^- content in rice (Wang *et al*. 2012) and *Achillea fragratissima* Forssk (Abd El-Azim and Ahmed 2009). Salinity induced a significant increase in proline concentration in rice and purslane (Rao *et al.* 2013 and Rahdari *et al.* 2012). Salinity stress enhanced sugar accumulation in alfalfa (Majid *et al.* 2012) and rice (Amirjani 2011).

Triticale cultivar was used as a plant material because reports on the effects of salinity on ion transport, sugar and proline content in triticale is very rare. In this paper, the effect of NaCl-salinity stress on the accumulation and distribution of K^+, Na^+, Cl^-, NO_3^- and reducing and total sugars and proline is reported.

Materials and Methods

Triticale (*Triticosecale* Wittmack), a hybrid between wheat and rye is tolerant to environmental stress and offers an important potential protein source for both human consumption and animal feed. Triticale seeds were collected through the courtesy of Bangladesh Agriculture Research Institute (BARI), Gazipur. Plants were grown in water culture to study the accumulation of ions (Na^+, K^+, Cl^- and NO_3^-), reducing and total sugar and proline. Seeds were surface sterilized according to Samad and Karmoker (2012). Then the seeds were spread over a cotton gauge placed in a lid having nine holes (3 cm dia.) and the lid with seeds was placed on a bucket containing 5 liter of modified half-strength Hoagland solution (Hoagland and Arnon 1950). The bucket was painted black to avoid the exposure of light to the roots. The buckets were kept in dark for 48 h to facilitate the germination of seeds. After germination, the buckets with the seedlings were placed in a light bank at a day/night temperature of 25ºC ± 1ºC/18ºC ± 1ºC and day/night length of 14 h/10 h and light intensity was 160 $\mu E/m^2/sec$. Relative humidity was 75% by day and 85% at

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night. Half-strength Hoagland solution was used as control. The salinity treatments were made by using 50, 100 and 150 mM NaCl solutions in half-strength Hoagland solution. Solutions of control and treatments were aerated continuously by an air compressor. After 3 days, seedlings were plugged to each hole of the lid with spongy foam for rendering support. Nutrient solution was completely replaced every 7 days. Distribution of ions was measured in roots and shoots of the seedlings after 7, 14 and 21 days of salinity treatments. Three replicates were used in each treatment. The tissues were dried in an oven at 80°C for 72 h to attain constant weight.

Na^+, K^+ and Cl^- were extracted from dry tissue by boiling in water bath with two changes of 10 ml distilled water contained in test tubes. Na^+, K^+ and Cl^- and NO_3^- contents were measured according to Begum (1993) and Cataldo *et al.* (1975) respectively.

Proline was measured following Bates *et al.* (1973). Reducing and total sugars were extracted by boiling fresh root and shoot tissue in two changes of 5 ml of 80% ethanol for five minutes in a hot water bath according to Karmoker and Van Steveninck (1979) and measured following Somogyi-Nelson method (Nelson 1944, Somogyi 1952) and Dubois *et al.* (1956).

Results and Discussion

NaCl stress (50 - 150 mM) caused a 12- to 16-fold increase in the accumulation of Na^+ in the root of Triticale seedlings at 7-day of treatment (Fig. 1a). At similar range of NaCl concentrations applied, a stimulation of Na^+ accumulation in the shoot by 2.4- to 3.3-fold was observed at 7-day of treatment (Fig. 1b). Similar stimulation of Na^+ accumulation in the root and the shoot of Triticale was observed at 14- and 21-day of treatment following 50 - 150 mM NaCl treatment (Figs 2a,b and 3a,b). Salinity was also found to increase the accumulation of Na^+ in rice (Wang *et al*. 2012).

K^+ accumulation in the root and the shoot was decreased by 93, 85 and 71% at 50, 100 and 150 mM NaCl treatment respectively at 7-day of treatment (Fig. 1a and 1b). Similarly all NaCl concentrations caused decrease in accumulation of K^+ in the root and the shoot at 14-day and 21-day of treatment (Figs 2a,b and 3a,b). Similar salinity- induced decrease in the accumulation of K^+ was recorded in barley (Tavakkoli *et al.* 2011).

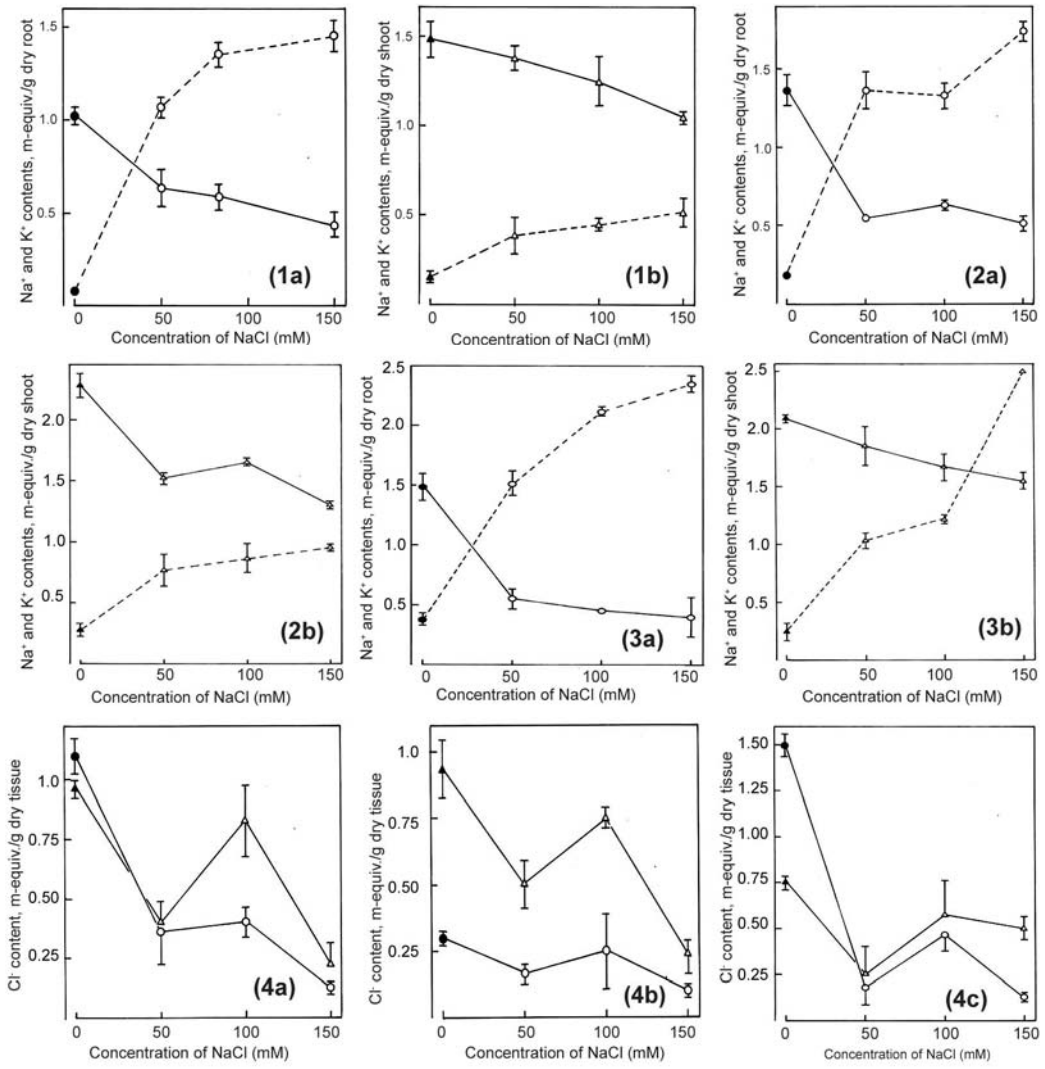
NaCl stress (50 - 150 mM) caused a 66 to 88% decrease in accumulation of Cl^- in the root of Triticali seedlings and a 14-76% decrease in Cl^- accumulation in the shoot at 7-day of treatment (Fig. 4a). A decrease of similar magnitude was recorded in the root and shoot at 14- and 21-day of treatment when seedlings were grown in 50-150 mM NaCl (Fig. 4b,c). On the contrary, salinity was found to increase Cl^- accumulation in rice (Wang *et al*. 2012). In Triticale, a decrease in uptake of chloride indicates that chloride exclusion pump might exist in plasmamembrane.

In the root, NO_3^- accumulation was decreased by 37 - 51% from 50 - 150 mM NaCl and the decrease was 35 - 65% in the shoot at 7-day of treatment (Fig. 5a). Accumulation of NO_3^- in the root and the shoot was also decreased following 50 to 150 mM NaCl treatment at 14-day and 21-day of treatment (Fig. 5b,c). This finding is in agreement with the work of Abd El-Azim and Ahmed (2009) who found that salinity decreased NO_3^- accumulation in *A. fragratissima* Forssk.

At all concentrations of NaCl, proline content caused a 3- to 27-fold increase in the root and a 3 - to 12- fold increase of that in the shoot at 7-day of treatment (Fig. 6a). Accumulation of proline was also increased in the root and the shoot at 14- and 21-day of treatment in plants grown in 50-150 mM NaCl (Fig. 6b,c). Similarly, an increase in proline content was observed in rice (Rao *et al.* 2013) and wheat (Khan *et al*. 2009) which synchronized with the increase in salinity level.

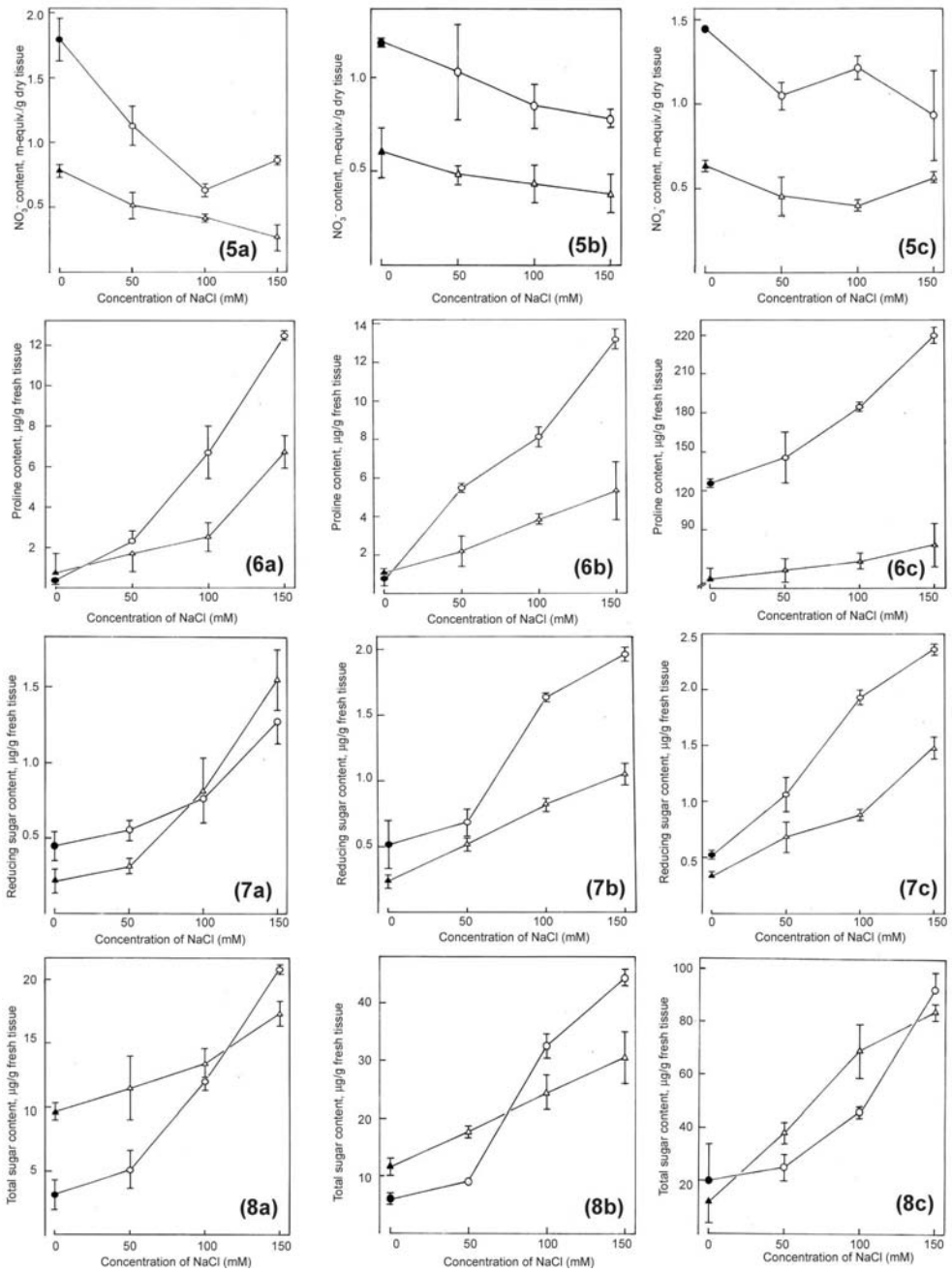
At 7-day of treatment, the accumulation of reducing sugar increased gradually from 1.2- to 2.8-fold in the root and 1.4- to 6.8-fold in the shoot when the seedlings were subjected to 50-150

mM NaCl salinity (Fig. 7a). Accumulation of reducing sugar was increased in the root and the shoot consistently with the increase in NaCl salinity stress at 14- and 21-day of treatment (Fig. 7b,c). This result is consistent with the work of Amirjani (2011) who found similar increase in the reducing sugar content in both the root and shoot when sugarcane was grown in high NaCl salinity.



Figs 1-4: 1. Effects of NaCl salinity stress on the accumulation of Na^+ and K^+ in the (a) root and (b) shoot of 7-day-old Triticale-I seedlings. O represents root and Δ represents shoot. Solid symbols represent control and open symbols represent treatment. Broken lines (---) represents Na^+ and solid lines (—) represents K^+. Each value is the mean of three replicates. Bars represent ± standard error of the mean value. 2. Effects of NaCl salinity stress on the accumulation of Na^+ and K^+ in the (a) root and (b) shoot of 14-day-old seedlings. Otherwise as Fig. 1. 3. Effects of NaCl salinity stress on the accumulation of Na^+ and K^+ in the (a) root and (b) shoot of 21-day-old seedlings. Otherwise as Fig. 1. 4. Effects of NaCl salinity stress on the accumulation of Cl^- in the root and shoot of (a) 7-day-old, (b) 14-day-old and (c) 21-day-old Triticale-I seedlings. O represents root and Δ represents shoot. Solid symbols represent control and open symbols represent treatment. Each value is the mean of three replicates. Bars represent ± standard error of the mean value.

(a)



Figs 5-8: 5. Effects of NaCl salinity stress on the accumulation of NO_3^- in the root and shoot of (a) 7-day-old, (b) 14-day-old and (c) 21-day-old seedlings. Otherwise as Fig. 4. 6. Effects of NaCl salinity stress on the accumulation of proline in the root and shoot of (a) 7-day-old, (b) 14-day-old and (c) 21-day-old seedlings. Otherwise as Fig. 4. 7. Effects of NaCl salinity stress on the accumulation of reducing sugar in the root and shoot of (a) 7-day-old, (b) 14-day-old and (c) 21-day-old seedlings. Otherwise as Fig. 4. 8. Effects of NaCl salinity stress on the accumulation of total sugar in the root and shoot of (a) 7-day-old, (b) 14-day-old and (c) 21-day-old seedlings. Otherwise as Fig. 4.

An increase in total sugar content in the magnitute of 1.6- to 6.5-fold was observed in the roots at 7-day of application from 50-150 mM NaCl treatment. Similarly, salinity at similar range increased total sugar accumulation in the shoot by 1.2- to 1.8-fold at 7-day of treatment (Fig. 8a). NaCl salinity stress caused a similar increase in accumulation of total sugar in the root and the shoot at 14- and 21-day of treatment (Fig. 8b,c). Similarly total sugar content was found to increase when tomato plants were grown in NaCl salinity (Yin *et al.* 2010).

Salinity-induced dramatic stimulation of accumulation of sugars and proline (Figs 6-8) in Triticale-I helps maintain the osmotic potential of cytoplasm of cells which is important for survival of plants under stress.

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