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INFLUENCE OF SODIUM CHLORIDE ON SEED GERMINATION AND SEEDLING ROOT GROWTH OF COTTON (*GOSSYPIUM HIRSUTUM* L.)

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Abstract

Response of cotton (*Gossypium hirsutum* L. cv. NIAB-78) to salinity, in terms of seed germination, seedling root growth and root Na^+ and K^+ content was determined in a laboratory experiment. Cotton seeds were exposed to increasing salinity levels using germination water with Sodium chloride concentrations of 0, 50, 100, 150 and 200 mM, to provide different degrees of salt stress. Germinated seeds were counted and roots were harvested at 24, 48, 72 and 96 h after the start of the experiment. It appeared that seed germination was only slightly affected by an increase in salinity (in most cases the differences between treatment were non-significant), whereas root length, root growth rate, root fresh and dry weights were severely affected, generally highly significant differences in these variables were found for comparisons involving most combinations of salinity levels, in particular with increased incubation period. K^+ contents decreased with increasing salinity levels, although differences in K^+ content were only significant when comparing the control and the 4 salinity levels. Na^+ content of the roots increased with increasing levels of NaCl in the germination water, suggesting an exchange of K^+ for Na^+. The ratio K^+/Na^+ strongly decreased with rising levels of salinity from around 4.5 for the control to ~ 1 at 200 mM NaCl.

Introduction

Cotton (*Gossypium hirsutum* L.) is one of the most important fiber crops which contributes about 60% of the world's total fiber. Many factors influence the yield of cotton, among them the variety grown, method of cultivation, environmental and climatic conditions, amount and application method of fertilizer, time of sowing and availability of irrigation water. However, one of the main factors affecting cotton yield is salinity (Szabolcs, 1994).

Soil salinity is a global problem posing a major threat to the sustainability of irrigated land in arid and semi-arid regions of the world where evapotranspiration greatly exceeds precipitation and the salts tend to accumulate in the topsoil. Moreover, the use of saline irrigation water, low soil permeability, inadequate drainage conditions, high water table and poor irrigation management also contribute to soil salinity. Salinity affects 7% of the world's land area, which amounts to 930 million ha (Szabolcs, 1994). The area is increasing; a global study of land use over the last 45 years found that 6% had become saline in that period. Current estimates indicate that 20-50% of all irrigated croplands are affected by high salt concentrations, resulting in worldwide economic losses of approximately US$ 12.6 billion per year (Ghassemi *et al.*, 1995).

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The root is a major component of the plant, both in terms of function and total dry weight; roots usually comprise about one third of the dry weight of the entire plant body. The roots absorb nutrients and water from the soil through root hairs and transport these to the stem and leaves. The salts present in the root environment adversely affect crop plants, exerting the following stresses simultaneously or one at a time: **a**) osmotically induced water stress, **b**) specific ion stress or toxicity, **c**) ion imbalance stress or induced nutrient deficiency and **d**) other secondary effects such as the damage caused by excess sodium to soil structure resulting in low permeability to water, poor aeration of roots and resistance to root penetration (Lone, 1988). Root growth, in contrast to leaf growth, can recover remarkably well from the addition of salt or other osmotica, but it depends on the level of osmotic stress and its duration (Hsiao & Xu, 2000; Frensch & Hsiao, 1995). The time taken and ability for roots to recover may depend on whether or not plasmolysis has occurred (Munns, 2002).

Cotton is considered a moderately salt tolerant crop, but its yield is markedly affected due to poor germination and subsequent abnormal plant development under severe saline conditions (Ashraf, 2002). Gausman *et al*., (1972) observed that cotton growth was retarded severely by salinity, through a decrease in the osmotic potential and reduced availability of nutrients. The plant height decreased significantly over a wide range of salinity (EC of 2 to 24 mmhos cm^{-1}). Longenecker (1974) reported that germinating cotton seedlings and young plants were severely affected by saline conditions. The occurrence of stunting reduced main stem height and biological yield which was probably due to a drop in osmotic potential of soil solution and non-availability of water.

The success of cotton production lies partly in cultivation of salt-tolerant cotton cultivars on salt affected soils. The present study was, therefore, undertaken in order to evaluate the effects of NaCl on the seed germination and early seedling root growth of cotton (cv. NIAB-78, a relatively salt-tolerant cultivar) under laboratory conditions. Although a considerable amount of study has been done on cotton plants under salinity stress and more have focus on the above-ground plant parts (Gausman *et al*., 1972, Jafri & Ahmad, 1994). In more detailed study where roots are considered, generally only a few aspects of root growth, e.g. germination (Javid *et al*., 2001) are considered. This study attempts to investigate a more comprehensive set of root parameters to gain more insight in the effect of salinity on early root growth of cotton.

Materials and Methods

The experiment was conducted at the Postgraduate Research Laboratory, Department of Plant Physiology and Biochemistry, Faculty of Crop Production, Sindh Agriculture University, Tando Jam, Pakistan. Cotton (cv. NIAB-78) was tested under the following levels of salinity: 0, 50, 100, 150 and 200 mM NaCl. Observations were recorded at incubation periods of 24, 48, 72 and 96 h, respectively. Within each treatment there were 4 times (for each incubation period) 3 replicates of 100 seeds each (split in 2 groups of 50 seeds).

Seed sterilization: The seeds (20 g) were placed in a 200 cm^3 beaker and approximately 4 cm^3 of concentrated sulphuric acid was added. The mixture of seeds and acid was stirred thoroughly with a glass rod for 3 minutes and left for 5 minutes. After a further 2 min of

stirring, the seeds were transferred to a Buckner funnel where they were washed with running tap water for 3 min. The seeds were then placed in 200 cm^3 1% Sodium bicarbonate solution for 10 min to neutralize remaining acid, and washed thoroughly with distilled water. The delinted seeds were soaked for an hour in 1000 cm^3 of distilled water. Thereafter, the floaters were discarded and the sinkers were placed in a 1.5% Na hypochlorite solution (0.1% available chlorine) for 20 min., for further sterilization. Finally, the seeds were washed eight times with sterile distilled water to remove the hypochlorite.

Germination procedure: The method adopted to germinate the seeds has been described by Chachar (1995). Fifty delinted and sterilized seeds were placed in 10 rows, with 5 seeds in each row, on a sheet of blotting paper (23 cm x 57 cm). The paper was moistened either with 40 cm^3 sterile distilled water (control) or with sodium chloride (NaCl) solution. It was then rolled up round a wooden stick (32 cm x 0.5 cm) and placed in a polyethylene bag (35 cm x 23 cm). The top of the bag was folded over and tied lightly to maintain sterile condition and allow air exchange. Finally, the bag and its contents were stood upright in a 500 cm^3 beaker and incubated in the dark at 30ºC for 24, 48, 72 and 96 h. After every 24 h, the bag and its contents were rolled gently to remoisten the seeds with water that had been collected at the bottom of the bags. For each treatment, and for each harvest period, 300 seeds were germinated in this way in the blotting paper rolls (6 rolls of 50 seeds; they were paired to provide three replicates of 100 seeds).

Analyses related to root growth: After germination periods of 24, 48, 72 and 96 h, respectively, the seedlings were harvested and counted to establish germination percentages. Ungerminated seeds were discarded and the remaining seedlings were dissected using a scalpel to remove their cotyledons and shoot epicotyls. This left the root and hypocotyls for use as the experimental materials. The root plus hypocotyls will be generally referred to as the root throughout this paper. The length of each root, L, was measured using a ruler. For fresh weight (W_f) determination, the roots were weighed on a high-precision electrical balance and then kept in an oven at 80°C for 48 h and reweighed to determine their dry weight (W_d). The root moisture content (M), expressed as a percentage, was calculated from W_f and W_d values: $M = 100(W_f - W_d)/ W_f$.

Sodium and potassium contents: Sodium and potassium contents of cotton roots were determined following Method No. 54 of the USDA Handbook No. 60 (Richards, 1954). For determination of Na^+ and K^+, 0.25 g of plant sample was taken from each treatment separately into a 100 mL conical flask. Ten mL of 1:5 concentrated nitric acid and 72% perchloric acid were added to the plant material and covered with a watch glass and allowed to stand overnight, until the initial reaction had subsided. Next, samples were gently heated on a hot plate and then shaken vigorously until a clear colorless solution appeared. The heat was discontinued when the volume had reduced to approximately 3 mL. Samples were then transferred to 50 ml volumetric flasks and the volume of the solution was made up to 50 mL by adding distilled water. This digested material was used for the determination of Na^+ and K^+, using a flame photometer (El) with an acetylene burner.

Statistical analysis: The data were subjected to a one-way analysis of variance and a Tukey-Cramer multiple comparison post-test. All statistical tests were conducted at $p<0.05$. Germination rates were arcsine corrected on beforehand.

Results

Seed germination: Fig. 1 shows that the percentage germination, G, decreased with an increase in salinity levels and increased with increasing incubation periods, but note that most of the germination had taken place during the first 24 h. Differences in G between the 0, 50 and 100 mM NaCl treatments were relatively small, at all incubation periods.

On the other hand, distinctly lower values (compared to the control) of *G* were observed for the seeds treated with 150 and 200 mM NaCl for incubation periods between 24 and 72 h. At the 150 mM NaCl level, *G* approached that of the lower salinity levels after 96 h of incubation, whereas at 200 mM NaCl, the final percentage of germination stayed below the other levels (78.7% compared to 86.8% on average for the other salinity levels). It is interesting to see that the rate of germination increased considerably during the last 24 h of the incubation period for the 150 and 200 mM salinity levels.

Despite the fact that the data in Fig. 1 present a clear pattern, differences between *G*-values for different salinity levels at 24 and 48 h were strictly speaking not statistically significant (Table 1). At 72 and 96 h, the differences in *G* between all salinity levels of 0, 50, 100, and 150 mM on the one hand and 200 mM on the other hand had become statistically significant (at 72 h the difference between *G* at 150 mM vs 200 mM was not yet significant). This implies that the adverse effect of the increased salt level on germination of this cotton cultivar takes some time to develop and a significant reduction in germination is only apparent at the highest salinity level.

Although a relatively clear increase in *G* (of about 2-8%) was observed during the entire experiment, according to the Tukey-Cramer test the difference in *G* between germination periods (i.e. within each salinity level) was only significant when comparing the values for 24 and 96 h for the 50 ($p<0.05$) and 150 mM ($p<0.01$) and for 48 and 96 h at the 150 mM ($p<0.05$) (data not shown).

Root length and root growth rate: Fig. 2a shows that the average root length, L, is clearly reduced for all treatments with salinity levels between 50 and 200 mM, compared to the control; at 200 mM NaCl, *L* is generally only about a quarter of the values at 0 mM salinity. Table 2 shows the results of the multiple statistical comparison. The longer the period of incubation, the more significant the differences in L between the treatments ($p<0.001$ for all salinity levels at 72 and 96 h, apart from 50 mM vs 100 mM at 72 h). For the first 2 incubation periods, the majority of the differences in *L* are significant. So, despite the fact that germination percentage is only slightly affected (and differences only become statistically significant at 200 mM), the growth of the roots is severely influenced by the increased salinity levels, including at the relatively low NaCl levels. Note that the increase of L with time is statistically highly significant ($p<0.001$) for all comparisons of incubation period (24 h vs 48h, 24 h vs 72 h, …..72h vs 96 h) for each salinity level.

During the final 24-h period of the incubation the rate of root length growth decreases for the 100 and 150 mM salinity levels (Fig. 2b). This decrease did not occur at the 200 mM level, although root growth rate did level of Fig. 2b shows that the rate of root growth varies considerably between and within treatments. For the control, the largest increase in root growth rate takes place between 24 and 48 h (steepest slope), after which the increase in growth rate decreased. Rates are clearly lower for the other treatments, with the rates of the 100 and 150 mM treatments falling below that of the 200 mM treatment.

Table 1. Results of a Tukey-Cramer multiple comparison test for the germination data. Column 1 indicates the various comparisons of salinity levels.

Comparison (*mM*)	Statistical significance			
	24 h	**48 h**	**72 h**	**96 h**
0 vs 50	ns	ns	ns	ns
0 vs 100	ns	ns	ns	ns
0 vs 150	ns	ns	ns	ns
0 vs 200	ns	ns	*	**
50 vs 100	ns	ns	ns	ns
50 vs 150	ns	ns	ns	ns
50 vs 200	ns	ns	*	**
100 vs 150	ns	ns	ns	ns
100 vs 200	ns	ns	*	*
150 vs 200	ns	ns	ns	*

ns: Non-significant ($p>0.05$), *: Significant at $p<0.05$, **: Significant at $p<0.01$, *** Significant at $p<0.001$.

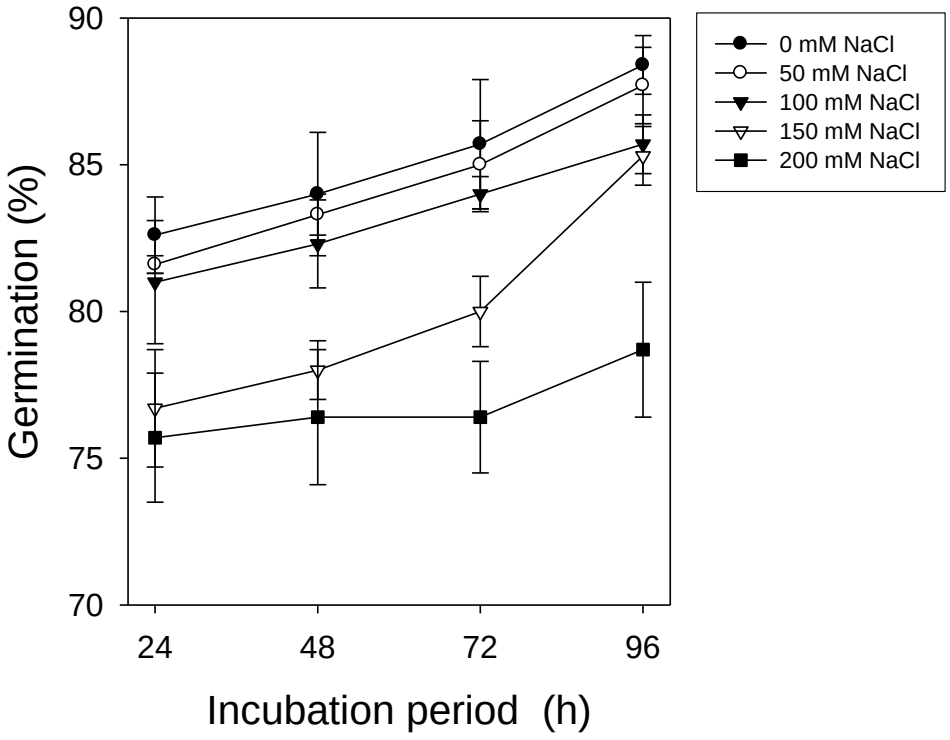

Fig. 1. Percentage germination of NIAB-78 cotton seeds when incubated in rolls of blotting paper moistened with sterile distilled water (control) or with different concentrations of sodium chloride (NaCl) solution (50, 100, 150 and 200 mM), at different times after the start of the experiment. Error bars represent standard errors (based on 3 replicates).

Table 2. Results of a Tukey-Cramer multiple comparison test for the root length data. Explanation of symbols etc., as in Table 1.

Comparison (*mM*)	Statistical significance			
	24 h	48 h	72 h	96 h
0 vs 50	ns	**	***	***
0 vs 100	**	***	***	***
0 vs 150	***	***	***	***
0 vs 200	***	***	***	***
50 vs 100	**	ns	ns	***
50 vs 150	***	ns	***	***
50 vs 200	***	***	***	***
100 vs 150	ns	ns	***	***
100 vs 200	**	***	***	***
150 vs 200	*	**	***	***

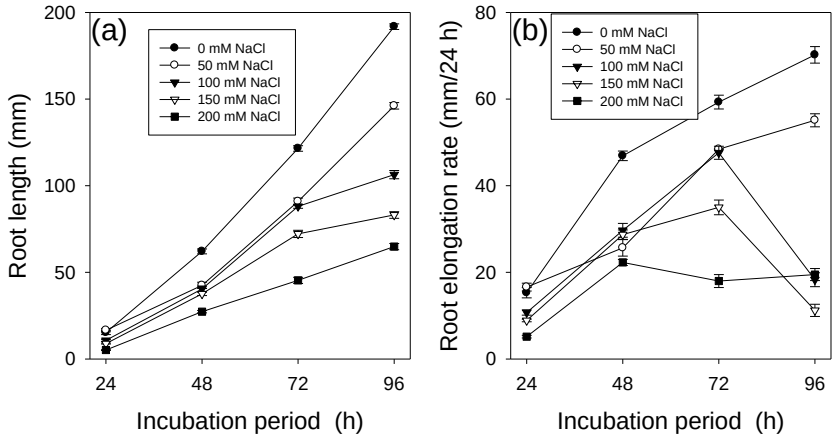

Fig. 2. Root length (a) and root elongation rate (b) of NIAB-78 cotton roots treated with different concentrations NaCl at different times after the start of the experiment.

Root fresh weight, dry weight and root moisture content: Fig. 3a shows that root fresh weight, W_f, was reduced in response to increasing salinity. The differences became larger with increased incubation period. In other words, an increase in incubation period increased W_f (i.e. the roots grew), but less so for higher NaCl levels. Salinity reduces the ability of roots to take up water, thereby causing a reduction in growth rate (as also indicated in Fig. 2). Table 3 shows that the values of W_f at the various salinity levels are not always significantly different from each other, especially not at 48 h (this is partly caused by a large SE for the 100 mM treatment), but on average they are.

During the final 24 h of incubation the change in W_f was very small for the salt concentrations of 100, 150 and 200 mM NaCl. On average, a decrease in root fresh weight for the 200 mM NaCl was observed during this period, which would indicate plasmolysis, but note the large error bars on the 96-h point. According to the Tukey test, changes of W_f over time for the 200 mM treatment were non-significant. For the other treatments they were highly significant for most of the time, apart from the change in W_f between 72 and 96 h for the 100 mM and 150 mM treatments and those from 24 to 48 h, and 48 to 72 h for the 100 mM NaCl treatment, which were non-significant.

Table 3. Results of a Tukey-Cramer multiple comparison test for the root fresh weight data. Explanation of symbols etc., as in Table 1.

Comparison (*mM*)	Statistical significance			
	24 h	48 h	72 h	96 h
0 vs 50	ns	*	**	*
0 vs 100	**	ns	***	***
0 vs 150	***	*	***	***
0 vs 200	***	**	***	***
50 vs 100	*	ns	ns	**
50 vs 150	***	ns	***	***
50 vs 200	***	ns	***	***
100 vs 150	ns	ns	*	ns
100 vs 200	**	ns	***	*
150 vs 200	ns	ns	ns	ns

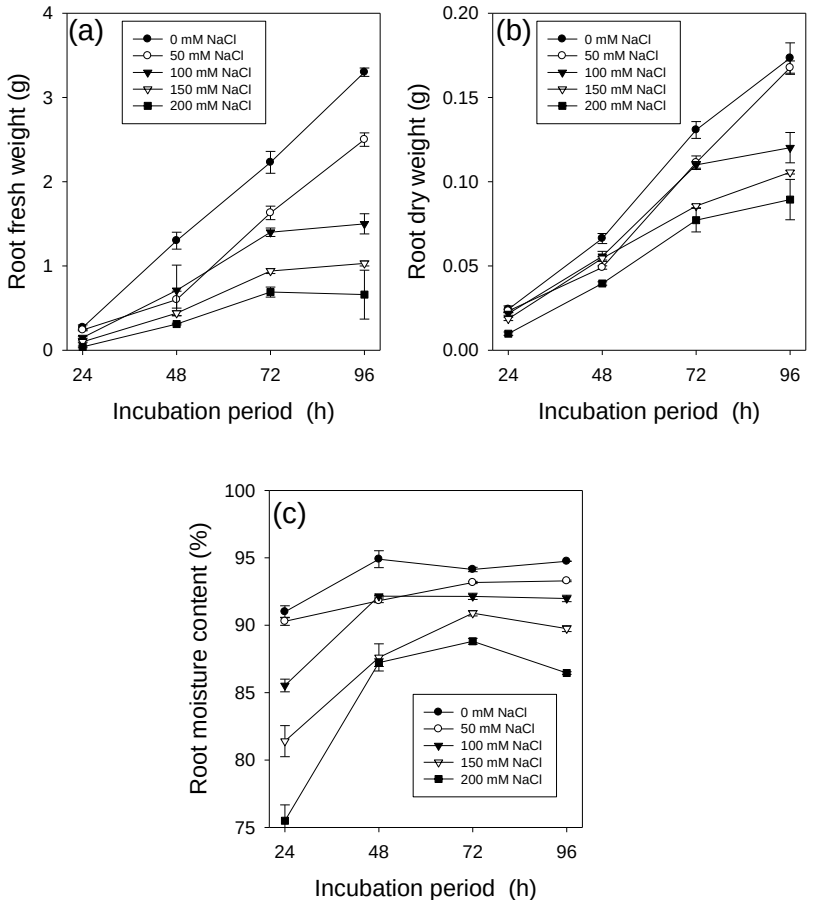

Fig. 3. Root fresh (a), dry weight (b) and root moisture content (c) of NIAB-78 roots treated with different concentrations of NaCl at different times after the start of the experiment.

Table 4. Results of a Tukey-Cramer multiple comparison test for the root dry weight data. Explanation of symbols etc., as in Table 1.

Comparison (*mM*)	Statistical significance			
	24 h	**48 h**	**72 h**	**96 h**
0 vs 50	ns	**	ns	ns
0 vs 100	ns	*	*	**
0 vs 150	ns	*	***	**
0 vs 200	***	***	***	***
50 vs 100	ns	ns	ns	*
50 vs 150	ns	ns	*	**
50 vs 200	***	ns	**	***
100 vs 150	ns	ns	*	ns
100 vs 200	***	**	**	ns
150 vs 200	**	**	ns	ns

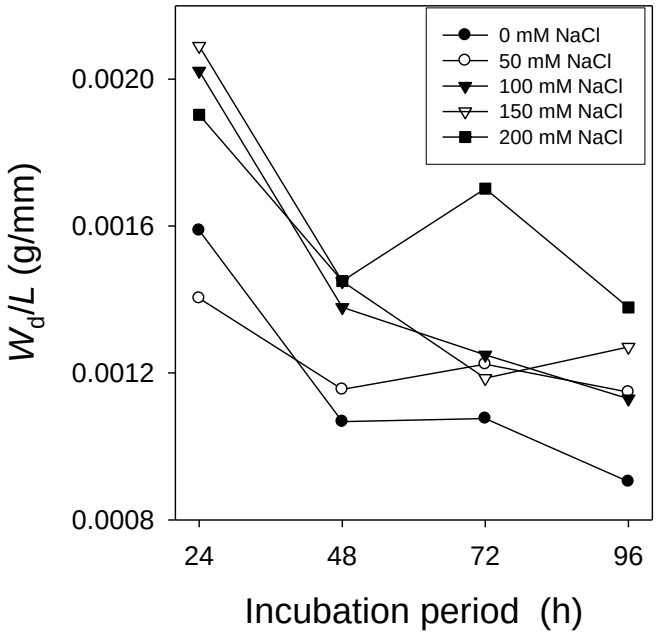

Fig. 4. The ratio of root dry weight and root length as a proxy for root diameter of NIAB-78 roots treated with different concentrations NaCl at different times after the start of the experiment.

Fig. 3b illustrates that a pattern similar to that in Fig. 3a can be observed for root dry weight, W_d, although the differences in W_d between the various treatments are less pronounced than those presented in Fig. 3a (Table 4, showing fewer significant differences and higher *P*-values than Table 3). Furthermore, the root growth rate during the final incubation period does not level off as much as for the W_f (at the 3 highest salinity levels). This means that the difference in W_f between these treatments is mainly caused by a decreased root moisture content, *M* (Fig. 3c). Furthermore, the levelling off (100 and 150mM) or decrease (200 mM) in W_f is largely caused by a reduced *M*, and less so by a decreased growth of root biomass.

Fig. 3c clearly shows that increasing salinity levels resulted in a decrease in *M*, data show that the uptake of water is clearly affected by the salt concentration in the growth medium: root soil moisture content goes down. During the experiment, the largest increase in root moisture content was recorded between 24 and 48 h of germination, for all treatments, but in particular at the higher salinity levels. Thereafter, *M* stayed approximately constant, and went down at the two highest NaCl levels, indicating the possibility of plasmolysis. The turgor of young root cells is about 0.6 Mpa. The osmotic pressure of a solution of about 150 mM NaCl is about 0.7 Mpa, so cells with a turgor less than this will plasmolyse if the plants are exposed 150 mM NaCl (Munns, 2002).

Changes in 'root diameter': Our study was not intended to test morphological modifications related to increased salinity levels and, hence, root diameter was not measured. However, the ratio of W_d and L was used as a proxy for root diameter (thinner roots will have less biomass per mm length of root and hence this ratio will be lower).

Fig. 4 shows the 'root diameter' as a function of incubation period for the different salinity treatments. W_d/L decreases over time. The main decrease occurs between 24 and 48 h, as generally expected in the early stages of root development, when roots are getting longer and narrower. However, it appears that, on average, 'root diameter' increases with increasing levels of NaCl. Neumann (1995) also found an increase in root diameter produced by salinity and suggested that a reduction in cell size, an increase in root diameter and a smaller plant size could be adaptive advantages for prolonged survival in saline or dry soils.

Sodium and potassium content: Fig. 5a shows that the Na^+ content in the roots was 0.15, 0.75 and 0.93% under non-saline conditions at 48, 72 and 96 h of incubation, respectively (note that unfortunately no data were available at 24 h). With an increase in incubation period, Na^+ content increased, although much less so during the final 24 h of the experiment. This emphasises the adequacy of seed reserves over the 96 h period.

The sodium content increased in response to increasing levels of salinity, especially for NaCl concentrations >100 mM. Note that for a concentration of 100 mM NaCl, the effect appears particularly pronounced after 72 and 96 h of incubation, so a delay appears to be present. It is not clear why that would happen in particular for this salinity level. Uptake of Na^+ levels stoped or goes down for the higher salinity levels, whereas the percentage Na^+ is still increasing for 0 and 50 mM, during the final 24 h of observation (although this increase appeared to be non-significant).

Table 5 shows that the difference in Na^+ between roots of the control level and all NaCl levels is significant at least at the $p<0.05$ level, but mostly at the $p< 0.001$ level. The same applies to the differences in root Na^+ between the 50 mM treatment and higher salinity levels (with the difference between 50 and 100 mM being the exception). For the intercomparison between Na^+ contents of roots of the higher salinity levels (100, 150, 200 mM), all differences are non-significant at 72 and 92 h and for the 150 v 200 mM also at 48 h. This means that at the end of the experiment Na^+ content is statistically the same for all salinity levels of ≥ 100 mM.

The increase of Na^+ with time is never significant at the 150 mM and 200 mM salinity levels. For all lower salinity levels and the control, changes in Na^+ were non-significant between 72 and 96 h, and between 48 and 72 h for the 50 mM level (statistics not shown).

Table 5. Results of a Tukey-Cramer multiple comparison test for the Sodium content data. Explanation of symbols etc., as in Table 1.

Comparison (*mM*)	Statistical significance		
	48 h	72 h	96 h
0 vs 50	*	*	*
0 vs 100	***	***	***
0 vs 150	***	***	***
0 vs 200	***	***	***
50 vs 100	ns	***	**
50 vs 150	***	***	**
50 vs 200	***	***	***
100 vs 150	**	ns	ns
100 vs 200	**	ns	ns
150 vs 200	ns	ns	ns

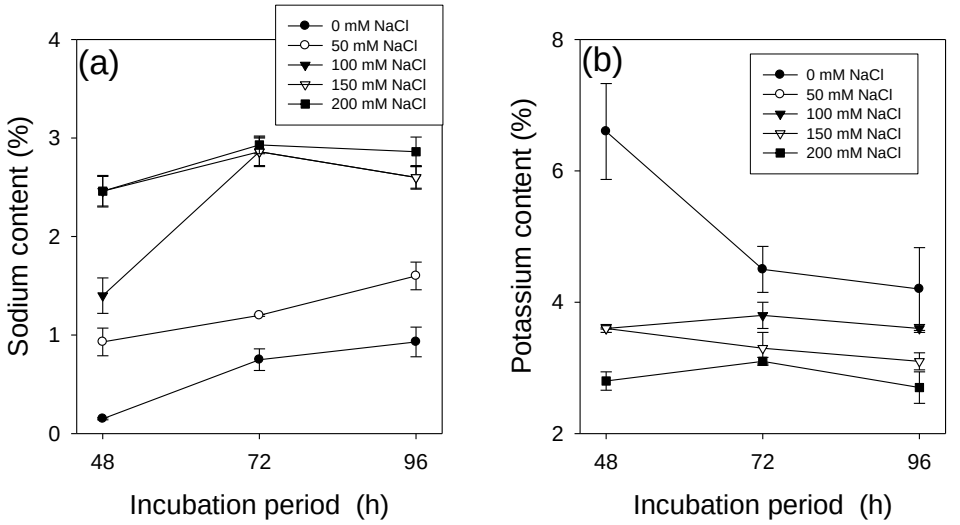

Fig. 5. Sodium content (a) and potassium content (b) of NIAB-78 roots treated with different concentrations of NaCl at different times after the start of the experiment.

The increase of Na^+ with higher salinity levels was accompanied by a decrease in K^+ (see Fig. 5b). It appeared that the introduction of salinity increasingly reduced the ability of plant roots to accumulate K^+ drawn from the seed reserve. Differences in K^+ between the control and the salinity treatments were highly significant at 48 h, but less so at 72 and 96 h (at 96 h, K^+ of 0 versus 150 mM only was significantly different). Differences among the salinity treatments (50 mM and higher) were non-significant at all incubation temperature.

At 96 h of incubation the ratio K^+/Na^+ decreased exponentially with an increase in salinity ($r^2 = 0.93$, n = 4). The ratio is 4.5 at 0 mM and close to unity at a salinity level of 200 mM. At this same harvest time, a strong positive correlation between K^+/Na^+ and root W_f was observed (logarithmic fit, $r^2 = 0.99$, n=4).

Table 6. Results of a Tukey-Cramer multiple comparison test for the Potassium content data. Explanation of symbols etc., as in Table 1.

Comparison (*mM*)	Statistical significance		
	48 h	72 h	96 h
0 vs 50	***	ns	ns
0 vs 100	***	*	ns
0 vs 150	***	**	*
0 vs 200	***	**	ns
50 vs 100	ns	ns	ns
50 vs 150	ns	ns	ns
50 vs 200	ns	ns	ns
100 vs 150	ns	ns	ns
100 vs 200	ns	ns	ns
150 vs 200	ns	ns	ns

Discussion

The seeds and seedlings studied were germinated and grown in a roll of moistened blotting paper. This is similar to the seed treatment used in Casenave *et al*., (1999) for their seedling growth experiment, who placed their seeds between two layers of germination paper saturated with distilled water, rolled the paper and then placed it vertically. However, in their germination experiment they placed their seeds between filter paper in 14 mm Petri dishes and added the NaCl solutions. In this way the seed environment would have stayed saturated throughout. The upright position of the plastic bag in which the seed rolls are placed in this experiment, causes drainage which may increase the salt concentration around the seeds and decrease the optimum moisture content (which explains our lower germination). Also, the solution is not replenished between rolling (see Tobe *et al.*, 2004), although re-rolling at 24, 48 and 72 h will add water again. Javid *et al*., (2001), placed their seeds in sand or soil after soaking them in suitable pre-treatment solutions (to disinfect them and prepare them for germination). Germination in moistened paper compared to sand generally gives considerably higher germination rate (e.g. at 0 salinity: 100% for Casenave *et al*., 1999, 90% in present study, and only about 25-30% for Javid *et al*., 2001).

Another difference between methods described in various papers concerns the different length of time during which germination is observed (e.g. 4 d in this case, 12 d for Casenave *et al.*, 1999). As an increase in salinity will not only affect the total germination, but also the rate of germination (lower rates at higher salinities), cutting off the experiment at an earlier time may lead one to believe that final germination is lower. This can be observed in Fig. 1 where the percentage of germination for the 150 mM treatment picks up considerably during the last 24 h. However, our data show that the trends of the effects of salinity on W_f and W_d generally become clear after 72 h. Therefore, a reversal of these effects at incubation periods >96 h is very unlikely.

Finally, the way in which the higher salinity levels are offered to the plants is important. In this case the seeds are brought into contact with the saline solution directly after pre-treatment and stay in this environment for the duration of the study. This is in contrast with the salt shock studies (salt on/salt off) described by Munns (2002), which allows plants to recover from low levels of salinity. Casenave *et al*., (1999), placed the seedlings (0, 100 and 275 mM NaCl) after they reached a root length of 3 cm. This may explain why their radicle length appeared to be less influenced by salinity than in our experiment.

The results clearly indicate that early seedling root growth (as expressed by root length, and fresh and dry root weight), to a lesser extent seed germination, and root Na^+ and K^+ content were affected by salinity.

Germination potential of seeds in saline conditions can be a simple and useful parameter for several reasons. First, salinity resistance at this stage has been shown to be a heritable trait which could be used as an efficient criterion for the selection of salt-resistant populations (Ashraf *et al*., 1987). Secondly, seeds and young seedlings are frequently confronted by much higher salinities than vigorously growing plants because germination usually occurs in surface soils which accumulate soluble salts as a result of evaporation and capillary rise.

Salt stress inhibits seed germination by limiting water absorption by the seeds (Dodd & Donovan, 1999), thereby arresting radicle emergence, although a toxic influence of salt cannot be excluded (Kurth *et al*., 1986). Furthermore, salt stress affects the mobilisation of stored reserves (Bouaziz & Hicks 1990) and the structural organisation or synthesis of protein in germinating embryos (Ramagopal, 1990). NIAB-78 is one of the most tolerant varieties of cotton at germination (along with PH-36). Indeed, germination (Fig. 1) seems to have been influenced considerably less than growth (Fig. 2b and 2c) or biomass production (Fig. 3a and 3b). The germination results are in broad agreement with those of Almansouri *et al*., (2001), Bajji *et al*., (2002) and Tobe *et al*., (2004) who also reported that germination of seeds for a variety of species decreased in response to increasing levels of salinity. Severity of the decrease strongly depended on plant species.

The reduction of the cotton seedlings' root length in response to increased salinity levels agrees with the findings of Almansouri *et al*., (2001) who showed that total wheat seedling length decreased with increasing concentrations of osmotic agents (NaCl and polyethylene glycol (PEG)), with dramatic effects at the highest NaCl concentration. Similar findings were also reported by Shalhevet *et al*., (1995) for maize and soybean seedlings, and Zhong & Lauchli (1993), for cotton seedlings.

Salt tolerance is often expressed as the percent biomass production in saline versus control conditions over a prolonged period of time. Salt tolerant species then have <20% reduction, cotton about 60%, and other species may be dead. Here we only have 96 h of data, but it clearly shows that the reductions in W_d vary between 3% (at 50 mM) and 48% (at 200 mM NaCl). These results are supported by the findings of Ashraf *et al*., (2002) and Katsuhara *et al*., (2003), who also reported a decrease in root fresh and dry weight with increasing salinity. Sairam & Srivastava (2002) reported that salinity decreased the relative water content of wheat roots.

Some studies have shown an increase in root growth in mild salinity (Jafri & Ahmad, 1994) for NIAB-78. Their results are not similar with our results. It may be related to the fact that these authors used soil as their growth medium; the interaction between the nutrients in the soil and the salts (e.g., reduced capacity to take up Ca^{2+} under saline conditions) may have played a role.

Various researchers (Kent & Lauchli, 1985; Cramer *et al*., 1987; Lleidi & Saiz, 1997) have investigated the effect of salinity on the content of K^+ in roots. Kent and Lauchli (1985) reported that contents of K^+ and Ca^{++} reduced as a result of an increase NaCl in both shoot and roots of cotton. Lleidi & Saiz (1997) found K^+/Na^+ ratios of ~ 1 for roots of cotton seedlings grown in salinised nutrient solutions, at 200 mM NaCl.

Mechanisms for salt tolerance vary widely (Munns, 2002). One mechanism involves exchanging K^+ for Na^+ by the cells lining the transpiration stream, which explains the high Na^+/K^+ ratios we found under increasing salt levels. Under normal physiological conditions plant cells have a relatively high K^+ concentration and low Na^+ concentration

in the cytosol (Bienzel *et al*., 1988), as shown by the results for the control in Fig. 5, which results from the selective uptake of potassium. In this case there is no potassium in the solution so the K^+ has to come from the seed reserve. The salt stress inhibits the mobilisation of stored reserves (Bouaziz & Hicks 1990).

Impaired K^+/Na^+ selectivity and sodium-induced K^+ deficiency are major factors involved in growth and yield reductions under saline conditions (Grattan & Grieve, 1999), which also explains the high Na^+/K^+ ratios we found under increasing salt levels. Salinity also impairs the uptake of Ca^{2+}, possibly by displacing it from cell membranes and altering membrane integrity (Lynch *et al*., 1987). In most plants accumulation of ions has a toxic effect and disturbs ion homeostasis (Hasegawa *et al*., 2000). The ion toxicity leads to irreversible damage to cellular membranes (Serrano & Gaxiola, 1994).

Conclusions

Despite the fact that NIAB-78 is a relatively salinity resistant cotton variety the results of this experiment illustrate that only its germination appears to be relatively unaffected by increased salinity levels. After 4 d of incubation, the germination rate is on average 86% for the 0, 50, 100, and 150 mM salinity levels (although percentage germination decreases with increased salinity, the differences are not statistically significant) and only significantly lower for the 200 mM NaCl treatment (77% germination).

Root length and biomass-related indicators of plant growth (such as fresh and dry weight) are seriously reduced, which shows that using germination as a sole indicator of resistance to salinity can be misleading.

Increased salinity levels lead to decreased K^+/Na^+ ratios, but note that this can not be caused by selective uptake of potassium from the solution, as the germination water contained no K^+. K^+ (and Na^+ for the control) were taken from the seed reserve.

The strong positive correlation between K^+/Na^+ and root fresh weight underlines the clear relationship that exists between this ion-ratio and root growth. Leidi & Saiz (1997) found a negative correlation between leaf growth and K^+/Na^+ ratio and interpreted this as a lack of significance of this parameter to cotton. The results presented here show that this parameter does play a significant role when considering root growth.

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