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Salt Tolerance of *Vigna angularis* during Germination and Early Seedling Growth

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The present study was undertaken to investigate the response to salinity and effect of plant growth regulators and proline under salinity stress on the germination and early seedling growth of *Vigna angularis*. The protective effect of external Ca^{2+} on root elongation under saline conditions was also investigated. The seed germination of *Vigna angularis* decreased with an increase in salinity. The growth regulators GA_3 and kinetin substantially alleviated the salinity- induced germination inhibition and GA_3 was more effective than kinetin. At a higher salinity, low concentrations of kinetin and high concentrations of GA_3 were more effective. The external application of proline and betaine improved germination under saline conditions. At a low salinity proline and betaine alleviated the salinity-induced inhibition of germination, yet at higher NaCl concentrations, proline and betaine were both ineffective. Exposure to salinity during germination was accompanied by an increase in the proline content, thereby suggesting that one compatible solute in the germinating seed would seem to be proline. The inhibition of germination by high NaCl concentrations was relatively more severe in scarified seeds than in intact seeds, indicating that the seed coat acts as a partial barrier to an Na^+ influx. A significant decrease in root elongation accompanied the response to external NaCl concentrations. Exogenous Ca^{2+} ameliorated the inhibition of root elongation, especially in the polyethylene glycol(PEG) and NaCl treatments. Elevated external levels of Ca^{2+} ameliorated the adverse effect of salinity stress.

Key words : calcium, germination, gibberellic acid, kinetin, proline, salinity stress, *Vigna angularis*

1. Introduction

Salinity stress is a widespread environmental problem that poses a severe threat to the growth and productivity of many plants(Boyer, 1982 ; Ashraf, 1994). Excessive soil salinity occurs in many semi-arid regions of the world, and the progressive salinization in irrigated land limits crop quality and yield. The metabolisms underlying salt stress in plants are complex not only physiologically but also genetically. Responses to salinity stress at the whole level are very variable and depend on a number of factors such as plant age, tissue organization, and environment.

Germination is a crucial stage in the life cycle of many plants, and salt tolerance during the germination stage is critical for the establishment of plants that grow in saline soil(Khan and Rizvi, 1993). The response of plants to salt stress depends on many factors, however, phytohormones are thought to be among the most important endogenous substances involved in the tolerance mechanisms or susceptibility of various plant species. Growth regulators such as gibberellic acid and kinetin are known to alleviate the inhibitory effect of salinity on germination(Khan and Ungar, 1997). However, the role of plant growth regulators in alleviating seed germination and dormancy induced by salt stress are not clearly understood.

The deletrious effects of salinity on plant growth

are attributed to a decrease in the osmotic potential of the growing medium, specific ion toxicity, and nutrient ion deficiency(Huang and Redmann, 1995 ; Munns *et al.*, 1995). The extent of injury to a plant by salinization varies with the salt composition, salt concentration, physiological stage of the plant when it is exposed to salinity, and plant species(Katembe *et al.*, 1998). Nonhalophytes exposed to salt stress must be able to respond to potential water deficit, ion toxicity, and nutrient balances(Greenway and Munns, 1980). However, not much information is available on the mechanism of salt injury and the relative extent of the osmotic and ionic components during the germination processes.

Plants have the ability to regulate their intracellular osmotic pressure in response to osmotic stress by accumulating various solutes(Sharp and Davies, 1985). Supplemental calcium plays a significant role in regulating the absorption of ions and can be expected to make a significant contribution to the osmotic adjustment in plant roots under salt stress by regulating the absorption of K^+(Nakamura *et al.*, 1990). It has been extensively reported that external Ca^{2+} ameliorates the long-term negative effects of NaCl on plant growth and protects against the adverse effects of salinity (Cachorro *et al.*, 1994). Salt tolerance resulting from the presence of external Ca^{2+} may be correlated with a regulation of the membrane permeability of plant cells by Ca^{2+}. However, a short-term effect of Ca^{2+} has also been observed when water relations are mainly involved in the plant response to saline shock(Ortiz *et al.*, 1994). Nevertheless, these early effects of Ca^{2+} have not been clearly established.

Many plants in saline habitats are known to accumulate organic solutes such as glycine-betaine, proline, and others(Poljakoff-Mayer *et al.*, 1987). Proline has been found to accumulate in some species as a symptom of a deleterious response to drought, salinity, or heavy metal stresses. This compound may act as a cytoplasmic osmoticum or nitrogen store, or it may protect the enzymes and membranes against salt or heat inactivation (Paleg *et al.*, 1985). Poljakoff-Mayer *et al.*(1994) reported that during germination in the presence of NaCl the betaine content decreased, while the proline content increased, thereby suggesting a possible role of proline during germination. The alleviation of the NaCl inhibition of growth by exogenous proline has been reported in other whole plants(Lehle *et al.*, 1992).

The aim of this study was to investigate the effect of NaCl on the germination and early seedling growth of *Vigna angularis* and determine whether the application of growth regulators and compatible osmotica, such as proline and betaine, could alleviate the germination induced by high salinity. The protective effect of Ca^{2+} on the elongation of intact roots under saline stress is also discussed.

2. Materials and Methods

2.1. Germination conditions

The seeds of *Vigna angularis* were sterilized with a 1% sodium hypochlorite solution for 1 min, then washed several times with distilled water. The germination experiments were carried out in petri dishes(9cm diam.) with two layers of filter paper discs containing 10 ml of the test solution. The seeds were then incubated in a growth chamber at a thermoperiod of 27 : 18℃(light : dark) and 12 h photoperiod($30\,\mu$mol photons $\cdot m^{-2} \cdot s^{-1}$). GA_3 concentrations of 0.1 and 1.0 mM, kinetin concentrations of 0.01 and 0.1 mM, proline and betaine concentrations of 0.1 and 1.0 mM, and NaCl concentrations of 100, 200, and 300 mM were used. Four replicates of twenty seeds were used for each treatment and the control. The seed germination was recorded daily for 5 consecutive days and the cumulative germination was determined. When the seeds were scarified, as small a piece as possible of the seed coat was cut off on the chalazal side. A seed was considered to have germinated upon the emergence of the radicle. The germination data were transformed before statistical analysis. An ANOVA analysis was used to determine if significant differences were present among the means. A Bonferroni test was carried out to determine if significant($P < 0.05$) differences occurred between the individual treatments(SPSS, 1994).

The experiments on germination in ion-osmotic solutions of salts were also carried out in a growth chamber. The control contained 10 mM KNO_3 plus 10mM NH_4NO_3, which is recommended for

promoting the germination of barley and mustard (Huang and Redmann, 1995). Salt solutions with similar osmotic potentials were prepared by adding salts to the control solution.

2.2. Growth measurements

For each harvest, the fresh and dry weights of the seedlings were determined, and the shoot and root lengths were recorded after the last day of germination measurements. The fresh weight was determined immediately after harvest. The dry mass production was determined by drying the seedlings at 80℃ for 48 h. The root elongation was estimated at intervals by measuring the distance from the reference point to the root tip. The proline was assayed according to Bates et al.(1973).

2.3. Plant growth conditions for Ca^{2+} effect

The seeds were imbibed in tap water in the dark for 24 h and then incubated in the growth medium (0.1mM KCl, 0.5mM $CaCl_2$, pH 5.8) for 24 h. After measuring their root lengths, the two-day-old seedlings were transplanted into a growth chamber containing the experimental medium, which consisted of 0.1mM KCl plus NaCl and $CaCl_2$ at various concentrations. Groups of twenty seedlings were grown in darkness in the growth chamber at 27℃ and the root lengths were measured at 4h intervals for 24h after the transplantation. The two-day old seedlings containing either 0.5 or 5mM Ca^{2+} were then treated with 100 mM NaCl or with an isosmotic solution of PEG 6000(Fluka, MW 5000-7000)

3. Results

3.1. Effect of salinity, growth regulators, and compatible osmotica on germination response

The germination of *Vigna angularis* deceased with an increase in salinity(Fig.1). The germination was substantially reduced in the 300mM NaCl treatment. Treatments higher than 300mM had little germination(data not shown). As expected the increased concentrations of NaCl delayed the germination. All concentrations of GA_3 and kinetin significantly promoted the germination of seeds in all salinity treatments(Figs. 2 and 3). Higher GA_3 and kinetin concentrations alleviated the high inhibitory salinity effects and promoted germination compared with the control. The growth regulators not only increased the percentage of germination of the seeds under saline conditions but also shortened the time required for germination. In the control and all NaCl treatments, increased GA_3 concentrations had a significant ($p<0.05$) effect on alleviating seed germination.

The inhibition of germination caused by NaCl salinity was progressively alleviated by increasing the GA_3 concentration(Fig. 2). A one-way ANOVA of germination for each salinity level revealed that GA_3 significantly affected the rate of germination of seeds at 0mM NaCl(0.00039), 100mM NaCl ($p<0.0016$), 200 mM NaCl($p<0.0059$), and 300mM NaCl($p<0.0054$). Kinetin also alleviated the germination in the non-saline controls and in the salinity treatments(Fig. 3). At a high(300mM) salinity, the low kinetin concentration(0.01mM) was more stimulatory than the high kinetin concentration (0.1mM). A one-way ANOVA for each salinity level revealed that kinetin significantly affected the rate of germination of seeds at 0mM NaCl ($p<0.0013$), 100 mM NaCl($p<0.0009$), 200mM NaCl($p<0.0203$), and 300mM NaCl($p<0.0149$). The treatment with GA_3 alone was more effective than combined with kinetin. Proline and glycine

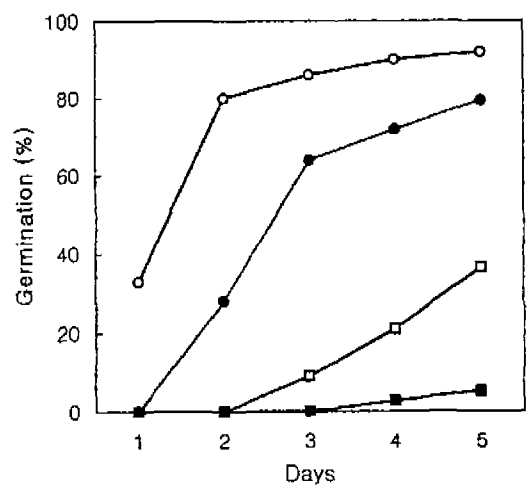

Fig. 1. Time course of germination of *Vigna angularis* in response to various concentrations of NaCl. ○, control ; ●, 100mM NaCl ; □, 200mM NaCl ; ■, 300mM NaCl.

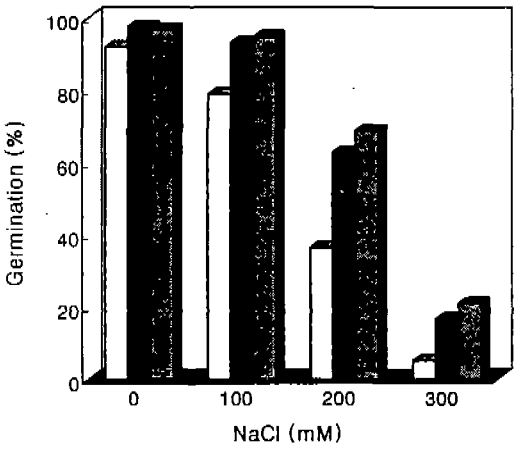

Fig. 2. Final germination percentages of *Vigna angularis* seeds after 10 days with various salinities and GA_3 regimes. □, control ; ■, 0.1mM GA_3 ; ▨, 1.0mM GA_3.

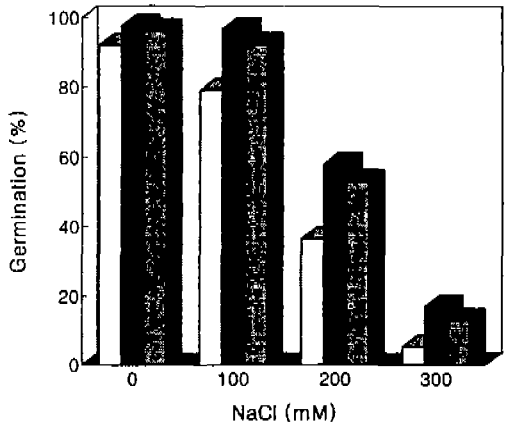

Fig. 3. Final germination percentages of *Vigna angularis* seeds after 10days with various salinities and kinetin regimes. □, control ; ■, 0.01mM kinetin ; ▨, 0.1mM kinetin.

betaine somewhat ameliorated the harmful effects of salinity(Figs. 4 and 5). However, with higher salinity treatments(300mM NaCl) neither of them were effective in alleviating the germination of the seeds. A one-way ANOVA of the rate of germination for each salinity level revealed that proline significantly increased the germination percentage of the seeds at 0 mM NaCl($p<0.0205$), 100 mM NaCl($p<0.0053$), and 200 mM NaCl ($p<0.0049$), whereas the effect of proline was not significant at 300 mM NaCl($p>0.2206$). The rate of germination in non-saline and a low quality level with betaine was substantially higher than in the controls. A one-way ANOVA for each salinity revealed that betaine increased the germination percentage of the seeds at 0 mM NaCl($p<0.0052$), 100 mM NaCl($p<0.0001$), and 200 mM NaCl ($p<0.004$), whereas the effect of betaine was not significant at 300 mM NaCl($p>0.3319$).

The content of proline(per seed) increased with the time of incubation, however, only in those seeds that germinated(Table 1). In the non-germinated seeds, the proline content remained very low, in some cases(after 24h) not higher than in the dry seeds, apparently depending on the degree of inhibition. In the non-germinated seeds, the radicle was on the verge of protrusion. Exposure to salinity was accompanied by an increase in the proline content.

Table 1. Effect of salinity on proline content of *Vigna angularis* seeds during germination

A. Germinated seeds

Duration of solution incubation(h)	Concentration of NaCl (mM) in external medium			
	0	50	100	200
24	3.2	-	-	-
48	4.1	4.6	5.2	-
72	5.9	5.8	6.1	6.7
96	5.7	6.2	6.8	7.5

B. Non-germinated seeds

Duration of solution incubation(h)	Concentration of NaCl (mM) in external medium			
	0	50	100	200
24	1.8	1.5	1.2	0.9
48	4.5	3.2	2.9	1.7
72	-	-	3.1	2.2
96	-	-	-	2.6

* Proline content is expressed as μg $seed^{-1}$. (-) designates that there are no seeds in this category ; either all have germinated or none were germinated.

The germination in iso-osmotic solutions of salts was reduced by NaCl and Na_2SO_4+$MgSO_4$(Table 2). Iso-osmotic PEG decreased the germination significantly, relative to the controls. The addition of calcium had little improving effect on germination relative to equivalent treatments without calcium. The PEG had a more severe effect on germination than the iso-osmotic salt solutions.

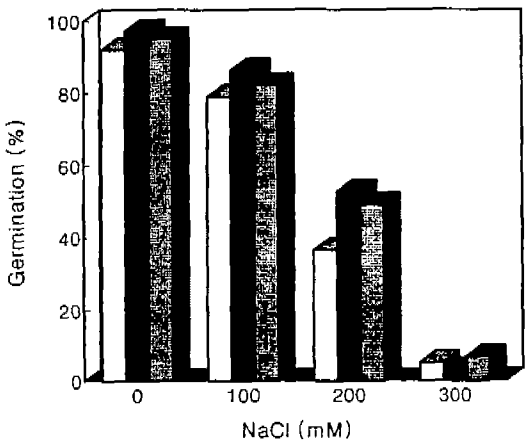

Fig. 4. Effect of proline on germination percentage of *Vigna angularis* seeds under salt stress. □, 0mM proline ; ■, 0.1mM proline ; ▨, 1.0mM proline.

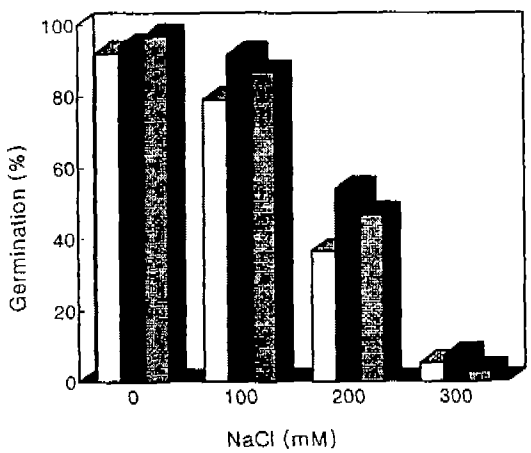

Fig. 5. Effect of betaine on germination percentage of *Vigna angularis* seeds under salt stress. □, 0mM betaine ; ■, 0.1mM betaine ; ▨, 1.0mM betaine.

Table 2. Final germination of *Vigna angularis* in iso-osmotic solutions of salts

Treatment(mM)	Germination(%)
Control(10 KNO_3+10 NH_4NO_3)	94.5
75 Na_2SO_4+150 $MgSO_4$	72.8
75 Na_2SO_4+150 $MgSO_4$ + $CaCl_2$	86.4
25% PEG 8000	30.7
25% PEG 8000+$CaCl_2$	55.6
179 NaCl	42.5
179 NaCl+$CaCl_2$	57.2

3.2. Effect of salinity and Ca^{2+} on growth

An attempt was made to differentiate between the osmotic and ionic effects of NaCl by germinating seeds in parallel in equiosmotic NaCl and mannitol solutions. In order to overcome the permeability problem, the response of intact and scarified seeds were compared. The germination of the scarified seeds was much faster than that of the intact seeds. In the intact seeds there was no difference between the NaCl and mannitol treatments and the percentage of germination was linearly related to the osmolarity of the medium (Fig. 6A). In the scarified seeds, the percentage of germination in the mannitol solution was also linearly related to the medium osmolarity(Fig. 6B).

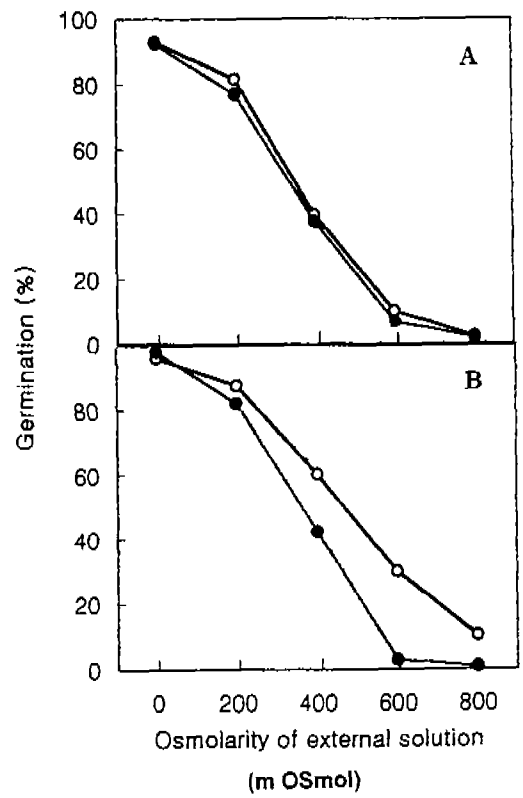

Fig. 6. Effect of NaCl and mannitol on germination related to osmolarity of external solution on (A) intact and (B) scarified seeds. ○, mannitol ; ●, NaCl.

However, in the NaCl solution, no linearity was observed and at concentrations above 300m Osmol

the germination was strongly inhibited. Salinity inhibited the growth of the seedlings. The elongation of the roots and shoots decreased in proportion to the external NaCl concentrations, and above 200mM NaCl, the growth rate was almost completely inhibited(Fig. 7). As can be seen from a comparison of the changes in the fresh and dry weights, the saline condition in *Vigna angularis* resulted in a decreased dry weight during the development(Fig. 8), although the differences were very slight, indicating that the seedlings were dehydrated with an increased concentration of NaCl.

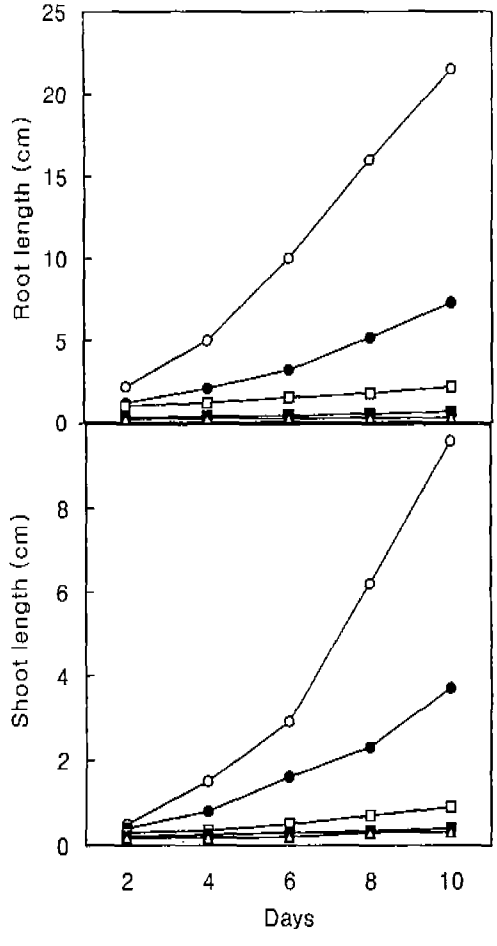

Fig. 7. Elongation of root(A) and shoot(B) of *Vigna angularis* seedlings exposed to different NaCl concentrations. ○, control ; ●, 50mM NaCl ; □, 100mM NaCl ; ■, 150mM NaCl ; △, 200mM NaCl.

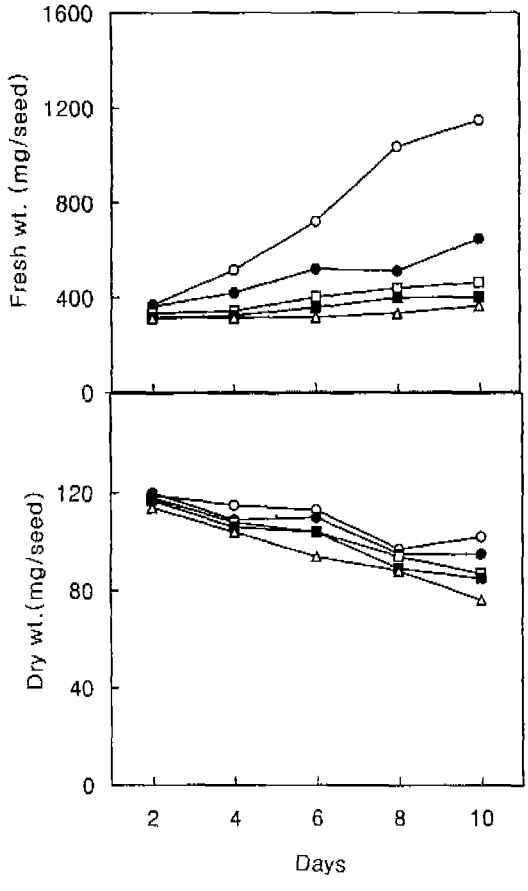

Fig. 8. Changes in (A)fresh weight and (B)dry weight of *Vigna angularis* seedlings with different NaCl treatments. ○, control ; ●, 50mM NaCl ; □, 100mM NaCl ; ■, 150mM NaCl ; △, 200mM NaCl.

3.3. Effect of external Ca^{2+} on root elongation under salinity stress

Fig. 9 shows the typical kinetics of the root elongation after the transplantation of the intact seedlings into the experimental media that contained 0mM and 50mM NaCl. The root elongation was suppressed by the addition of 100 mM NaCl. In particular, in the absence of external Ca^{2+}, there was a significant inhibition of root elongation in 100mM NaCl. The root elongation was significantly inhibited under high salinity conditions in the absence of Ca^{2+}. The rate of elongation was reduced with concentrations of 50mM NaCl and above in a concentration-

dependent manner in both the absence and presence of Ca^{2+}(Fig. 10). The rate of root elongation under 100mM NaCl conditions increased with an increased concentration of Ca^{2+}(Fig. 11). The root elongation was suppressed under the osmotic pressure of a 200 mM solution of mannitol, which corresponds to that of 100mM NaCl(Table 3).

When compared with unstressed plants, the root elongation was reduced by treatment with either NaCl or PEG(isosmotic potentials)(Table 4). The growth of the salinity-treated samples pre-incubated with a high Ca^{2+} concentration was only slightly affected. The salinity-treated plants with a low Ca^{2+} concentration along with the PEG-treated samples showed growth rates that were more effected by osmotic shock. With a low Ca^{2+} concentration during the experiments, the root elongation of the plants in PEG was always lower than that of the salinized plants. The root elongation of the plants grown in PEG with high Ca^{2+} concentrations were slightly higher than those receiving PEG and a low Ca^{2+} treatment, although the difference between them was less than when under NaCl stress.

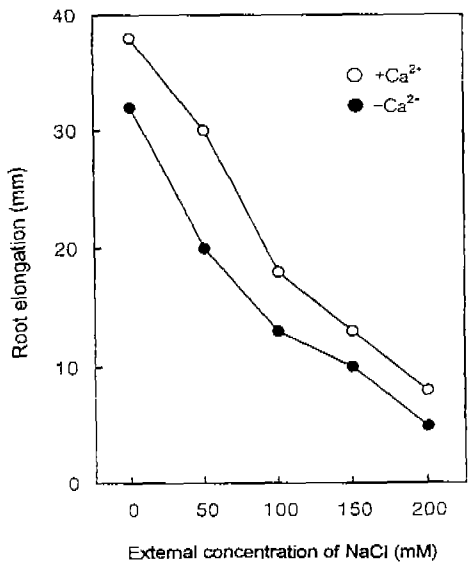

Fig. 10. Root elongation over course of 24h with different NaCl treatments with and without 0.5mM Ca^{2+}.

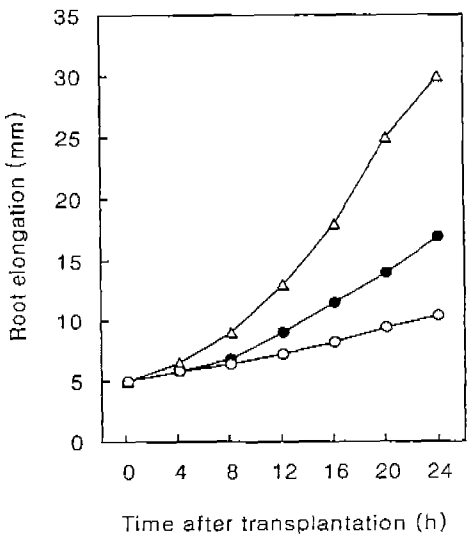

Fig. 9. Kinetics of root elongation during 24h with 0mM NaCl, 0.5mM Ca^{2+}(control), and 50mM NaCl with and without 0.5mM Ca^{2+}. △, 0mM NaCl(+Ca^{2+}) ; ●, 50mM NaCl(+Ca^{2+}) ; ○, 50mM NaCl(-Ca^{2+}).

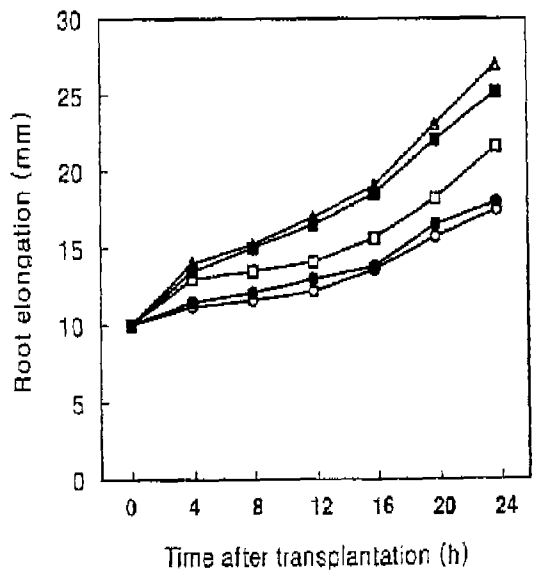

Fig. 11. Effect of external concentration of Ca^{2+} on root elongation during 24h with 100mM NaCl. ○, +0.5 mM Ca^{2+} ; ●, +1.0mM Ca^{2+} ; □, +4.0mM Ca^{2+} ; ■, +5.0mM Ca^{2+} ; △, +7.0mM Ca^{2+}.

Table 3. Growth of roots of *Vigna angularis* after 24h with mannitol

Treatment	Root elongation(mm)
Control	30.5
100mM mannitol	20.7
100mM mannitol+0.5mM $CaCl_2$	30.4
200mM mannitol	13.6
200mM mannitol+0.5mM $CaCl_2$	18.5
300mM mannitol	10.2
300mM mannitol+0.5mM $CaCl_2$	13.5
400mM mannitol	5.7
400mM mannitol+5.0mM $CaCl_2$	8.5

Table 4. Effect of osmotic treatment on root length of *Vigna angularis* under stress

Treatment	Root elongation(mm)
Control, 0.5mM $CaCl_2$	22.5
Control, 5.0mM $CaCl_2$	24.7
0.5mM $CaCl_2$+100mM NaCl	16.3
5.0mM $CaCl_2$+100mM NaCl	19.6
0.5mM $CaCl_2$+12% PEG	14.8
5.0mM $CaCl_2$+12% PEG	15.5

4. Discussion

Salinity is known to adversely affect plant growth and metabolic processes. Lower levels of salinity delay germination and higher levels reduce the final percentage of germination(Sharma, 1983). The germination of *Vigna angularis* was decreased with an increase in salinity(Fig. 1). The germination was substantially inhibited at 300mM NaCl. The plant growth regulators, GA_3 and kinetin, were found to be very effective in alleviating the innate dormancy along with the salinity-induced dormancy in many plants(Khan, 1991 ; Khan and Rizvi, 1993). At a lower salinity these growth regulators not only alleviated the effect of salinity but also promoted germination in comparison with the control(Fig. 2 and 3). GA_3 was more effective than kinetin on stress. Similar effects have also been observed in other plants(Khan and Ungar, 1985 ; Khan and Weber, 1986). It has been reported that gibberellins play a primary role in the control of germination, whereas the roles of cytokinins and inhibitors are secondary and preventive and permissive, respectively. Kinetin can affect inhibition by increasing the permeability of the membrane, thereby selectively enhancing the movement of GA_3 in and out of the embryonic tissues(Kabar, 1987). The effects of NaCl on seed germination are both ionic and osmotic(Ungar, 1991). Compatible osmotica such as proline and betaine, which acclimatize plant tissue to high salt concentrations, may be of significance in seeds. Poljakoff-Mayber *et al.*(1994) studied the proline and betaine levels in *Kostelletzkya virginica* seeds during the process of germination. They suggested that proline could be a compatible osmoticum for germinating seeds. The external application of proline and betaine to *K. virginica* seeds had no effect on germination in both the control and the saline medium. The current study indicates that exogenous proline and betaine alleviate the germination of *Vigna angularis* seeds(Fig. 4 and 5). At a low salinity 0.1 mM proline and betaine were more effective in alleviating the salinity-induced inhibition of germination. Both proline and betaine failed to show any effect on alleviating germination at higher salinity concentrations. These results are similar to those of Khan and Ungar (1997), who observed no effect of compatible osmotica at a high salinity, yet substantially promoted germination at low a salinity with both proline and betaine. The content of endogenous proline was very low in the dry seeds and this increased as the germination advanced(Table 1). Exposure to salinity was accompanied by an increase in the proline content ; thus proline may be considered as a compatible solute. These data indicate that compatible osmotica can overcome drought or ionic stress in seeds, whereas growth regulators directly stimulate germination.

The physical process of water uptake leads to the activation of metabolic processes as the dormancy of the seed is broken following hydration. The main impairment for water entry into a seed during imbibition appeared to be the low water permeability of the seed coat ; the scarified seeds imbibed freely and germinated 100%. It has also been shown that permeability to water increases with time during prolonged storage (Poljakoff-Mayber *et al*, 1994). A comparison of the effects of NaCl and mannitol solutions of equal osmotic concentrations(Fig. 6) showed a clear

difference between the percentage of germination in the equiosmotic concentrations of NaCl and manitol. In both cases the germination percentage decreased with an increased osmolarity. In the scarified seeds, however, the effect of the two solutes was different. In mannitol, similar to the intact seeds, the percentage of germination decreased with an increased external osmolarity, yet more gradually. However, the NaCl solutions drastically inhibited the germination of the scarified seeds. These results suggest that the seed coat not only substantially impaired the water entry into the intact seeds, but also controlled the ion entry. In the scarified seeds, where there was no barrier to solute entry, a strong inhibition of germination by NaCl occurred. An elevated salinity showed a lower water uptake by the seeds, which, therefore, inhibited their germination and root elongation. With a low osmotic potential, both NaCl and PEG inhibited the processes of inhibition, germination, and root elongation of *A. prostrata*(Katembe *et al*, 1998). Ungar(1978) reported that inorganic ions are not more inhibitory than mannitol and PEG in several halophytes, thereby demonstrating that seeds are mainly affected by osmotic stress rather than specific ion toxicities. For all of the treatments, PEG inhibited germination to a greater extent than the iso-osmotic salt solutes(Table 2), probably because the salt ion uptake reduced the osmotic stress on the seedlings. PEG and NaCl were equally effective in reducing germination in *Vigna angularis*. Huang and Redmann(1995) reported a similar result in the *Hordeum* and *Brassica* species.

Early seedling growth was significantly reduced with an increased salinity(Fig. 7). The seedling growth was highest in the controls, which indicates that salinity is not necessary for the optimal growth of *Vigna angularis*. Khan and Rizvi(1993) showed a progressive decrease in dry weight with an increase in salinity in *Atriplex* spp.. Similar results were obtained in the present study(Fig. 8). Salinity affects the growth of plants by decreasing the availability of water to the roots due to the osmotic effect of external salt and through the toxic effects of the excessive salt accumulation within the plant (Munns *et al.*, 1995). An increase in the external concentration of NaCl has both an osmotic and an iso-specific effect on plant growth. However, the root elongation was not affected by the osmotic stress generated by the addition of 200mM mannitol to the medium(Table 3), which is iso-osmotic with 100 mM NaCl. Accordingly, the reduction in the rate of root elongation at concentrations of NaCl up to 100mM is due rather to the ion-specific effect of NaCl(Itoh *et al.*, 1986).

Supplemental calcium had some appreciable effect on the final germination in *Vigna angularis*. Kent and Lauchli(1985) reported that the addition of calcium has no effect on germination yet improves root growth in cotton. Ca^{2+} plays an important role in the response of plants to externally imposed saline or non-ionic osmotic shock. The ameliorative effect of Ca^{2+} on growth was previously observed in barley(Suhayda *et al.*, 1992), bean plants(Ortiz *et al.*, 1994), and bean germination(Cachorro *et al.*, 1994). The results of these studies indicate that calcium responsiveness may vary with the developmental stage and be characteristic of only certain species. In the current study, external Ca^{2+} seemed to have a significant effect on root elongation during a 24 h incubation under saline conditions(Fig. 9 and 10). The rate of root elongation was increased with an increased concentration of Ca^{2+}(Fig. 11). Thus, Ca^{2+} overcomes the inhibited root elongation under NaCl stress via the antagonism of Ca^{2+} against the effects of NaCl. It has been proposed that the protective effect of Ca^{2+} in salinized plants is due to its role in maintaining the structural integrity of the plasma membrane(Cramer *et al.*, 1985) and modulating the lipid composition of the root cell membranes (Cachorro *et al.*, 1993).

The presence of a high concentration of Ca^{2+} in PEG-stressed plants slightly increased the growth rate of the roots(Table 4), indicating that Ca^{2+} exerts a beneficial effect on plants under osmotic shock. High external levels of Ca^{2+} were able to overcome the ion-specific effects, yet not offset the osmotic effects on root elongation under salt stress. These results clearly demonstrate that ion-specific stress and osmotic stress act on different aspects of the mechanisms of plant growth. Accordingly, it should be considered that the lack of growth restoration in the PEG-stressed plants may be due to in part to the non-osmotic effects sometimes ascribed to PEG. In the current study, no deleterious effects were observed in plants growing in high quality PEG 6000, therefore, the previously reported

growth inhibition of roots, as observed in beans (Fernandez-Ballester *et al.*, 1997), would not appear to be due to a necrotic reaction. The fact that PEG inhibits plant growth seems to indicate that water deficit rather than salt toxicity is the limiting factor of plant growth.

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