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Water Uptake and Germination Pattern of Rice Seeds under Iso-osmotic Solutions of NaCl and Peg, Different Concentrations of $CaCl_2$ and Combinations of NaCl and $CaCl_2$

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Abstract: Water uptake and pattern of germination of two rice cultivars differing in seed size were determined in iso-osmotic solutions of NaCl and polyethelene glycol, different concentrations of $CaCl_2$ and combinations of NaCl and $CaCl_2$. The results demonstrated that full imbibition of rice seeds occurred at around 30% moisture content (mc) and the critical mc for germination was around 25-30%. Although rates of water uptake in rice seeds were reduced with increasing salinity, rice seeds attained full imbibition by 48 h up to 150 mM salinity and reached at least critical mc by 72 h up to 250 mM salinity. Water uptakes in NaCl solutions were greater than in iso-osmotic solutions of PEG and rice seeds did not attain even critical mc in PEG solutions lower than -0.232 MPa even after 72 h. When Ca was added in combination with NaCl, water uptake in rice seeds increased. Water uptake in smaller seed was less during the first 12 h of imbibition than larger seeds. However, equilibrium mc was attained within 48 h in both large and small seed. The onset of germination declined with increasing concentrations of NaCl and in iso-osmotic solutions of PEG, particularly below-0.457 MPa osmotic potential. Large and small grain rice seed differed significantly in their response to salt and osmotic stress. Supplemental Ca (3 to 9 mM) significantly increased germination percentage compared to no Ca salt stress. Three mM Ca completely offset the deleterious effects of 150 mM NaCl and 6 mM Ca partially offset the deleterious effects of 225 mM NaCl on rice seed germination. Nine mM Ca significantly increased germination in large seed cultivar but not in the small grain cultivar compared to 6 mM Ca.

Key words: Rice, water uptake, germination, calcium and PEG

Introduction

Soil water potential is an important environmental factor controlling germination and seedling establishment (Falleri, 1994). The regulation of seed germination by environmental factors is important in both ecological and agricultural contexts. The interaction between environmental stresses and endogenous dormancy mechanisms determine whether a particular seed will germinate under given conditions (Bradford *et al*., 1992). Fully-imbibed, non dormant seeds can be expected to initiate radicle growth after a lag period related to the temperature. If the water potential of the imbibition medium is reduced, germination will be delayed or prevented depending upon the extent of reduction in water potential (Hegarty, 1978).

Excessive soil and water salinity are environmental stresses in many south and southeast Asian countries that inhibit germination, growth and yield of rice. Germination in rice production is of prime importance as it determines stand establishment and in some cases the yield of the crop (Hadas, 1976). Seed germinations under simulated iso-osmotic solutions of polyethylene glycol (PEG) and NaCl have been found to be quite different (Alam, 2001; Katembe *et al*., 1998) indicating soil osmotic potentials developed by droughtness and osmotic potentials developed by soil and water salinity would effect seed germination differently. The effect of salt stress on germination is particularly interesting because NaCl may readily cross the cell membrane into the cytoplasm of the cell. Entering ions lower the seed osmotic potential which facilitates hydration of the seed by allowing a higher seed metric potential than the osmotic potential of the solution surrounding the seed (Katembe *et al*., 1998). Under natural drought conditions or in PEG, however, hydrolysis of seed storage compounds could lower the internal osmotic potentials of the seed sufficiently for water entry, which rarely happens (Hampson and Simpson, 1990). Therefore, imbibition rate and critical and optimum moisture content for germination of rice seed under saline conditions needs to be quantified for successful rice cultivation in saline areas.

On the other hand, the precise mechanisms by which excessive salinity inhibit germination and growth of crop plants are still not fully understood but may include osmotic effects, as well as the direct toxicity caused by certain ions, nutritional imbalance or a combination of any

of these adverse factors. A notable feature of sodium toxicity is its amelioration by supplemental calcium. In salt conditions, Ca has been reported to improve germination and plumule emergence in rice (Khan *et al*., 1997) and in barley (Bliss *et al*., 1986), root elongation in maize (Zidan *et al*., 1990; Azaizah *et al*., 1992) and in callus cultured cotton (Akhtar *et al*., 1999). They suggested that NaCl has adverse effects on water transport parameters of plant cells. Extra calcium could, in part, compensate for these effects through considerable apoplasmic and cell-to-cell water flow. However, there is no documentation of increased water uptake in salt-stressed germinating rice seeds under supplemental calcium application conditions. In the present study, pattern of water uptake and initiation of germination of two rice cultivars differing in seed size were determined in iso-osmotic solutions of NaCl and PEG, different concentrations of $CaCl_2$ and combinations of NaCl and $CaCl_2$. The purpose was to quantify imbibition rate and critical and optimum moisture content for rice seed germination under different osmotica and whether there is significant variability with individual seed size. The other objective was also to quantify the pattern of water uptake in salt stressed rice seed in the presence of $CaCl_2$.

Materials and Methods

Two rice (*Oryza sativa* L.) cultivars V1 (BR5331-93-2-8-3) and V2 (BR5842-15-4-8) were used in this experiment. These cultivars had different mean seed weights, V1c 21 g and V2 c 16.5 g per thousand seeds.

Water uptake was measured in different concentrations of NaCl, $CaCl_2$, PEG 4000 and NaCl/$CaCl_2$ combinations. Three replicates of 50 seeds were imbibed in petri-dishes with germination paper in 6 ml of NaCl (0. 50, 100, 150, 200 and 250 mM), PEG (0, 8.27, 14.79, 19.62, 23.04 and 24.77% giving the same osmotic potential of NaCl solution), $CaCl_2$ (3, 6, 9 and 12 mM) or a combination of NaCl and $CaCl_2$. Seeds were removed from the petri-dishes at regular intervals, quickly surface-dried with a paper tissue and weighed and returned to the original conditions. Measurements were taken at 6, 12, 18, 24, 33, 48 and 72 h. Reduced water potentials were imposed using different concentrations of NaCl and polyethylene glycol 4000 (PEG 4000) (Table 1).

Germination was assessed using four replicates of 25 seeds in a RBD testing combinations of four levels of salinity (0, 75, 150 and 250 mM NaCl) and four levels of Ca (0, 3, 6 and 9 mM $CaCl_2$) in 90 cm diameter petri-dishes. The seeds were placed in rows without any germination paper. Because germination paper has been found to contain Ca, which might miss lead the results. Five ml of treatment solution was used in each petri-dish. Four petri-dishes were placed in a plastic tray, covered with a moistened jey cloth and kept in a tightly closed polythene bag. The trays were placed in a germination room in the dark at 21±1°C. Germination was recorded every day for 9 days. Seeds were considered germinated when both the plumule and radicle were extended to more than 2 mm.

Table 1: Water potentials of NaCl solution and PEG 4000 at 22°C in terms of mM NaCl and %PEG 4000

Osmotic potential (MPa)	NaCl (mM)	% PEG 4000 (Based on average molecular weight of 3350)
0.0	0	0.0
-0.232	50	8.267
-0.457	100	14.789
-0.677	150	19.624
-0.906	200	23.036
-1.129	250	24.770

(Adapted from Mexal *et al*., 1975 and Naylor, 1991)

The data were analyzed using analysis of variance in GENSTAT. All percentage germination data were transformed to arc sine values before statistical analysis. The statistical significance of differences between pairs of treatments was determined by student's t-test. Plumule and radicle growth were analyzed using analysis of co-variance to allow for the different germination times and therefore different opportunities for growth of the various treatments.

Results

Initial seed moisture contents (mc) of V1 and V2 were respectively 9.68 and 9.44%. Rapid increases in seed mc were observed during the first 12 h (17-23%) in all the treatments. Thereafter there was a more gradual increase in mc. The seeds reached an equilibrium mc (around 30%) in deionized water after about 48 h although germination did not begin until 60 h. Germination was initiated in most seeds in deionized water within 72 h, by which time the seed mc reached about 34% (Fig. 1a and 3b).

Increasing concentrations of NaCl significantly reduced water uptake in both the cultivars and at any specific period from 6 to 72 h the seed mc in deionized water was significantly higher than in any salt concentrations except for 50 mM at 72 h (Fig. 1a, b). After 48 h of imbibition, seed mc in 50, 100 and 150 mM salt attained about 30% and germination was initiated after about 72 h. In 200 and 250 mM salt concentrations, seed mc was less than 29% even after 72 h and none of the seeds started to germinate. Water uptake was significantly less in the small grain cultivar V2, than the large grain cultivar V1 up to 18 h in deionized water as well as in most salt solutions. However, at later times (24 h and onwards), water uptakes in both the cultivars were very similar.

Calcium concentrations of 3 to 12 mM did not significantly reduce water uptake in the large grain cultivar V1 (Fig. 2a, b). However, it did reduce water

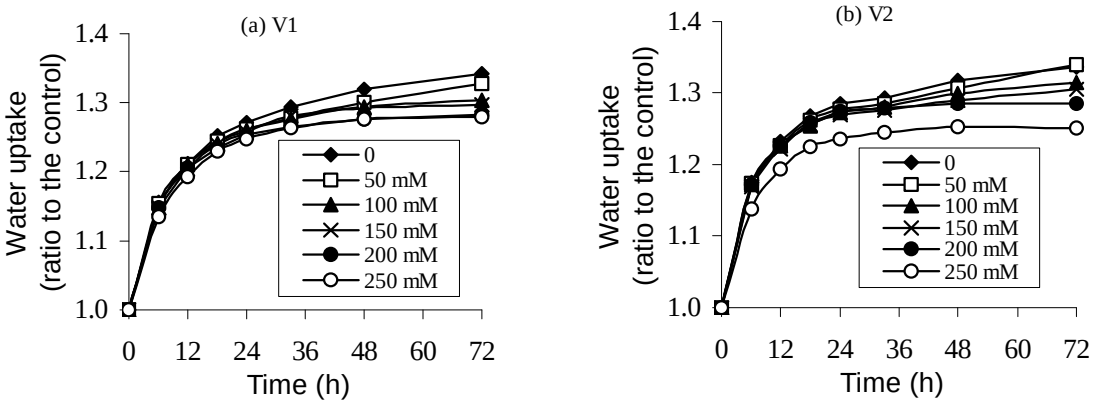

Fig. 1a, b: Effect of NaCl on water uptake of V1 (small grain) and V2 (large grain)

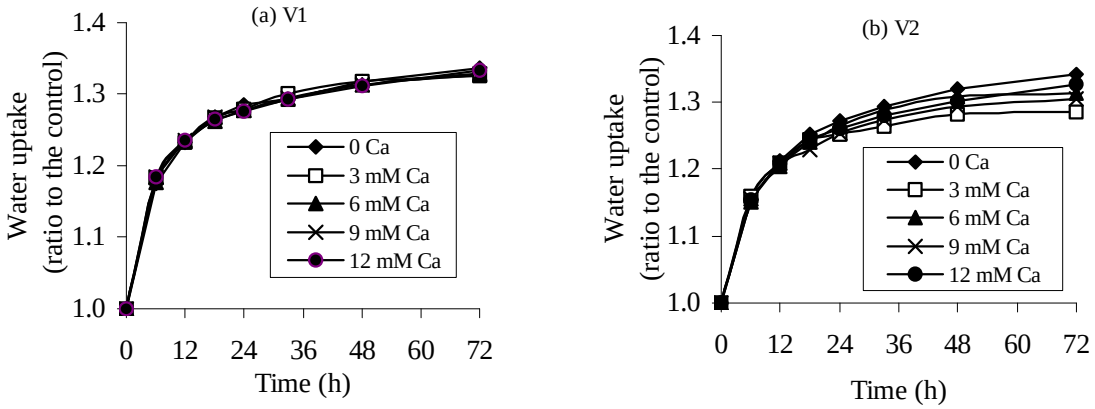

Fig. 2a, b: Effect of $CaCl_2$ on water uptake of V1 (small grain) and V2 (large grain)

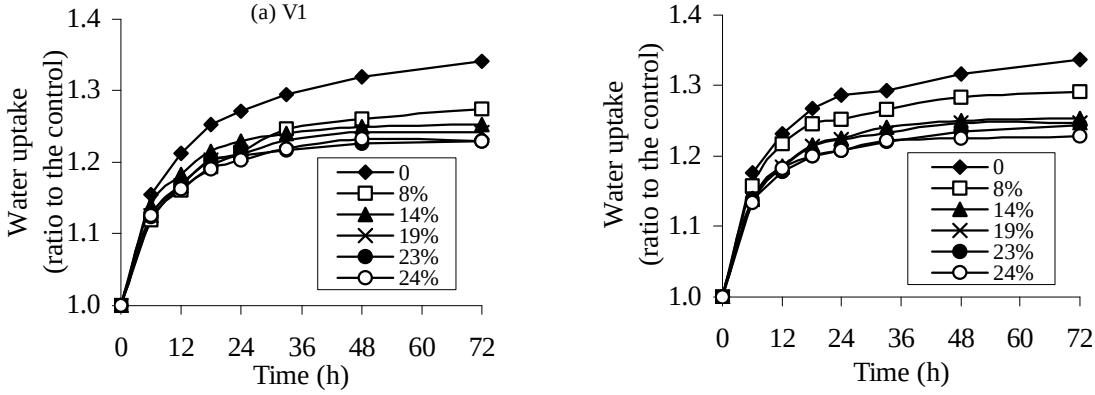

Fig. 3a, b: Effect of PEG on water uptake of V1 (small grain) and V2 (large grain)

uptake in small grain cultivar V2 and the effects were pronounced after 24 h of imbibition. Both cultivars attained about 30% mc within 48 h of imbibition in most Ca concentrations and germination was initiated in most seeds within 60 h as was the case in deionized water.

Like NaCl, increasing concentrations of iso-osmotic PEG solutions significantly reduced water uptake in both the cultivars and mc at any PEG concentrations did not attain 30% even after 72 h of imbibition (Fig. 3a and b). In 8.27% PEG solution (which is equivalent to 50 mM NaCl), mc in V1 and V2 were respectively about 29 and 27% after 72 h, when germination was initiated in both the cultivars. In 14.79% PEG solution (which is equivalent to 100 mM NaCl), the mc of both the cultivars were similar about 25% after 72 h and none of the seeds had started to germinate. The time of onset of germination up to -0.232 MPa (= 50 mM NaCl or 8% PEG) was similar to that of control (4 days) both in NaCl and PEG in both the cultivars and

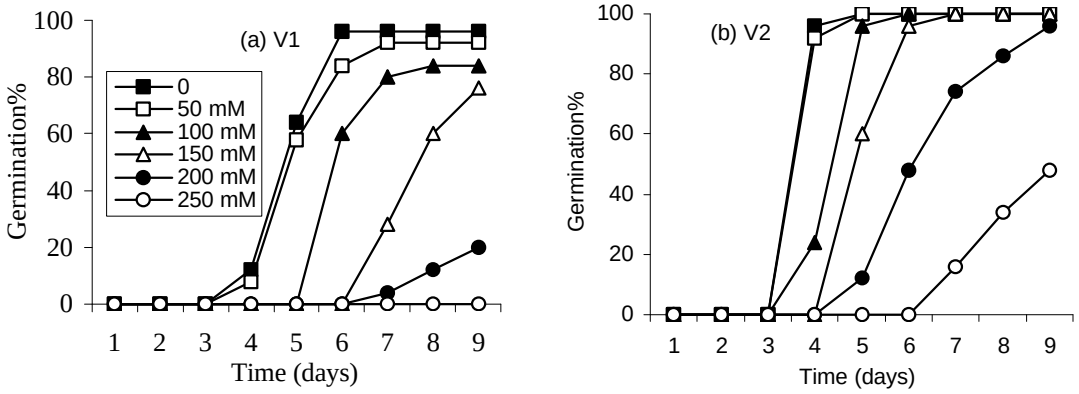

Fig. 4a, b: Pattern of germination of V1 (large grain) and V2 (small grain) in different levels of salinity

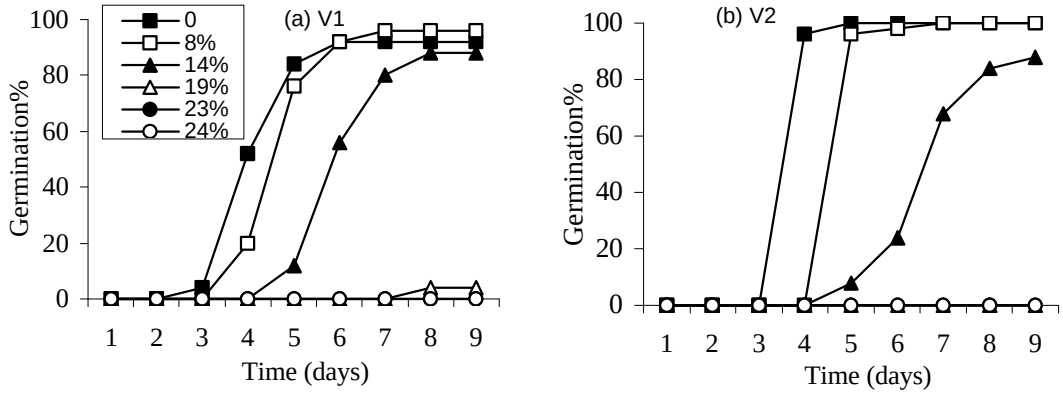

Fig. 5a, b: Pattern of germination of V1 (large grain) and V2 (small grain) in isosmotic solutions of PEG

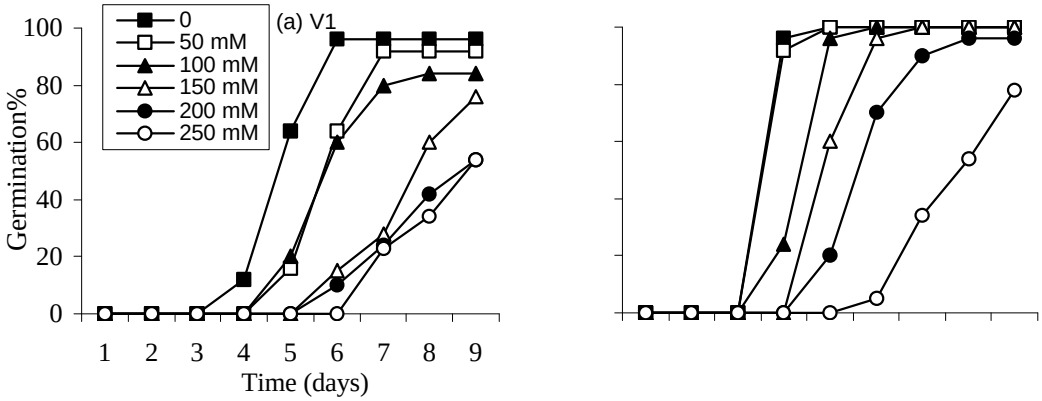

Fig. 6a, b: Pattern of germination of V1 (large grain) and V2 (small grain) under different levels of salinity with 6 mM added Ca

maximum germinations were attained with the next two days. However, when osmotic potentials were lowered beyond –0.232 MPa pronounced differences were observed between cultivars in response to osmotic regarding onset of germination and as well as in final germination. At osmotic potential –0.457 MPa (= 100 mM NaCl or 14% PEG) V1 took 6 days to initiate germination in NaCl and as high as about 80% germination was attained by 8 days (Fig. 4a). While at this osmotic potential V2 took only 4 days to initiate germination in NaCl and the very next day it attained as high as cent percent germination indicating better tolerance of V2 to salinity (Fig. 4b). In contrast, in the comparable osmotic solution of PEG, V1 took 5 days to initiate germination and by 8 day maximum germination of about 90% was attained. While V2 took 5 days to initiate germination maximum

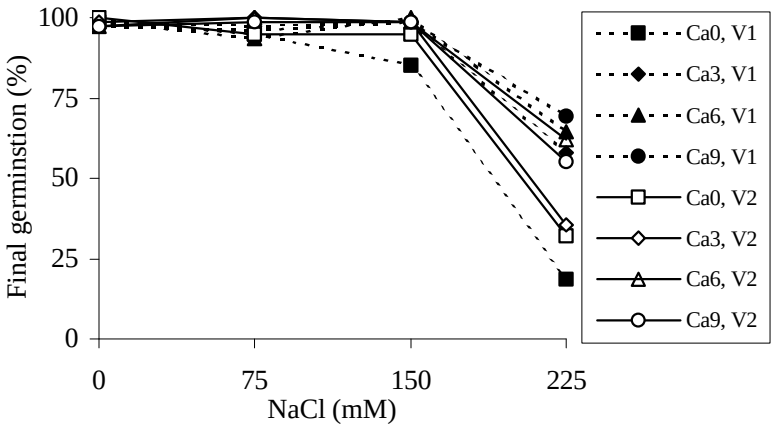

Fig. 7: Effect of $CaCl_2$ and NaCl on germination of V1 (large grain) and V2 (small grain)

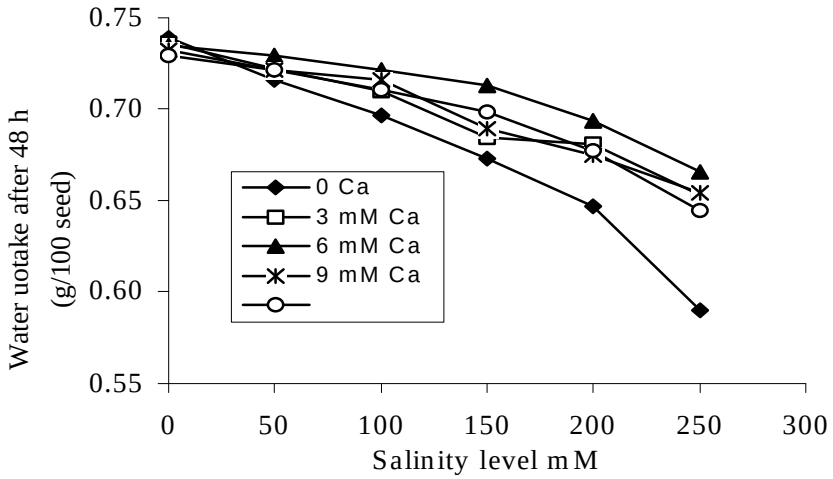

Fig. 8: Interaction effect of $CaCl_2$ and NaCl on water uptake of V1 (large grain) and V2 (small grain)

germination of about 80% was attained by 9th day indicating poor tolerance of V2 to water potential than V1. Further lowering of osmotic potentials completely inhibited germination in PEG in all cultivars. However, germination in NaCl continued with progressive delay till the last day of the count (9th day). At –0.906 MPa (= 200 mM NaCl) V1 took 7 days to initiate germination and by 9th day maximum germination was as high as about 20%. In V2 however, germination was initiated at 5th day more than 90% germination was attained by 9th day. At –1.129 MPa (=250 mM NaCl) V1 completely failed to germinate while V2 took 6 days to initiate germination and as high as about 50% germination was attained by 9th day confirming better tolerance of V2 to salt than V1.

Addition of 6 mM Ca with different levels of NaCl had pronounced effect on onset of germination as well as on final germination on V1 specially at higher levels of salinity (>150 mM). While effect of 6 mM Ca on V2 was not pronounced. Initial pattern of germinations in V1 were similar up to 100 mM salinity regardless of addition of 6 mM Ca. At 150 and 200 mM salinity, V1 took 6 and 7 days, respectively to initiate germination and maximum germinations were about 70 and 20%, respectively. At 250 mM salinity it completely failed to germinate (Fig. 4a). While in contrast, when 6 mM Ca was added, V1 took equally 6 days to initiate germination at 150 and 200 mM salinity and 7 days at 250 mM salinity and maximum germinations were attained about 50% both at 200 and 250 mM salinity (Fig. 6a). Effect of additional 6 mM Ca on V2 was not pronounced (Fig. 6b).

Increasing concentrations of NaCl significantly reduced final germination and increasing concentrations of supplemental Ca significantly reduced the extent of this effect in both the cultivars (Fig. 7). The greatest effect was on V1. A salt concentration of 75 mM and Ca concentrations of up to 9 mM alone had no deleterious effects on final germination. In V1, germination was significantly reduced by about 15% at 150 mM salinity

when no Ca was added. However, no reductions in germination were observed at this level of salinity when Ca was applied. At 225 mM salinity, germination was reduced by about 80% in V1 when no Ca was applied. However, the reductions in germination were about 40, 35 and 30% when 3, 6 and 9 mM Ca was applied respectively. In V2, germination was not reduced at 150 mM salinity, however, at 225 mM salinity germination was reduced by about 70% when no Ca was applied. When 3, 6 and 9 mM Ca were applied the reductions were about 65, 40 and 45%, respectively.

The interaction between NaCl and $CaCl_2$ on water uptake by V1 after 48 h of imbibition is shown in Fig. 8. Increasing concentrations of NaCl gradually reduced water uptake both with and without Ca. However, at any level of salinity, when Ca was added in combination with NaCl, water uptakes were significantly higher than without Ca. Significantly higher water uptake was observed with 6 mM Ca but water uptake with 3, 9 and 12 mM Ca were very similar.

Discussion

The seeds reached an equilibrium mc (around 30%) in deionized water after about 48 h and germination was initiated in most seeds within 72 h by which time the seed mc reached about 34%. This suggests that equilibrium and attainment of the optimum mc for germination of rice seeds occur at around 30%. The further gain (34% mc) in seed fresh weight might have resulted from protrusion of the radicle and plumule. In 8.27% PEG solution, the mc of V2 and V6 were about 29 and 27%, respectively after 72 h when germination was initiated in both the cultivars. In 14.79% PEG solution, mc of both the cultivars were about 25% after 72 h but none of the seeds germinated. This was suggested that the critical moisture content for germination of rice seeds was around 25-30% and it may differ between cultivars with different seed sizes. Water content in any of the iso-osmotic solutions of PEG did not reach 30% in any cultivars and in the highest concentration of PEG (24.77%) was only about 23% after 72 h. In iso-osmotic solutions of up to 150 mM NaCl, water uptake reached about 30% within 48 h and in 250 mM NaCl (equivalent of 24.77% PEG) was about 26%. Thus water uptakes in NaCl solutions were clearly greater than in iso-osmotic solutions of PEG. These findings agreed with Azaizeh *et al*. (1992) and Akhtar *et al*. (1999). Water uptake in smaller seed was less during the first 12 h of imbibition than larger seeds. However, with time the differences were reduced. The results were consistented with Gurmu and Naylor (1991).

When additional Ca was not added, germination of V1 was reduced by about 15 and 80% at 150 and 225 mM salinity, respectively. In V2, no reduction in germination was observed at 150 mM salinity, but at 225 mM salinity about 70% reduction in germination was recorded when no Ca was added. This indicated the differences in salt tolerance between these two cultivars during germination. Addition of Ca completely offset the deleterious effects of 150 mM NaCl on germination in V1. The results here confirm that Ca partially ameliorated the deleterious effects of NaCl on germination of rice seed. These ameliorative effects of Ca on germination and early seedling growth have also been reported by Bliss *et al.* (1986) and Nakamura *et al.* (1990). Germination of V1 was more responsive to supplemental Ca than V2. This might be genetic in origin or due to the better seed quality of V1. In addition, it might simply reflect the greater responsiveness of V1 to Ca giving a bigger opportunity for amelioration.

At higher salt concentrations (>150 mM), onset of germination and also final germination were higher with supplemental Ca than salt solution alone in both cultivars indicating better hydration in Ca added treatments than salt solution alone. These results were in agreed with Bliss *et al.* (1986), Cramer *et al.* (1986), Nakamura *et al.* (1990) and Zidan *et al.* (1990) who also found that Ca^{2+} improved germination in salt affected seed. Better hydration might be due to the increased hydraulic conductivity of the cell membranes in presence of Ca^{2+}. Azaizeh *et al.* (1992) demonstrated that NaCl has adverse effects on water transport in root cells and supplemental Ca^{2+} could in part compensate for these effects. They concluded that NaCl and $CaCl_2$ affect root hydraulic conductivity and cell hydraulic conductivity differently. NaCl caused more pronounced reductions in cell hydraulic conductivity values compared with root hydraulic conductivity and $CaCl_2$ had ameliorative effects when salt stress was imposed. Water content of the callus cultures decreased significantly in NaCl and increased on a $CaCl_2$ supplemented medium (Akthar *et al*., 1999). Better hydration in presence of Ca has also been shown in Fig. 8.

The results illustrate the beneficial effects of supplemental Ca on germination of rice under NaCl stress. Although Ca was proved to be inefficient at higher salt concentrations (>200 mM) up to 150 mM salinity germinations were as better as in the absence of salt stress and at any salt level, germinations were significantly higher with added Ca than salt solution alone.

Following germination, the endosperm still supplies nutrients to the growing seedlings, but for cytoplasmically immobile ions, such as Ca^{2+} the rapidly growing radicle must be supplied with an adequate

amount from the environment to prevent leakage of solutes and maintain other processes in which Ca^{2+} is essential (Lauchli and Epstein, 1970). Kurth *et al.* (1986) concluded that the enhancement of root elongation in salt stressed cotton seedlings provided with supplemental Ca^{2+} was the result of two distinct processes; cell elongation (favoured at the expense of radial cell expansion) and maintenance of high rates of cell production. These processes were probably mediated by interactions of Na^{+} and Ca^{2+} with the cell wall, plasmalemma and cytoskeleton. Sodium ions inhibit elongation of root cells. Comparison of the number of cells per file per unit length in the cortex with root length suggested that supplemental Ca^{2+} enhanced the rate of cell production by approximately 20 to 30% at salinities ranging from 25 to at least 100 mM NaCl, while salinity diminished the rate of cell production considerably at low Ca^{2+} levels (Kurth *et al.*, 1986). Salinization of nutrient media sharply reduced Ca^{2+} activity in the medium (Cramer *et al.*, 1986) as well as Ca^{2+} transport and content in cotton plants, specially at low Ca^{2+} concentrations.

With reducing osmotic potentials, both NaCl and PEG delayed the onset of germination and reduced final percent germination. Such responses have been reported by many workers over a wide range of halophytic and glycophytic plant species (Hampson and Simpson, 1990; Falleri, 1994; De and Kar, 1995; Rogers *et al.*, 1995; Huang and Redmann, 1995; Katembe *et al.*, 1998). The general view is that a decrease in water potential gradient between seeds and their surrounding media adversely affects seed germination and subsequent growth processes. The physical process of water uptake leads to the activation of metabolic processes as the dormancy of the seed is broken following hydration (Katembe *et al.*, 1998). Elevated NaCl and PEG concentrations slowed down water uptake by seeds, thereby inhibiting germination process (Werner and Finkelstein, 1995). Reduced fresh weight in elevated iso-osmotic solutions of both NaCl and PEG (data are not presented) also indicated reduced water uptake by the germinating seeds.

The results thus showed that for all the treatments, PEG was found to be more inhibitory to germination compared to iso-osmotic NaCl solutions. These results agree with those of Hampson and Simpson (1990) for wheat, as well as De and Kar (1995) for mungbean, Huang and Redmann (1995) for barley and *Brassica*, Gulzar and Khan (2001) for *Aeluropus lagopoides*. However, such findings are not universal. Roundy *et al.* (1985) and Katembe *et al.* (1998) observed the opposite in wild rye, wheatgrass and *Atriplex* species probably due to the tolerance of these plant species (halophytic) to high osmotic stress. The explanation of the higher inhibitory effect of PEG than NaCl lies in ion or solute entry into the seed. The first phase of water uptake by seeds involves movement of water into the free spaces (apoplast) and does not depend on the osmotic potentials of the surrounding solutions (Bewley and Black, 1994). The second slower phase of water uptake involves the movement of water across cell membranes into the cells of the seeds and is determined by the difference between osmotic potentials of the seed and that of the medium (Bewley and Black, 1994). Unlike PEG, NaCl may readily cross the cell membrane into the cytoplasm of the cell unless an active metabolic pump prevents accumulation of the ions (Katembe *et al.*, 1998). Entering ions lower the seed osmotic potential, which facilitates hydration of the seed by allowing a higher seed matric potential than the osmotic potential of the solution surrounding the seed (Roundy *et al.*, 1985). In PEG, hydrolysis of storage compounds could lower the internal osmotic potentials of the seed sufficiently for water entry (Hampson and Simpson, 1990).

The results reported herein thus show that equilibrium mc of rice seeds occurs at around 30% mc and the critical mc for germination is around 25-30%. Although rates of water uptake in rice seeds under salinity stress reduced with increasing salinity, rice seeds attained equilibrium mc by 48 h up to 150 mM salinity and reached at least critical mc by 72 h up to 250 mM salinity. Water uptakes in NaCl solutions were greater than in iso-osmotic solutions of PEG and rice seeds did not attain even critical mc under PEG solutions beyond 8.27%
(= -0.232 MPa) even after 72 h. Water uptake was faster in large seeds than small seeds under calcium concentrations of up to 12 mM. However, equilibrium mc was attained within 48 h in both large and small seed. Water uptake in rice seeds increased when Ca was added in combination with NaCl.

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