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Tolerance of Grasses to Calcium Chloride, Magnesium Chloride and Sodium Chloride

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Abstract : The tolerance of six cool-season grasses and six warm-season grasses to three kinds of salt was examined in solution culture. Among the cool-season grasses, tall fescue (*Festuca arundinacea* Schreb.) was the most tolerant to all three salts. Among the warm-season grasses, bermudagrass (*Cynodon dactylon* (L.) Pers.) was the most tolerant to excess calcium chloride and sodium chloride, while bahiagrass (*Paspalum notatum* Flugge) was the most tolerant to excess magnesium chloride. A positive and significant correlation was found between estimates of the concentration at which plant growth decreases by 50% (C_{50}) in the presence of excess $CaCl_2$ and those in the presence of excess NaCl. The C_{50} estimates in excess $MgCl_2$, however, were not correlated with those in the other two salts. The results suggest that common physiological mechanism confers tolerance to both excess $CaCl_2$ and excess NaCl, but a different mechanism to excess $MgCl_2$.

Key words : Calcium, Co-tolerance, Grass, Magnesium, Salinity, Sodium.

Salinity is one of the major environmental and agricultural problems. As grasses are major components of the floras of saline areas (Gorham, 1992), many reports exist of the salinity tolerance of grasses (Greub et al., 1985; Marcum and Murdoch, 1990; Rogers et al., 1996). However, most of them have been concerned with sodium. By contrast, there are only a few reports about the tolerance to other cations, such as calcium and magnesium (Ashraf et al., 1989; Dvořák and Ross, 1986). For instance, Oizumi et al. (1979) found calcium to be the main cation in soil with salt accumulation in a plastic greenhouse, and attempted the cultivation of grasses as a remedy for excessive salt accumulation. Magnesium is the second major cation in seawater (ca. 50 mM) and has been detected at potentially inhibitory concentrations for grasses at a salt marsh and seashore (Hodson et al., 1981; Wu, 1981).

Do NaCl-tolerant plants also have tolerance to other salts? Some reports have found coincidental tolerance (co-tolerance) to certain ions including Na^+. Ashraf et al. (1989) reported that NaCl-tolerant lines had greater root growth in $CaCl_2$ solutions than did unselected lines in each of the four grass species. In *Medicago sativa* L., the callus derived from NaCl-selected suspension cultures was more tolerant to the chlorides of alkali metals than the non-selected callus (Shah et al., 1993). If this phenomenon, in which the selection of tolerance to Na^+ confers tolerances to other cations, is also general among different plant species, there probably are some relationships between the tolerance to Na^+ and tolerance to other cations in various grasses.

In this study, we examined the tolerance to excess $CaCl_2$ and $MgCl_2$ in 12 common commercial grasses in order to identify these species most suitable for cultivation in soils with high levels of these salts. We also examined the tolerance of grasses to NaCl and compared the tolerance to each of the three salts.

Materials and Methods

The experiment was conducted in a growth chamber. Air temperature in the growth chamber was maintained at 23/15 °C or 28/20°C (12-h

Table 1. Plant materials.

Common name	Classification	Cultivar
Cool-season grass		
Italian ryegrass	*Lolium multiflorum* Lam.	Nioudachi
Kentucky bluegrass	*Poa pratensis* L.	Award
Orchardgrass	*Dactylis glomerata* L.	Okamidori
Perennial ryegrass	*Lolium perenne* L.	Friend
Redtop	*Agrostis alba* L.	commercial [S)]
Tall fescue	*Festuca arundinacea* Schreb.	Southern cross
Warm-season grass		
Bahiagrass	*Paspalum notatum* Flugge	commercial [S)]
Barnyard millet	*Echinochloa utilis* Ohwi et Yabuno	Shirohie
Bermudagrass	*Cynodon dactylon* (L.) Pers.	Giant bermuda cough
Finger millet	*Eleusine coracana* (L.) Gaertn.	commercial [T)]
Rhodesgrass	*Chloris gayana* Kunth	Asatsuyu
Weeping lovegrass	*Eragrostis curvula* Nees	commercial [S)]

[S)] Seeds were obtained from Snow Brand Seed Co., Ltd.
[T)] Seeds were obtained from Takii Seed Co., Ltd.

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day/12-h night cycle) for cool- or warm-season grasses, respectively. Light was provided from high-pressure sodium vapor and metal halide lamps at 480 μmoles m^{-2} s^{-1} at plant height.

Seeds of each grass species (Table 1) were germinated on 0.4% agar plates, one plate per species. When plant height reached 2 to 3 cm, six seedlings of each cool-season grass species or each warm-season grass species were transplanted into a polystyrene tray (30 cm × 45 cm) in a plastic tank (36 plants/tank). The tank contained 15 L of a one-third-strength Hoagland's no. 2 solution (Hoagland and Arnon, 1950) supplemented with 30 μM Fe-EDTA, 0.1 mM NaCl and 25 μM Na_2SiO_3. Therefore, the culture solution contained 1.3 mM Ca^{2+}, 0.67 mM Mg^{2+} and 0.15 mM Na^+. The solution pH was monitored daily and maintained at 5.2 by adding either 1 M HCl or 1 M NaOH. The solution was completely aerated and changed at 10 days after transplanting.

At 7 days after transplanting, three plants of each species in each tank were harvested and excess salinity treatments commenced. The excess salinity treatments included six concentrations with two replications in each salt ($CaCl_2$: 0, 15, 30, 45, 60, 75 mM (1.0, 3.9, 6.5, 8.9, 11.1, 13.1 dS m^{-1}), $MgCl_2$: 0, 15, 30, 45, 60, 75 mM (1.0, 3.7, 6.1, 8.2, 10.2, 12.1 dS m^{-1}), NaCl: 0, 60, 90, 120, 150, 210 mM (1.0, 6.6, 9.1, 11.4, 13.6, 17.7 dS m^{-1})). After the 10-day salinity treatment, the remaining plants were harvested. The harvested plants were dried at 60 °C for 72 hours and weighed.

Growth during the treatment was calculated as the difference between the dry weight of the whole plant at 7 days and 17 days after transplanting, for each grass in each tank. In this study, the relative growth (the growth under saline/the growth under non-saline) was used to compare the salinity tolerance of grasses, because the grass species used in this study are diverse in dry weight (Table 2).

To obtain parameters characterizing salinity tolerance, a sigmoidal growth response model proposed by van Genuchten and Hoffman (1984) was used. This model is of the form:

$$Y = Ym / [1 + (C/C_{50})^p],$$

where Y is plant yield, Ym is the yield under nonsaline condition, C_{50} is the salinity concentration at which plant yield decreases by 50%, and p is an empirical constant. In this study, Y is replaced by the relative growth. The model parameters were estimated by nonlinear least squares techniques with JMP statistical computer software (version 4.0.5J, SAS Institute Inc.). The reliability of each salinity-growth response function was assessed by the criteria of Royo and Aragues (1993, 1999): (a) significance of the correlation coefficients between the observed and estimated Y values, (b) C_{50} estimates significantly different from zero, and (c) standard errors of the C_{50} estimates lower than 25% of the C_{50} estimates.

Results

In this experiment, we used a sigmoidal model

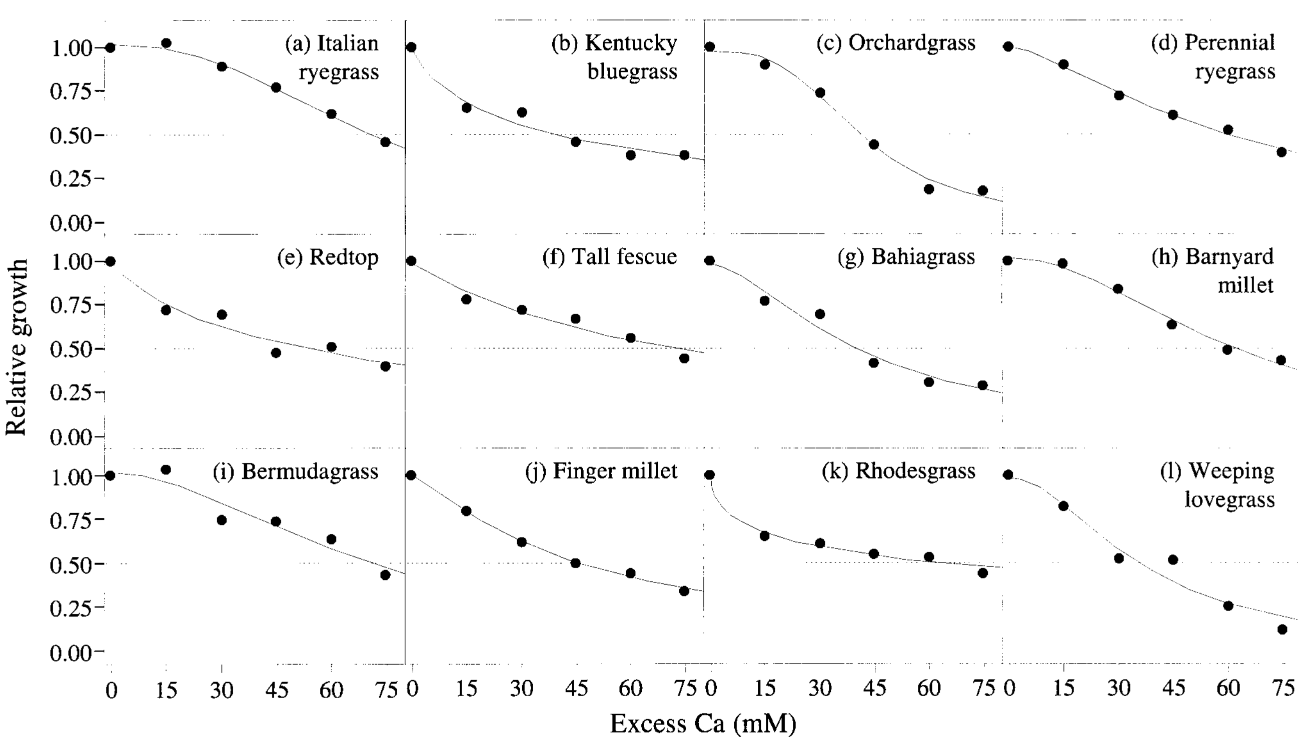

Fig. 1. Response functions between excess $CaCl_2$ concentration (mM) and relative growth in cool-season grass ((a)-(f)) and warm-season grass ((g)-(l)).

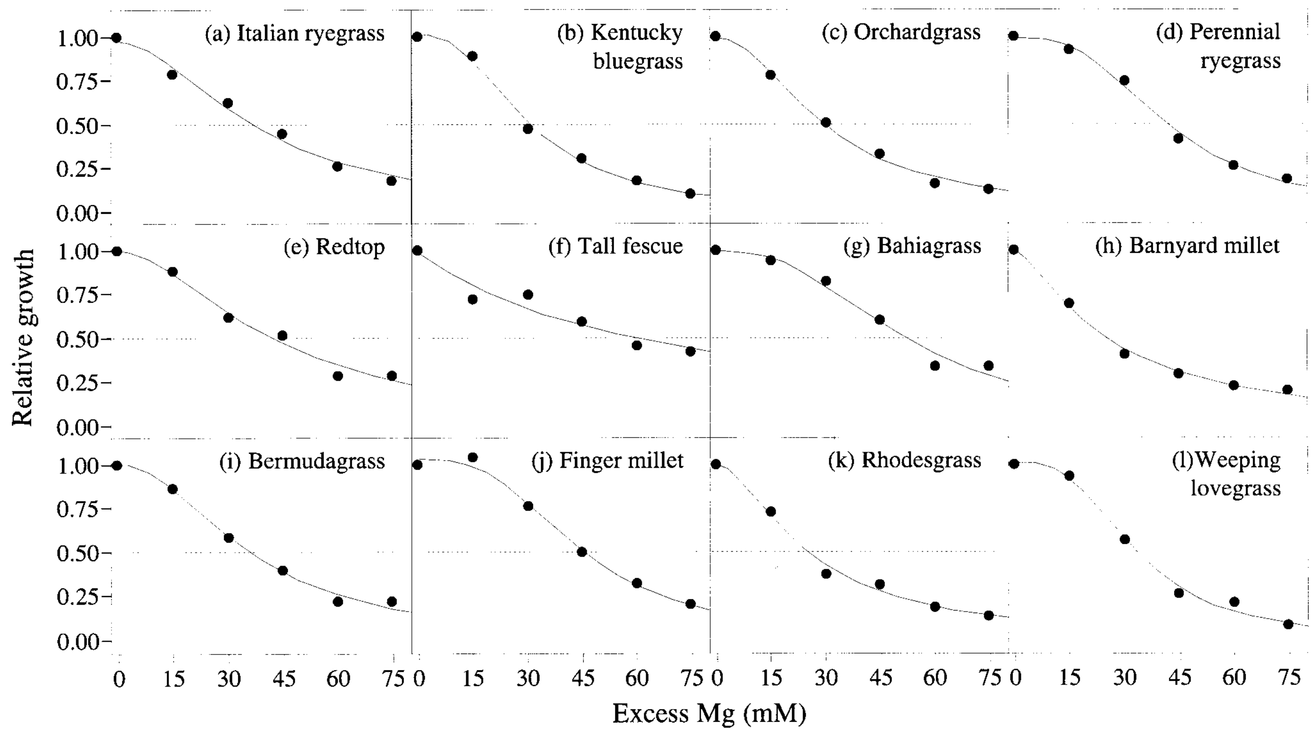

Fig. 2. Response functions between excess $MgCl_2$ concentration (mM) and relative growth in cool-season grass ((a)-(f)) and warm-season grass ((g)-(l)).

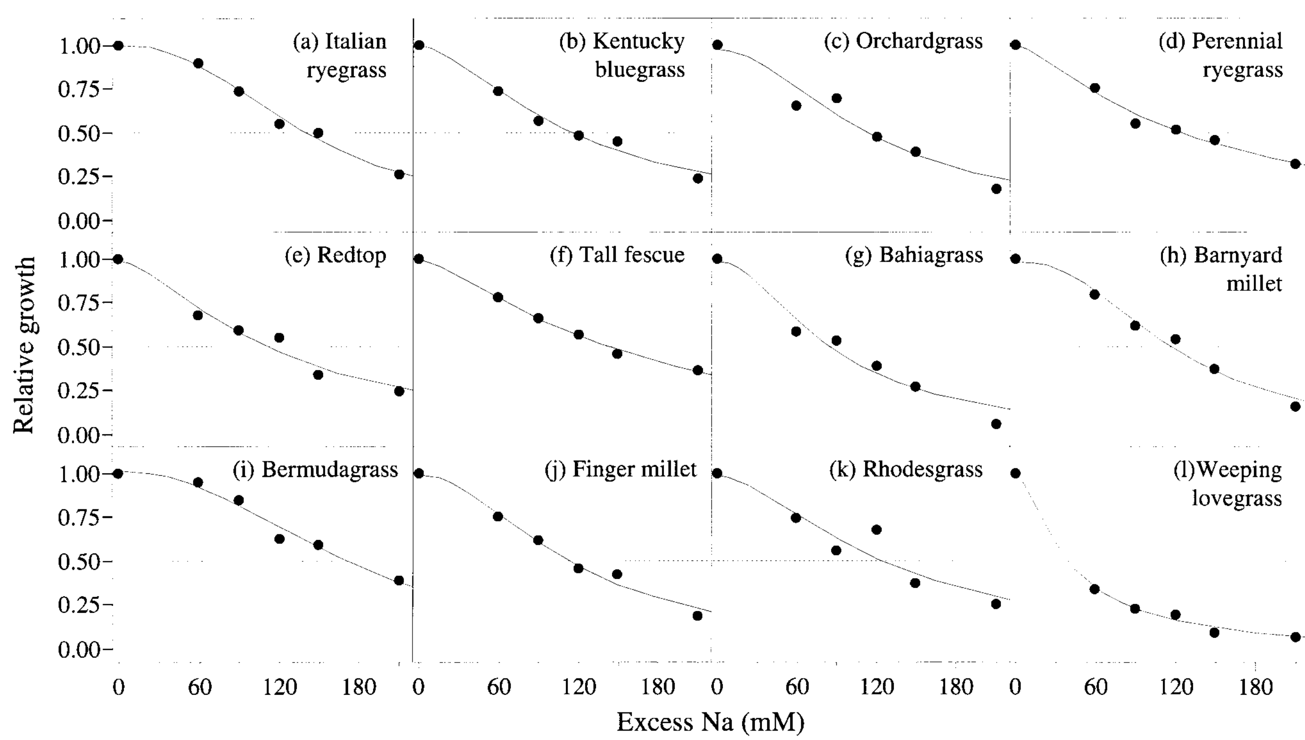

Fig. 3. Response functions between excess NaCl concentration (mM) and relative growth in cool-season grass ((a)-(f)) and warm-season grass ((g)-(l)).

developed by van Genuchten and Hoffman (1984) to describe the salinity-growth response. All grasses in all salinity treatments had high coefficients of determination ($R^2 > 0.89$, Table 2) and significant r values at $P < 0.01$. All estimated C_{50} values were significantly different from zero ($P < 0.05$), which was judged with the statistical software. All estimated standard errors of C_{50} were below 25% of the C_{50}

Table 2. Plant dry weights in control, and calculated values of C_{50} (estimated standard error) and R^2 in excess $CaCl_2$, $MgCl_2$ and NaCl.

Grass	Dry weight in control*	$CaCl_2$		$MgCl_2$		NaCl	
	(mg $plant^{-1}$)	C_{50} (mM)	R^2	C_{50} (mM)	R^2	C_{50} (mM)	R^2
Cool-season grass							
Italian ryegrass	298	70.4 (2.1)	0.994	37.9 (3.2)	0.987	139.0 (6.0)	0.991
Kentucky bluegrass	28	39.7 (7.0)	0.972	30.5 (1.6)	0.995	115.4 (9.2)	0.984
Orchardgrass	102	41.9 (2.4)	0.989	30.0 (1.5)	0.996	115.6 (17.3)	0.941
Perennial ryegrass	171	60.0 (2.8)	0.994	42.1 (1.6)	0.996	122.7 (10.2)	0.984
Redtop	31	53.3 (11.1)	0.948	42.0 (3.6)	0.984	111.9 (13.2)	0.968
Tall fescue	94	74.4 (13.1)	0.954	61.0 (13.0)	0.933	141.0 (4.5)	0.997
Warm-season grass							
Bahiagrass	134	40.7 (5.1)	0.970	52.0 (3.4)	0.981	85.9 (12.5)	0.960
Barnyard millet	2039	60.8 (2.9)	0.989	25.0 (1.7)	0.995	118.1 (7.5)	0.986
Bermudagrass	294	69.4 (9.7)	0.921	35.9 (1.9)	0.994	168.3 (11.3)	0.975
Finger millet	1082	45.9 (1.8)	0.997	44.4 (2.1)	0.992	114.3 (8.1)	0.985
Rhodesgrass	449	64.1 (12.7)	0.983	25.1 (2.2)	0.991	126.1 (25.1)	0.897
Weeping lovegrass	85	35.9 (5.9)	0.955	33.0 (1.7)	0.994	39.9 (4.7)	0.997

*Each value shows the mean of the dry weights under non-saline condition in three excess salt treatments.

(Table 2). These results indicated that the growth-salinity response values obtained in this experiment fit the sigmoidal response model well.

Fig. 1 and Table 2 show the effects of excess $CaCl_2$ on the relative growth of each grass. In the cool-season grasses, tall fescue had the best relative growth under excess $CaCl_2$, followed by Italian ryegrass, perennial ryegrass, redtop, orchardgrass and Kentucky bluegrass. With C_{50} estimates as the reference parameter, tall fescue (74.4 mM) was about 1.9 times as tolerant as Kentucky bluegrass (39.7 mM). In warm-season grasses, bermudagrass had the best growth under excess $CaCl_2$, followed by rhodesgrass, barnyard millet, finger millet, bahiagrass and weeping lovegrass. Taking C_{50} estimates as the reference parameter, bermudagrass (69.4 mM) was about 1.9 times as tolerant as weeping lovegrass (35.9 mM).

Fig. 2 and Table 2 show the effects of excess $MgCl_2$ on grass growth. In cool-season grasses, tall fescue had the best growth, followed by perennial ryegrass, redtop, Italian ryegrass, Kentucky bluegrass and orchardgrass. With C_{50} estimates as the reference parameter, tall fescue (61.0 mM) was about twice as tolerant as orchardgrass (30.0 mM). In warm-season grasses, bahiagrass had the best growth under excess $MgCl_2$, followed by finger millet, bermudagrass, weeping lovegrass, rhodesgrass and barnyard millet. Taking C_{50} estimates as the reference parameter, bahiagrass (52.0 mM) was about 2.1 times as tolerant as barnyard millet (25.0 mM).

Fig. 3 and Table 2 show the effects of NaCl on grass growth. In the cool-season grasses, tall fescue had the best growth, followed by Italian ryegrass, perennial ryegrass, orchardgrass, Kentucky bluegrass and redtop. With C_{50} estimates as the reference parameter, tall fescue (141.0 mM) was about 1.3 times as tolerant as redtop (111.9 mM). In the warm-season grasses, bermudagrass had the best growth, followed by rhodesgrass, barnyard millet, finger millet, bahiagrass and weeping lovegrass. With C_{50} estimates as the reference parameter, bermudagrass (168.3 mM) was about 4.2 times as tolerant as weeping lovegrass (39.9 mM).

In order to investigate the relationships among the tolerance to the three salts, we compared the C_{50} estimates in each salt to salt (Fig. 4). The C_{50} estimates in $CaCl_2$ and in NaCl were positively and significantly correlated at a 1% probability level ($r = 0.783$, $P = 0.0026$). By contrast, the C_{50} estimates in excess $MgCl_2$ were not correlated with that in $CaCl_2$ or with that in NaCl ($P > 0.05$).

Discussion

This experiment revealed distinct differences in the tolerance of different grasses to the three salts, indicating that some grasses are more suitable than others for cultivation in each salt affected soil. Among the cool-season grasses examined, tall fescue was the most tolerant to all three salts, which suggests that tall fescue is the best cool-season grass for various kinds of salt. Among the warm-season grasses used, bermudagrass was the most tolerant to excess $CaCl_2$

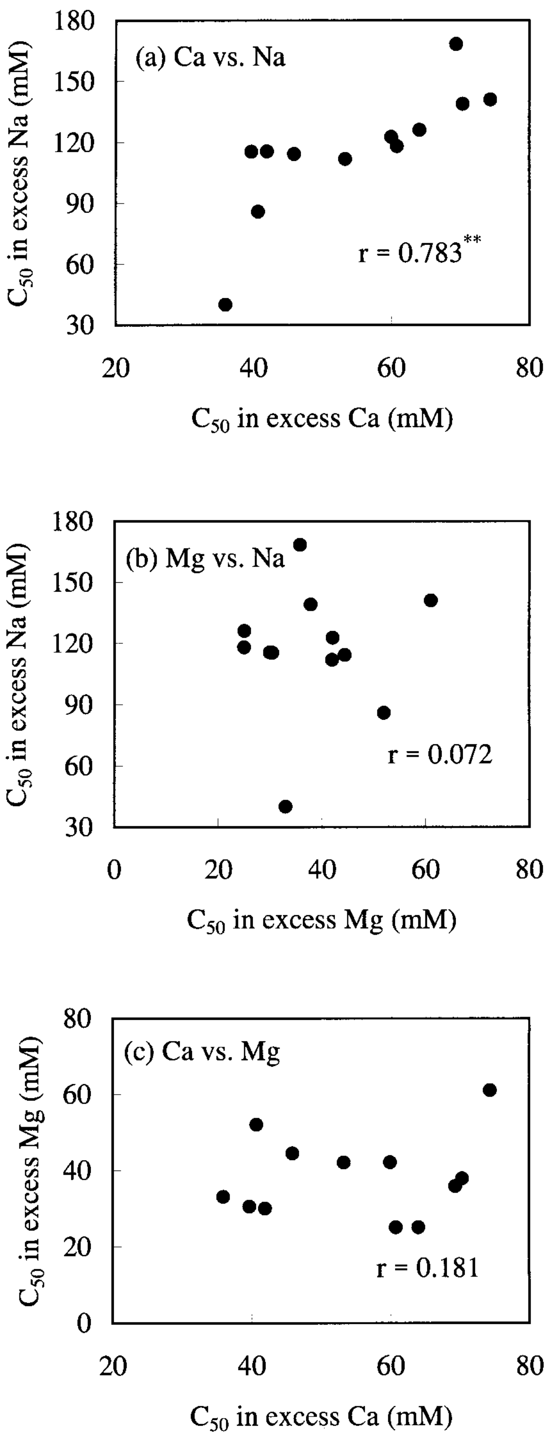

Fig. 4. Relationships among C_{50} estimates in excess $CaCl_2$, $MgCl_2$ and NaCl.
**Significance established at the 0.01 probability level.

and NaCl, but its tolerance to $MgCl_2$ was weak. By contrast, bahiagrass was the most tolerant to excess $MgCl_2$ but showed weak tolerance to $CaCl_2$ and NaCl.

In order to understand the relationships among the tolerance to the three salts, we compared the C_{50} estimates of grasses in the three salts (Fig. 4). The C_{50} estimates in $CaCl_2$ and in NaCl were positively and significantly correlated ($P < 0.01$), indicating that the tolerance to excess $CaCl_2$ may be related to the tolerance to excess NaCl.

One such relationship between the tolerance to different ions is the coincidental tolerance (Hodson et al., 1981; Shah et al., 1993). Cox and Hutchinson (1979) reported a population of *Deschampsia cespitosa* growing on soils contaminated with copper, nickel or aluminum had tolerance to lead and zinc, which were not elevated in the soils. That study suggested that some physiological mechanisms for metal tolerance are commonly held; in other words, the presence of mechanisms conferring tolerance to one metal might also confer tolerance to other metals. Likewise, grasses may share common physiological mechanisms conferring tolerance to both $CaCl_2$ and NaCl, which might explain the correlation between the tolerances to $CaCl_2$ and NaCl in this report. This idea is supported by reports that the NaCl-tolerant lines of four grass species or citrus cells had greater tolerance to excess $CaCl_2$ than did unselected lines (Ashraf et al., 1989; Ben-Hayyim et al., 1987). In the study of Citrus cells, internal K^+ concentration was suggested to play a key role in determining growth capacity in both increasing NaCl and $CaCl_2$ concentrations (Ben-Hayyim et al., 1987).

Tolerance to excess $MgCl_2$ was not correlated with tolerance to $CaCl_2$ or NaCl (Fig. 4), indicating that most of the mechanisms of tolerance to excess $MgCl_2$ are different from those to excess $CaCl_2$ or NaCl. This was supported by the report that the NaCl-tolerant line had greater tolerance to excess $MgCl_2$ than did the unselected line in only one of the four grass species (Ashraf et al., 1989). In addition, low Ca^{2+} concentration was suggested to cause subclinical growth depression in $MgCl_2$-treated Eucalypts seedlings (Marcar and Termaat, 1990).

The present study demonstrates that there are distinct differences in the tolerance to $CaCl_2$, $MgCl_2$ and NaCl with the grass species, and that the tolerance to $CaCl_2$ and NaCl are related, whereas the tolerance to $MgCl_2$ was different from the others. These results indicate that sodium-tolerant grasses can be introduced to calcium-affected soil but not to magnesium-affected soil. As Ammal et al. (1999) and Wu (1981) reported, magnesium is the second major cation in seawater (ca. 50 mM) and seawater increases both sodium and magnesium content in soil and ground water. Through this study, it can be concluded that the investigation of magnesium tolerance is important as well as that of sodium tolerance when examining seawater tolerance.

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