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

Hidekazu Kobayashi, Setsuro Sato and Yoshikuni Masaoka*

(National Agricultural Research Center for Western Region, NARO, Ohda, Shimane 694-0013, Japan; *Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8528, Japan)

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% (C50) in the presence of excess CaCl2 and those in the presence of excess NaCl. The C50 estimates in excess MgCl2, however, were not correlated with those in the other two salts. The results suggest that common physiological mechanism confers tolerance to both excess CaCl2 and excess NaCl, but a different mechanism to excess MgCl2.

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; Dvorak 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 CaCl2 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 CaCl2 and MgCl2 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 L. | Niodachi |
| Kentucky bluegrass | Poa pratensis L. | Award |
| Orchardgrass | Dactylis glomerata L. | Okamidori |
| Perennial ryegrass | Lolium perenne L. | Friend |
| Redtop | Agrostis alba L. | commercial 5|
| Tall fescue | Festuca arundinacea Schreb. | Southern cross |
| **Warm-season grass** |
| Bahiagrass | Paspalum notatum Flugge | commercial 5|
| Barnyard millet | Echinochloa crus-galli Ohwi et Yabuno | Shirohie |
| Bermudagrass | Cynodon dactylon (L.) Pers. | Giant bermuda couch |
| Finger millet | Eleusine coracana (L.) Gaertn. | commercial 7|
| Rhodesgrass | Chloris gayana Kunth | Asatsuyu |
| Weeping lovegrass | Ergonanis curvula Nees | commercial 5|

5 Seeds were obtained from Snow Brand Seed Co., Ltd.
7 Seeds were obtained from Takii Seed Co., Ltd.
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 Na₂SiO₃. Therefore, the culture solution contained 1.3 mM Ca²⁺, 0.67 mM Mg²⁺ 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 (CaCl₂: 0, 15, 30, 45, 60, 75 mM (1.0, 3.9, 6.5, 8.9, 11.1, 13.1 dS m⁻¹), MgCl₂: 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:

\[ Y = \frac{Y_m}{1 + \left(\frac{C}{C_{50}}\right)^p} \]

where \( Y \) is plant yield, \( Y_m \) 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 CaCl₂ concentration (mM) and relative growth in cool-season grass ((a)-(f)) and warm-season grass ((g)-(l)).](image-url)
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 $C_{50}$.
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 Italian ryegrass, perennial ryegrass, orchardgrass, Kentucky bluegrass and redtop. With C$_{50}$ estimates as the reference parameter, tall fescue (61.0 mM) was about 2.1 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 (69.4 mM) was about 4.2 times as tolerant as weeping lovegrass (35.9 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$_2$. 

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* (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.
and NaCl, but its tolerance to MgCl₂ was weak. By contrast, bahiagrass was the most tolerant to excess MgCl₂ but showed weak tolerance to CaCl₂ and NaCl.

In order to understand the relationships among the tolerance to the three salts, we compared the C₅₀ estimates of grasses in the three salts (Fig. 4). The C₅₀ estimates in CaCl₂ and in NaCl were positively and significantly correlated (P < 0.01), indicating that the tolerance to excess CaCl₂ 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 CaCl₂ and NaCl, which might explain the correlation between the tolerances to CaCl₂ 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 CaCl₂ 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 CaCl₂ concentrations (Ben-Hayyim et al., 1987).

Tolerance to excess MgCl₂ was not correlated with tolerance to CaCl₂ or NaCl (Fig. 4), indicating that most of the mechanisms of tolerance to excess MgCl₂ are different from those to excess CaCl₂ or NaCl. This was supported by the report that the NaCl-tolerant line had greater tolerance to excess MgCl₂ than did the unselected line in only one of the four grass species (Ashraf et al., 1989). In addition, low Ca²⁺ concentration was suggested to cause subclinical growth depression in MgCl₂-treated Eucalypts seedlings (Marcar and Termaat, 1990).

The present study demonstrates that there are distinct differences in the tolerance to CaCl₂, MgCl₂ and NaCl with the grass species, and that the tolerance to CaCl₂ and NaCl are related, whereas the tolerance to MgCl₂ 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 groundwater. 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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