Effects of Excess Magnesium on the Growth and Mineral Content of Rice and Echinochloa

Hidekazu Kobayashi, Yoshikuni Masaoka* and Setsuro Sato

(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 three cultivars of rice (Oryza sativa L.) and three species of the genus Echinochloa to excess magnesium was examined in solution culture. In Echinochloa species, excess MgCl₂ or MgSO₄ in the culture solution (30 mM) reduced the growth to 33-42% of that in the control plants and caused symptoms resembling those of calcium deficiency. In rice cultivars, however, excess Mg in the culture solution reduced the growth only to 54-67% of that in the control and did not cause the symptoms like those of Ca deficiency. The effect of excess Mg on the mineral contents of plants differed between rice (Nipponbare) and Echinochloa oryzicola. The Mg content of the whole plants in rice increased in proportion to MgCl₂ concentration in the culture solution up to 30 mM, while that in E. oryzicola leveled off when MgCl₂ concentration exceeded 10 mM. The excess MgCl₂ treatment greatly reduced the calcium content of the whole plants in E. oryzicola and slightly in rice. In rice, the excess Mg treatment increased the Mg content of shoots and roots, and the potassium and chloride contents of roots, but slightly decreased the Ca and K contents of shoots. In E. oryzicola, the excess Mg treatment increased the K and Cl contents of shoots and the Mg and K contents of roots, and slightly increased the Mg content of shoots, but greatly decreased the Ca content of shoots. These results indicate that rice is more tolerant than Echinochloa to excess Mg and that the tolerance is related to Ca deficiency.

Key words: Barnyard grass, Early watergrass, Japanese millet, Magnesium, Rice, Salinity tolerance.

One of the main constraints to plant growth and development is salinity, and the responses to salinity have been investigated in various plants. Some investigators have studied the salinity tolerance of Echinochloa crus-galli (L.) Beauv. (barnyard grass) and of E. oryzicola Vasing (early watergrass), both of which are noxious weeds in rice fields (Aslam et al., 1987; Bilski and Foy, 1988; Rahman and Ungar, 1994; Kim et al., 1999). Bilski and Foy (1988) reported that E. crus-galli was exceptionally tolerant to NaCl and Na₂SO₄ among seven weed species examined. Kim et al. (1999) indicated that E. oryzicola was more tolerant to NaCl than rice, and that tolerance to NaCl was related to the ability in limiting Na uptake and its transport to shoots, and to maintain K in shoots under saline conditions. However, they investigated only sodium salts. To our knowledge, no reports have compared the tolerance to other cations such as magnesium of Echinochloa weeds with that of rice. Magnesium is the second major cation in seawater and the tolerance to MgCl₂ was suggested to be different from that to NaCl (Kobayashi et al., 2004).

In a previous report, a cultivated species of the genus Echinochloa, Echinochloa utilis Ohwi et Yabuno (Japanese millet), was found to have the least tolerance to excess MgCl₂ among 12 grass species tested (Kobayashi et al., 2004). On the other hand, rice was reported to be more tolerant to excess magnesium (Mg) than other crops (Tanaka et al., 1976). From these reports, we expected that Echinochloa weeds, such as E. crus-galli and E. oryzicola, would be less tolerant than rice to excess Mg. If confirmed, this finding could lead to the development of a new method for controlling Echinochloa weeds in rice fields. Indeed, interspecific differences in the responses to Mg were reported in relation to species occurrence and distribution at ultramafic site (Nagy and Proctor, 1997).

In the present study, we examined the difference between rice and Echinochloa in their tolerance to excess Mg and investigated the effects of excess Mg on the mineral content of the plants.

Materials and Methods

1. Plant materials
We used three cultivars of rice (Oryza sativa L., cv. Koshihikari, Milyang 23, and Nipponbare) and three species of the genus Echinochloa (E. crus-galli (L.) Beauv. var. crus-galli, E. oryzicola Vasing, and E. utilis Ohwi et Yabuno cv. Shirohie). E. crus-galli was collected in Ohda city, Shimane prefecture. E. oryzicola was collected in Yawara village, Ibaraki prefecture. E. utilis cv. Shirohie was obtained from Snow Brand Seed Co., Ltd.

2. Growth conditions
The experiments were conducted in a growth
MgCl₂ and MgSO₄. The experiments with MgCl₂ and Echinochloa
Experiment 1
3. height.
MgSO₄ were conducted separately.

chamber. Air temperature in the growth chamber was
maintained at 25/20°C (14-h day/10-h night cycle).
Light was provided from high-pressure sodium vapor
and metal halide lamps at 480 µmol s⁻¹ at plant

3. Experiment 1
Three cultivars of rice and three species of
Echinochloa were examined for their tolerance to excess
MgCl₂ and MgSO₄. The experiments with MgCl₂ and
MgSO₄ were conducted separately.

Seeds were sterilized with NaClO (available Cl
concentration: 2.5%) for 10 minutes and germinated
on 0.4% agar plates. At seven days after sowing,
four seedlings from each cultivar or species were
transplanted into a polystyrene tray (30 cm×45 cm)
in a plastic tank (24 plants/tank). Six tanks were used
in a single experiment. The tank contained 15 L of
a nutrient solution (Table 1). The solution pH was
monitored daily and adjusted to 5.2 by adding either 1
M HCl or 1 M NaOH. The solution was renewed every
4 or 5 days.

Excess MgCl₂ treatment commenced at 10 days
after transplanting. The treatment consisted of four
levels of excess MgCl₂ with three replications. MgCl₂
was progressively added at a rate of 10 mM per day
until treatment levels of 0, 10, 20, and 30 mM of
excess MgCl₂ were reached (1.2, 3.2, 4.9, and 6.5 dS
m⁻¹, respectively). At 40 days after transplanting, the
plants were harvested, washed with distilled water and
separated into shoots and roots. The plant materials
were dried at 60°C for 72 hrs and weighed. Mg and Ca
contents were determined by an inductively coupled
plasma spectrophotometer (Nippon Jarrell-Ash, ICAP-
575II). K content was determined by flame emission
spectrophotometry (Tokyo Photoelectric, ANA135).
Cl content was determined by ion chromatography
(TOSOH, column: TSKgel IC-Anion-PW 4.6mmI.D. ×
5cm, detector: UV-8000 (230nm)).

Results
1. Experiment 1
Both treatments with 30 mM MgCl₂ and MgSO₄
inhibited growth to a greater extent in Echinochloa
species than in rice cultivars (Table 2). The excess
MgCl₂ treatment reduced the growth of Echinochloa
species to 33-40% of that in the control, while the
growth of the rice cultivars was maintained at 54-62%.
The excess MgSO₄ treatment reduced the growth of
Echinochloa species to 36-42% that of the control, while
the growth of rice cultivars was maintained at 65-67%.

In Echinochloa species, leaves emerging after the
start of the excess Mg treatment were bleached,
serrated and withered (Fig. 1), which are similar to the
symptoms of Ca deficiency (Islam et al., 1987; Kawasaki
and Moritsugu, 1979). In contrast, the symptoms like
those of Ca deficiency were not observed in rice tested
with excess Mg.

2. Experiment 2

(1) Growth under excess MgCl₂ treatment
Excess MgCl₂ treatment reduced growth to a
greater extent in E. oryzicola than in rice Nipponbare
(Fig. 2). The weight of the whole plant in E. oryzicola
was greatly decreased as concentration of MgCl₂
increased, falling to 22% of that in the control at 30
mM of excess MgCl₂. In contrast, the weight of rice
under the 30 mM MgCl₂ treatment was maintained at
52% that of the control. Two-way ANOVA indicated
significant differences among plant species and MgCl₂
treatment (P<0.001). Plant species ×MgCl₂ treatment
interaction was also significant (P<0.001).

Among plant parts, excess MgCl₂ inhibited root
growth more than shoot growth in both rice and E.
oryzicola (Fig. 2 (b), (c)). At 30 mM of excess MgCl₂,
the root weights were 32% and 16% of the control
in rice and E. oryzicola respectively, while the shoot

Table 1. Culture solution.

|                | Concentration   | Concentration        |
|----------------|-----------------|----------------------|
| NH₄NO₃        | 1.5 mM          | Fe-EDTA              |
| NaH₂PO₄       | 0.2 mM          | H₂BO₄⁺               |
| K₂SO₄         | 0.5 mM          | MnSO₄⁺               |
| CaCl₂         | 1 mM            | (NH₄)₂MoO₄            |
| MgSO₄         | 1 mM            | CuSO₄⁺               |
| Na₂SO₄        | 1 mM            | ZnSO₄⁺               |

*These materials were dissolved in 10% H₂SO₄.
The excess Mg treatment in this experiment caused symptoms similar to those of Ca deficiency more severely than occurred in Experiment 1, as the treatment period was longer in Experiment 2. In *E. oryzicola*, the symptoms were observed even at 10 mM of excess MgCl₂. In rice, the Ca-deficiency-like symptoms were observed at 30 mM, but were not observed in Experiment 1.

(2) Mineral contents of the whole plant

Fig. 3 shows the Mg, Ca, K, and Cl contents of the whole plants in rice (Nipponbare) and *E. oryzicola* treated with excess MgCl₂. The excess MgCl₂ treatment increased the Mg content of the whole plants in both species (Fig. 3 (a)). The Mg content of the whole plants increased as MgCl₂ concentration in the culture solution increased in *E. oryzicola*, but not in rice.

The effect of excess MgCl₂ treatment on the K content of shoots and roots differed between the two species (Fig. 4). In *E. oryzicola*, excess MgCl₂ treatment greatly increased the K content of roots but only slightly increased the K content of shoots. In rice, excess MgCl₂ treatment greatly increased the Mg content of shoots and roots.

The effect of excess MgCl₂ treatment on the Ca content decreased with increasing MgCl₂ concentration in the culture solution in both shoots and roots of both rice and *E. oryzicola* (Fig. 4). The decrease was prominent in the shoots of *E. oryzicola*, in which the Ca content of the shoots in the solution with 30 mM MgCl₂ was only 18% of that in the control. On the other hand, the Ca content of the shoots in rice was maintained at 59% of the control even in the solution with 30 mM MgCl₂.

The effect of excess MgCl₂ treatment on the Cl contents of shoots and roots also showed differences between the two species (Fig. 4). The excess MgCl₂ treatment increased the Cl content of shoots and steeply decreased the Cl content of roots in *E. oryzicola*, but had no effect on the Cl content of shoots and slightly increased the Cl content of roots in rice.

Discussion

The present study revealed that excess Mg treatment reduced growth to a greater extent in *Echinochloa* than in rice (Table 2, Fig. 2). This was consistent with the report that *E. utilis* was the least tolerant to excess MgCl₂ among 12 grass species tested (Kobayashi et al., 2004) and that rice was the most tolerant to excess Mg among 20 crops examined (Tanaka et al., 1976). On the other hand, Kim et al., (1999) reported that *E. oryzicola* was more tolerant to NaCl than rice. These contrasting observations suggest that the mechanism underlying tolerance to excess Mg differs from that underlying tolerance to excess Na. This idea was supported by our previous study, in which tolerance to excess MgCl₂ was not correlated with tolerance to...
excess CaCl₂ or NaCl in twelve grass species (Kobayashi et al., 2004). The excess MgCl₂ treatment had various effects on the Mg, Ca, K, and Cl contents of plants. Mg content was increased by excess MgCl₂ treatment in both shoots and roots of both rice (Nipponbare) and *E. oryzicola* (Fig. 3 and 4). In barley, excessive accumulation of Mg, resulting from high-Mg treatment, was suggested to reduce growth (Grant and Racz, 1987). In the present study, rice accumulated Mg in proportion to the MgCl₂ concentration in culture solution, and the Mg content of the plants in the solution with 30 mM excess MgCl₂ exceeded 200% of that in the control (Fig. 3 and 4). In contrast, Mg content of the shoots in *E. oryzicola* treated with 10 mM excess MgCl₂ was 143% of that in the control but that treated with 30 mM MgCl₂ was only 116% of the control (Fig. 4). These results suggest that excess Mg accumulation does not play a main role in determining tolerance to excess Mg in rice and *Echinochloa*.

Ca content of plants was decreased by excess Mg treatment greatly in *E. oryzicola* and slightly in rice (Fig. 3 and 4). In previous studies reduced plant growth under high Mg conditions was associated with Ca deficiency (Ehret et al., 1990; Kurita, 1990; Marcar and Termaat, 1990). In the present study, also the Ca-deficiency-like symptoms were observed in *E. oryzicola* even at 10 mM excess MgCl₂, and Ca content of *E. oryzicola* decreased dramatically as MgCl₂ concentration increased (Fig. 3 and 4). In rice, however, the symptoms were observed only at 30 mM of excess MgCl₂, and the Ca content was maintained at more

---

Fig. 1. Symptoms like calcium deficiency in *Echinochloa* with excess Mg.

The picture shows the symptoms observed in *E. utilis* with 30 mM of MgSO₄.

Fig. 2. Effects of excess MgCl₂ treatment on the growth of rice (Nipponbare) and *E. oryzicola*.

Data are means±S.E. of three pots; where no error bars appear, they were smaller than the symbols.

Each pot contains eight plants of rice or three plants of *E. oryzicola*. 
than a half of that in the control even in the solution with 30 mM MgCl₂ (Fig. 3 and 4). In addition, rice has been reported to have a low critical content of Ca without Ca deficiency in growing leaves (Tanaka and Tadano, 1973). These results indicate that decreased Ca content plays an important role in the response to excess Mg.

The effects of excess MgCl₂ on K content differed between rice and *E. oryzicola* (Fig. 3 and 4). In rice, the K content of shoots decreased with increasing MgCl₂ concentration, while that of roots increased,
which indicates that excess Mg inhibits translocation of K from roots to shoots in rice. In E. oryzicola, excess Mg treatment increased K content in both shoots and roots. The stimulation of K uptake was also observed in E. crus-galli under NaCl stress (Aslam et al., 1987). Yamanouchi et al. (1990) indicated that the effects of NaCl on cation content of plants were determined by the ability to exclude Na⁺ and Cl⁻; if the ability to exclude Na⁺ is greater than that to exclude Cl⁻, the absorption of other cations is stimulated to neutralize the excess of Cl ion in the plants. In the present study, Cl content of E. oryzicola increased in proportion to the concentration of MgCl₂ in the culture solution up to 30 mM, while Mg content leveled off when the MgCl₂ concentration exceeded 10 mM (Fig. 3). These results suggest that the ability to exclude Mg is stronger than that to exclude Cl in E. oryzicola, and this may be why the K content of the plant increased with increasing MgCl₂ concentration.

The effects of excess MgCl₂ on Cl content differed between rice and E. oryzicola (Fig. 3 and 4). The most conspicuous response to excess MgCl₂ was a steep reduction of Cl content in the roots of E. oryzicola. The reason for this drop was unclear in our experiment; further experiments, including other organic or inorganic anions, may be needed. However, since excess MgSO₄ also reduced plant growth in a similar manner (Table 2), the increase of Cl concentration in culture solution may not be the main cause of the reduced plant growth.

The present study clarified that rice is more tolerant than Echinochloa to excess Mg and that this tolerance seems to be related to Ca deficiency. The result that a crop species is more tolerant than its companion weed species is more tolerant than its companion weed seems to be related to Ca deficiency. The result that a mineral analysis.

Acknowledgments

We thank Yoshiaki Kawana, National Agricultural Research Center, for providing the seeds of E. oryzicola, and Kei Matsushita, National Agricultural Research Center for Western Region, for providing the rice seeds. We are grateful to Sada Ando and Yasuhiko Nishiguchi, National Agricultural Research Center for Western Region, and Ryuichi Suwa, Hiroshima University, for useful advice and excellent assistance in mineral analysis.

References

Aslam, Z., Salim, M., Qureshi, R.H. and Sandhu, G.R. 1987. Salt tolerance of Echinochloa crusgalli. Biologia Plantarum 29: 66-69.

Bilski, J.J. and Foy, C.D. 1988. Differential tolerances of weed species to aluminum, manganese and salinity. J. Plant Nutr. 11: 93-105.

Ehret, D.L., Redmann, R.E., Harvey, B.L. and Cipywenyk, A. 1990. Salinity-induced calcium deficiencies in wheat and barley. Plant Soil 128: 143-151.

Grant, C.A. and Racz, G.J. 1987. The effect of Ca and Mg concentrations in nutrient solution on the dry matter yield and Ca, Mg and K content of barley (Hordeum vulgare L.). Can. J. Soil Sci. 67: 857-865.

Islam, A.K.M.S., Asher, C.J. and Edwards, D.G. 1987. Response of plants to calcium concentration in flowing solution culture with chloride or sulphate as the counter-ion. Plant Soil 98: 377-395.

Kawasaki, T. and Morisugu, M. 1979. A characteristic symptom of calcium deficiency in maize and sorghum. Commun. Soil Sci. Plant Anal. 10: 41-56.

Kim, Y.H., Shim, I.S., Kobayashi, K. and Usui, K. 1999. Relationship between Na content or K/Na ratio in shoots and salt tolerance in several gramineous plants. J. Weed Sci. Tech. 44: 293-299.

Kobayashi, H., Sato, S. and Masaoka, Y. 2004. Tolerance of grasses to calcium chloride, magnesium chloride and sodium chloride. Plant Prod. Sci. 7: 30-35.

Kurita, Z. 1990. Screening for tolerance to magnesium chloride on guayule, Parthenium argentatum Gray. Jpn. J. Trop. Agr. 34: 84-91.

Marcar, N.E. and Termaat, A. 1990. Effects of root-zone solutes on Eucalyptus camaldulensis and Eucalyptus bicostata seedlings: Responses to Na⁺, Mg²⁺ and Cl⁻. Plant Soil 125: 245-254.

Nagy, L. and Proctor, J. 1997. Soil Mg and Ni as causal factors of plant occurrence and distribution at the Meikle Kilrannoch ultramafic site in Scotland. New Phytol. 135: 561-566.

Rahman, M. and Ungar, I.A. 1994. The effect of competition and salinity on shoot growth and reproductive biomass of Echinochloa crus-galli. Aquatic Botany 48: 343-353.

Tanaka, A. and Tadano, T. 1973. Comparative plant nutrition. Comparison of adaptability to bases among crop plants. Part 2. Plant characters resulting in difference of the critical Ca concentration of culture solution inducing Ca deficiency symptoms. J. Sci. Soil Manure, Japan (Nippon Dojohiryogaku Zashhi) 44: 572-576.

Tanaka, A., Tadano, T. and Hitsuda, K. 1976. Comparative plant nutrition. Comparison of adaptability to bases among crop plants. 5. Adaptability to magnesium. J. Sci. Soil Manure, Japan (Nippon Dojohiryogaku Zashhi) 47: 361-366.

Yamanouchi, M., Fujyama, H., Kimura, Y. and Nagai, T. 1990. The effects of sodium chloride on the absorption and the translocation of several ions in sugar beets, rice plants, soy beans, azuki beans, and kidney beans. Jpn. J. Soil Sci. Plant Nutr. 61: 173-176**.

* In Japanese.

** In Japanese with English abstract.