Variation of Salinity Tolerance in Zoysia Clones Collected from Different Habitats in Taiwan

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Abstract: One hundred and fifteen clones of Zoysia spp. collected around the coast of Taiwan were examined for the variation of salinity tolerance. Zoysia spp., native of Taiwan, showed a wide range of salinity tolerance among the clones collected from various regions. The clones from the regions with higher annual rainfall and limestone showed lower NaCl tolerance, and some of them showed 100% shoot mortality under 3% NaCl within 3 weeks. On the contrary, some clones from the regions with lower rainfall retained a few green leaves even at 7.5% NaCl for 3 weeks. A positive correlation was observed between the percentages of 100% shoot mortality in clones treated with 6% NaCl and the rainfall of the regions where the clones were collected. However, based on the same level of rainfall, the clones collected from the limestone region showed higher percentages of shoot mortality than those collected from the other regions. Using four clones including NaCl-tolerant and NaCl-sensitive, the effect of Ca on the salinity stress was further examined. Addition of CaCl₂ to the nutrient solution could alleviate the leaf firing and electrolyte leakage from the leaf of Zoysia clones caused by NaCl. The geographical variation of salinity tolerance in Zoysia might be caused by the differences in the salt and calcium concentrations in soil, which were affected by rainfall and geology of their habitats.

Key words: Calcium, Habitat, Rainfall, Salinity tolerance, Turfgrass, Zoysia.

Zoysia spp., perennial C₄ grasses, distributed from China, Japan, tropical Asia to New Zealand, with well-developed stolon and short culm, can form a dense lawn. Some of them include a number of interfertile species that are important turf grasses (Kitamura, 1989). Recently, low water input has been a tendency for turf management. Thus, there is an increasing need for the development of salinity-tolerant turfgrass cultivars (Harivandi et al., 1992). Marcum et al. (1998) reported that zoysiagrass species had been considered as salinity-tolerant turfgrasses, with a broad range of salinity tolerance among cultivars and clones. Akiyoshi et al. (1998) also reported that interspecific variation on sea water tolerance of zoysiagrasses was obvious.

In Taiwan (21° 55’ N-25° 18’ N), Zoysia spp. is distributed widely in various environments of littoral regions. They showed a great variation in morphology when they were transplanted to pots and grown on the campus of National Chung-Hsing University. These variations might be caused by differentiation of genes for adapting to the environments of the habitats. For example, the clones collected from a rocky seashore have lying leaves and short internodes, and those from a sandy seashore have erect leaves and long internodes, possibly to avoid being covered by sand (Weng et al., 1995).

Rainfall is an important environmental factor affecting plant growth. Among the littoral regions of Taiwan, there is a wide variation in rainfall ranging from 1200 to 3300 mm per year. Some regions mainly consist of limestones. Calcium has been reported to reduce the adverse effects of NaCl salinity in some plants (Kauss, 1987; Nakamura et al., 1990; Suhayda et al., 1992). It could be that both rainfall and geology would influence the concentrations of salt and calcium in soil, thus the Zoysia spp. collected from different regions may show different salinity tolerance. In this study, 115 clones of Zoysia spp. collected from the coastal regions around Taiwan and Penghu Island were used to examine the variation of salinity tolerance.

Materials and Methods

During 1993-1994, 115 clones of Zoysia collected from 59 sites around the coasts of Taiwan and the Penghu Island (located in Taiwan Strait) (Fig. 1) were used as materials. Collected rhizomes were transplanted in pots (15 cm in diameter) filled with soil and sand (1:1), and placed outdoors on the campus of the National Chung-Hsing University, Taichung, Taiwan (24° 10’ N, 78 m). In May, 1995, the current-year rhizomes of length about 11 cm with shoots were transplanted to a tank (200 cm (L) × 30 cm (W) × 30 cm (H)) and grown in a glasshouse by hydroponic culture (Hyponica fertilizer, Kyowa Co Ltd, Osaka, Japan. 791 ppm NO₃⁻, 177 ppm PO₄³⁻, 128 ppm Ca²⁺, 312 ppm K⁺, 30 ppm Mg²⁺, EC = 2.0 mS cm⁻¹, pH = 6.0). Fifty-seven or 58 clones were planted randomly in each nutrient tank, and each clone had 3 replications with 3 rhizomes per replication. The tanks were constantly aerated by pumping air.

The nutrient solution of hydroponic culture was supplemented with NaCl from three weeks after transplanta-
Fig. 1. A diagram showing the regions and sites in Taiwan where the clones of *Zostera* spp. were collected. •: collected site, ○: no *Zostera* plants were found in the collection, △: meteorological station, ■: limestone area. N: north coast; WN and WS: northern and southern part of west coast, respectively; EN and ES: northern and southern part of east coast, respectively; PH: Penghu. The numerals in parentheses are the number of clones tested.

Fig. 2. The percentage of clones with 100% shoot mortality to all clones collected from each of the six regions after the treatment with NaCl at various levels for 3 weeks. ▲: north coast; ■ and □: northern and southern part of east coast, respectively; ● and ○: northern and southern part of west coast, respectively; ▼: Penghu. The numbers of the clones tested in each region are shown in Fig. 1.

classes. The two NaCl–sensitive clones, HB and KE, were collected from the northern and southern part of the east coast, respectively; and two tolerant clones, LRM and LCG, from the southern part of west coast, and Penghu Island, respectively. The current–year rhizome of about 11 cm in length with shoots were transplanted to a pot (42 cm (L) × 32 cm (W) × 12 cm (H)) and grown in a growth cabinet (400 μmol s⁻¹ m⁻², 30 /25°C and 12/12 h day/night) by hydroponic culture using full strength modified Hoagland nutrient solution.

Three weeks after transplantation, 0.5% NaCl was added to the nutrient solution together with 0, 6 or 12 mM of CaCl₂, and the salinity level was gradually increased by the increments of 0.5% NaCl every 2 days up to the designated concentration. All the treatments had four replications. The numbers of green leaves in 10 shoots in each replication were recorded at the day before NaCl treatment and the 2nd day after addition of NaCl up to 1.5, 3, 4.5 and 6%.

In addition, electrolyte leakage of leaves of the plants grown under 1.5% and 3% NaCl together with 6 mM or without CaCl₂ were determined. Detached leaves 0.5 g in fresh weight were rinsed with deionized water, cut into 5 mm length and placed in a test tube containing 15 mL of deionized water. The tube was covered with paraffin film and maintained at 28°C for 1 hr. Then the electric conductivity of the extract was measured with a pH/conductivity meter (model PCT-403, Extech, USA).

**Results**

The ratios of clones with 100% shoot mortality to all clones from each region are shown in Fig. 2. The clones collected from the north coast and the northern part of the east coast showed higher sensitivity to NaCl than the others. The clones from both regions included one clone showing 100% shoot mortality even at 3% NaCl, most of
the other clones showed 100% shoot mortality at 4.5% or 6% NaCl. The clones collected from the southern part of the west coast and Penghu Island showed higher tolerance to NaCl, and about 50% of them did not show 100% shoot mortality even at 6% NaCl. Furthermore, one clone from the southern part of the east coast and Penghu Island, respectively, and 2 clones from the southern part of the west coast still survived at 7.5% NaCl for 3 weeks. Remarkable clonal differences in the NaCl tolerance were observed at 4.5% and 6% NaCl levels, more clearly at 6% than at 4.5% NaCl.

A positive regression line was obtained between the % of shoot mortality at 6% NaCl and the annual rainfall in the regions where the clones have been collected, and a positive regression curve between the % of shoot mortality at 6% NaCl and the rainfall in the dry season (Oct. - Mar.) was observed also (Fig. 3). Under the same level of rainfall, the clones collected from the northern part of the east coast showed a higher % of shoot mortality than those collected from the other regions.

The percentage of the number of green leaves remaining on the shoots after applying NaCl to that before NaCl application (% of green leaves) varied with the clone (Fig. 4). At 1.5% NaCl, the % of green leaves in the most NaCl-sensitive clone, HB, collected from the limestone region, was only 15% and that in another NaCl-sensitive clone, KE, was 85%. All of them showed 100% or nearly 100% leaf firing at 3% NaCl. The NaCl-tolerant clones, LRM and LCG, remained 100% green leaves at 1.5% NaCl, and 40% green leaves survived at 6% NaCl. Also, the addition of 6 and 12 mM CaCl₂ to the nutrient solution could reduce the leaf firing in two NaCl-sensitive clones, but a significant effect was found.

Fig. 3. The relationship between the percentage of 100% shoot mortality at 6% NaCl and the annual rainfall (left), and rainfall in the dry season (October- March, right) of the regions where the clones were collected. N: north coast; WN and WS: northern and southern part of west coast, respectively; EN and ES: northern and southern part of east coast, respectively; PH: Penghu. (**: P < 0.01, O only).

Fig. 4. Effects of various levels of NaCl and CaCl₂ on the percentage of green leaf number on the shoots of Zostoa clones. The percentage of green leaves is the % of the green leaf number after the treatment to that before treatment. Data are means ±SE (n=4).

Fig. 5. Effects of various levels of NaCl and 6 mM CaCl₂ on the leaf electrolyte leakage of Zostoa clones. Data are means ±SE (n=4).
only under 12 mM CaCl₂ in two NaCl-tolerant clones (Fig. 4).

The electrolyte leakage from leaves was also affected by NaCl and CaCl₂ almost in parallel with leaf firing (Fig. 5). The most NaCl-sensitive clone, HB, collected from the limestone region, showed the highest electrolyte leakage under NaCl treatment, followed by another NaCl-sensitive clone, KE. The tolerant clones were less affected by 1.5% and 3% NaCl treatment. Addition of 6 mM CaCl₂ to the nutrient solution reduced the electrolyte leakage of the two NaCl-sensitive clones under NaCl treatment, but no significant effect of CaCl₂ was found in the two tolerant clones.

Discussion

The genetic relationships among tested clones in the present study were analyzed by random amplified polymorphic DNA (RAPD), but the same genotype could not be found (Lin, 2000). Marcum et al. (1998) reported that zoysiagrass species showed a broad range of salinity tolerance among cultivars and clones, ranging from 19 to 80% relative leaf firing at 400 mM NaCl. The present study also indicated that some NaCl-sensitive clones showed 100% leaf firing at 3% (512 mM) NaCl within 3 weeks, and the most NaCl-tolerant clone maintained some green leaves even at 7.5% NaCl for 3 weeks (Fig. 2). The differences in NaCl tolerance of clones collected from various regions in Taiwan were related to the rainfall of their habitats (Fig. 2, 3). Most of the clones collected from the region with a higher rainfall (throughout the year or dry season) showed lower NaCl tolerance, and those from the region with lower rainfall showed higher NaCl tolerance. The rainfall might reduce the NaCl concentration in the soil in the coastal regions.

Na⁺ can displace Ca²⁺ from the plasma membrane, resulting in a change in membrane permeability that can be detected by the leakage of ions from the cells (Cramer et al., 1985). The calcium could reduce the Na⁺ uptake and its transport to the shoots (Mass and Griver, 1987; Cramer et al., 1989; Davenport et al., 1996), as well as reduce the Na⁺-induced displacement of membrane-associated calcium (Cramer et al., 1989). Davenport et al. (1996) reported that Ca²⁺ could reduce the uptake of Na⁺ in both salt-tolerant and salt-sensitive species. Fig. 4 and Fig. 5 show that adding CaCl₂ to nutrient solution reduced both leaf firing and leaf electrolyte leakage caused by NaCl in Zoysia, especially in NaCl-sensitive clones.

The clones from the regions with higher rainfall showed lower NaCl tolerance (Fig. 3). However, based on the same level of rainfall, the clones collected from the northern part of the east coast had higher percentages of 100% shoot mortality than those collected from the other regions. The northern part of the east coast of Taiwan mainly consists of limestone (Fig. 1). Zoysia clones with lower NaCl tolerance grown there might be protected from NaCl stress by calcium.

As mentioned above, Zoysia spp. native of Taiwan showed a wide range of salinity tolerance among the clones collected from different regions. Most clones collected from the region with a higher annual rainfall and limestone showed lower NaCl tolerance. It was concluded that the geographical variation of salinity tolerance in Zoysia might be caused by the difference in salt and calcium concentrations in soil, which were affected by rainfall and geology of their habitats.

References


*In Japanese.

**In Chinese with English summary.