The Physiological Responses of Zoysia Clones to NaCl Stress

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ABSTRACT

Zoysia spp., a perennial C₄ grass species with well-developed stolon and short culm, can form a dense lawn. Some varieties of this species are important turfgrass and show a great variation in salinity tolerance. NaCl-tolerant clones of Z. sinica and Z. matrella (LRM and LCG, could retain a few green leaves at 7.5% NaCl for 3 weeks) and two sensitive clones of Z. matrella (HB and KE, showed 100% and near 100% leaf necroses in 3% NaCl) were used for this study. Plants were grown in nutrient solution supplemented with NaCl up to 3% under controlled conditions. The most sensitive clone (HB) showed higher Na⁺ accumulation than the other sensitive clone (KE) and two tolerant clones, due to both lower rates of Na⁺ secretion from leaves and Na⁺ exclusion in shoots. Moreover, compared with the same level of Na⁺ accumulated in shoot, two NaCl-sensitive clones showed higher degree of leaf electrolyte leakage than those of two tolerant clones. Thus, Zoysia clones with NaCl-tolerant ability could not only restrict saline ion accumulation in shoots but also maintain membrane integrity under NaCl treatment.

Key words: Genotype, NaCl stress, Salinity tolerance, Zoysia.
INTRODUCTION

Zoysia spp., a perennial C₄ grass species with well-developed stolon and short culm, can form a dense lawn. Their distribution across China, Japan and Tropical Asia to New Zealand, and some of which, including a number of interfertile species, are important turfgrass. Previously we found that Zoysia spp. are widely distributed in various environments of littoral regions in Taiwan, and showed a great variation in morphology and salinity tolerance even when they were transplanted to the campus of the National Chung-Hsing University (Weng et al. 1995, Weng and Chen 2001).

Leaf necroses percentage under salt stress has been used as an indicator for relative salt injury of Zoysia spp. (Marcum et al. 1998, Qian et al. 2000). Marcum et al. (1998) reported that Zoysiagrasses have been considered salinity tolerant turfgrasses, while there was a broad range of salinity tolerance among the cultivars and clones, from 19 to 80% relative leaf necroses in response to 400 mM NaCl. Same tendency was found in our previous paper (Weng and Chen 2001), where the NaCl-sensitive clones showed 100% and near 100% leaf necroses in 3% NaCl within 3 weeks. On the other hand, the most NaCl-tolerant clones could retain a few green leaves at 7.5% NaCl for 3 weeks. Thus, some Zoysia spp. may be considered as halophytes.

The ability of salinity tolerance is an important characteristic for cultivation and eco-physiological study. Because Zoysia spp. are widely distributed in various environments of littoral regions in Taiwan, it is important to elucidate the possible mechanisms of broad range of salinity tolerance among Zoysia clones. The ability of salinity tolerance among turfgrass species and cultivars has been associated with the restriction of saline ion accumulation in shoots (Marcum and Murdoch 1990, Wu and Lin 1994, Qian et al. 2000). Marcum et al. (1998) and Marcum (1999) also reported that NaCl-tolerant Zoysia spp. and other subfamily of Chloridoideae were associated with the salt gland secretion in the leaf. In fact, tolerance to salinity may be associated with other factors, such as osmotic adjustment, ion exclusion, ion compartmentation, favourable ion balance and maintenance of membrane integrity (Flowers et al. 1977, Cheeseman 1988, Bradley and Morris 1991, Dionisio-Sese and Tobita 1998, Borsani et al. 2003, Martínez-Ballesta et al. 2004, Koyro 2006). However, except the gland secretion, other mechanisms related to salinity tolerance of Zoysia spp. are not clear at this point.

In order to understand the overall mechanisms of salinity tolerance for Zoysia spp., four clones, two sensitive and two tolerant, were used in this study.

MATERIALS AND METHODS

Plant Materials

Two NaCl-sensitive (HB and KE) and two tolerant clones (LRM and LCG) were selected as experimental materials. Among them, HB and KE are Z. matrella collected from Hanben (Ilan Hsien) and Kentin (Pingtung Hsien), and showed 100% and near 100% leaf necroses in 3% NaCl, respectively. While LRM is Z. sinica, collected from Luermen (Tainan Hsien), and LCG is Z. matrella, collected from Penghu (Penghu Hsien). Both clones could retain a few green leaves at 7.5% NaCl for 3 weeks (Weng and Chen 2001).

Leaf Necroses, Electrolyte Leakage and Solute Accumulation

The newly-emerged rhizomes (about 11 cm in length) with shoots were transplanted to a pot (42 cm (L) × 32 cm (W) × 12 cm (H)] and grown in growth cabinet (400 μmol m⁻² s⁻¹ PAR, 30/25 ℃ and 12/12 h day/night) with hydroponic culture in full strength modified Hoagland nutrient solution. Sodium chloride was added 3 weeks after transplanting. To avoid salinity shock, salinity levels were gradually increased by the increments of 0.5% NaCl every 2 days up to 3%. At the day before NaCl treatment and the 2nd day after adding NaCl up to 1.5 and 3%, the numbers of green leaves for 10 shoots in each replication were recorded, and the averaged values of these 10 shoots were used as statistical parameter of each replication. The sampled plants

不耐鹽之營養系其莖葉所累積之脯胺酸較耐鹽者高。但是，鹽逆境對各營養系體內K⁺累積均無顯著之影響。

關鍵詞：基因型、鹽逆境、耐鹽性、結縷草。
were then harvested for further examination.

For leaf electrolyte leakage analysis, 0.5 g of leaves in each replication were sampled and rinsed with deionized water, cut into 5 mm long, and then added with 15 ml of deionized water for 1 hr in 28°C. The electron conductivity of extract was measured by a pH/conductivity meter (model PCT-403, Extech, USA).

For solute analysis, the samples were divided into shoots and rhizome + root, and rinsed for 20 sec in deionized water. After dried in a forced-air dryer at 70°C, each part was ground in a mill with 40-mesh screen.

To determine the total solute accumulation at dry weight base, 0.1 g of ground sample was put into a 10 ml test tube and added with 0.5 ml deionized water. After shake, tube was centrifuged at 1860 x g for 15 min. The osmolality of supernatant was measured with a vapor pressure osmometer (model 5500, Wescor, USA). Soluble sugar was extracted from 0.1 g ground sample with 10 ml 80% ethanol at 70°C for 1 hr. The extract was then centrifuged at 1600 x g for 10 min and determined with anthrone reagent (Hansen and Moller 1975). Proline was extracted from 1 g ground sample with 10 ml 3% sulphosalicylic acid, and determined according to Bates et al. (1973). For K⁺ and Na⁺ analysis, 0.1 g ground sample was dissolved in concentrated H₂SO₄ and 3% H₂O₂ (Watson and Isaac 1990), and determined by atomic absorption spectrophotometry (model 170-30, Hitachi, Japan).

**Leaf Na⁺ Secretion from Salt Gland**

The newly-emerged rhizomes (about 11 cm in length) with shoots were transplanted in nutrient solution for 3 weeks. Ten shoots in each replication were rinsed by spraying deionized water, and then cultured with 1.5% NaCl containing nutrient solution for 24 hr. The leaves were rinsed with deionized water again, and the Na⁺ content of rinsed water was determined by atomic absorption spectrophotometry (model 170-30, Hitachi, Japan). The leaf area was measured with a CI-420 leaf area imager (CID Inc., USA). All the treatments had four replications.

**Na⁺ Exclusion in Shoots**

The Na⁺ exclusion of Zoysia clones was estimated by the Na⁺ accumulation in the rhizome, culm and leaf sheath when leaf blades were removed to block the Na⁺ secretion. The newly-emerged rhizomes (about 11 cm in length) with shoots were transplanted in nutrient solution for 3 weeks. After leaves were removed, the remained parts of Zoysia clones were cultured with 1.5% NaCl containing nutrient solution for 48 hr in very low irradiance (ca. 20 μmol m⁻² s⁻¹ photon flux density at day time), and the Na⁺ of rhizome + culm + leaf sheath was determined. Four replications were tested with each replication containing 8 rhizomes.

**RESULTS**

In the treatment of 1.5% NaCl, the NaCl-sensitive clones, HB and KE, decreased their green leaf number to 15 and 85% (before treatment as 100%), respectively, and 100% or near 100% leaf necroses occurred under 3% NaCl (Fig. 1). On the contrary, the NaCl-tolerant clones, LRM and LCG, did not decrease their green leaves significantly under 1.5% NaCl, and retained their green leaves near 50-60% under 3% NaCl (Fig. 1). The most NaCl-sensitive clone, HB, showed the highest electrolyte leakage under NaCl treatment, followed by the other sensitive clone, KE. The two tolerant clones were less affected by NaCl treatment. The Na⁺ secretion rate from leaf was lower in the most NaCl-sensitive clone, HB, and higher in another NaCl-sensitive clone and two tolerant clones (Fig. 1).

The accumulated total solutes and Na⁺ increased with NaCl level in both shoots and rhizome + root, while shoots accumulated higher total solutes than rhizome + root (Fig. 2). Among four tested clones, the most NaCl-sensitive clone, HB, accumulated higher total solutes and Na⁺ in shoot under both 1.5% and 3% NaCl than the other clones, but not in rhizome + root. The K⁺ content in both shoot and rhizome + root was not significantly influenced by the NaCl in most treatments. Therefore, Na⁺/K⁺ ratio was influenced mainly by Na⁺ accumulation (Fig. 2). When leaf blades were removed to block the Na⁺ secretion, two higher Na⁺ secretion clones (KE and LCG) showed significant higher Na⁺ accumulation in culm + leaf sheath than those of no leaf-removed ones, but not significantly
Fig. 1. The rate of Na⁺ secretion from leaf, percentage of green leaf number (before NaCl treatment as 100%), and leaf electrolyte leakage of Zoysia clones under NaCl treatments. I: se (n= 4). Leaf Na⁺ secretion was determined at 1.5% NaCl only.
Fig. 2. The variation of total solute, Na\(^+\) and K\(^+\) contents in shoot and rhizome + root of Zoysia clones under NaCl treatments. I: se (n= 4).
different with low Na⁺ secretion clone, HB. Moreover, the NaCl-tolerant clone (LCG) showed lower Na⁺ accumulation in culm + leaf sheath than those of sensitive clones in both the leaves removed and no leaf-removed treatments (Fig. 3).

The soluble sugar content of Zoysia in shoot increased significantly in one NaCl-sensitive clone (HB) and two NaCl-tolerant clones at 3% NaCl, but only LCG increased significantly under 1.5% NaCl in shoot (Fig. 4). On the contrary, soluble sugar content in rhizome + root was lower than that in shoot, and only two clones, LCG and KE, increased with the increasing NaCl level (Fig. 4). In Fig. 4, it was also found that NaCl treatment could promote proline accumulation in both shoot and rhizome + root. However, no significant difference of proline content could be found in rhizome + root between 1.5% and 3% NaCl treatments. Besides, two NaCl-sensitive clones showed more proline accumulated in shoot than those of two tolerant clones.

**DISCUSSION**


It was reported that some NaCl-tolerant plants, including turfgrasses, accumulated lower Na⁺ than sensitive plants under NaCl treatment (Marcum and Murdoch 1990, Pérez-Alfocea et al. 1996, Qian et al. 2000). Marcum et al. (1998) and Marcum (1999) also reported that the lower Na⁺ accumulation in NaCl-tolerant Zoysia spp. were associated with the salt gland secretion in the leaf. Same tendency was observed in the present study that the most NaCl-sensitive clone, HB, showed lower Na⁺ secretion from the leaves and higher Na⁺ accumulation in shoots or in culm + leaf sheath (Fig. 1 to Fig. 3).

However, Na⁺ accumulation in plants not only related to the NaCl secretion by the gland in shoot but NaCl exclusion by the root and the shoot (Flowers et al. 1977, Cheeseman 1988, Bradley and Morris 1991). The NaCl exclusion of Zoysia clones was estimated by removing the leaf blade to block the Na⁺ secretion. Because leaves were fed by the transpiration stream and could receive large quantities of sodium (Winter 1982), to minimize the effects of transpiration, plant materials, both leaf-removed and no leaf-removed, were put in very low irradiance. Result indicated that the NaCl-tolerant clone (LCG) showed lower Na⁺ accumulation than those of sensitive clones in

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**Fig. 3.** The Na⁺ content in rhizome + culm + leaf sheath of Zoysia clones under 1.5% NaCl treatments for 48 hr. I: se (n= 4).
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Fig. 4. The variation of soluble sugars (molecule wt. as sucrose) and proline contents in shoot and rhizome + root of Zoysia clones under NaCl treatments. I: se (n= 4).

both the leaf-removed (Na⁺ secretion from leaves were blocked) and no leaf-removed treatment (Fig. 3). Results suggest that lower Na⁺ accumulation in the shoots of some Zoysia clones was due also to the higher Na⁺ exclusion in shoots.

Maintenance of membrane integrity is an important mechanism for NaCl-tolerant ability (Flowers et al. 1977, Dionisio-Sese and Tobita 1998). From Fig. 5, the relationship between leaf electrolyte leakage and Na⁺ accumulation could be divided into two groups, one group was the NaCl-tolerant clones (LRM and LCG) and the other was the NaCl-sensitive clones (HB and KE). Under the same level of Na⁺ accumulation in shoot, two NaCl-sensitive clones showed higher degree of leaf electrolyte leakage than those of two NaCl-tolerant ones. The most NaCl-sensitive clone, HB, showed higher Na⁺ accumulation and membrane injury under NaCl treatment, while the other sensitive clone, KE, showed lower Na⁺ accumulation but higher membrane injury (Fig. 1 and Fig. 2). Thus, it appeared that the NaCl-tolerant Zoysia clones exhibited a lower saline ion accumulation in shoots and better membrane integrity.

Declined K⁺ content with increased salinity had been reported for both halophytes (Ungar 1996, Qian et al. 2000) and glycophytes (Chow et al. 1990, Botella et al. 1997). Frequently, salt tolerant plants were found to preferentially absorb K⁺ over Na⁺ (Flower et al. 1977, Bradley and Morries 1991). In this study, the K⁺ content of four tested Zoysia clones in both shoot and rhizome + root were not significantly influenced by NaCl in most treatments (Fig. 2). It indicated that less competitive inhibition of K⁺ uptake at higher Na⁺
Fig. 5. The relationships between leaf electrolyte leakage and Na⁺ accumulation in shoot of four tested Zoysia clones under 1.5% and 3% NaCl treatments. ***: P < 0.001.

contents (Flowers et al. 1977, Bradley and Morries 1991) in both NaCl-sensitive and NaCl-tolerant Zoysia clones.

The accumulation of soluble sugar in response to environmental stresses was assumed to be associated with osmotic adjustment (Morgan 1992) and/or with osmoprotection (Guy 1990, Leprince et al. 1993). It was reported that carbohydrates of low molecular weight were the main organic solutes involved in osmotic adjustment to saline and osmotic stresses in glycophyte (Pérez-Alfocea et al. 1993, Balibrea et al. 1997, Kerepesi et al. 1998). However, in some crops and halophytes, the content of total sugar declined with increasing levels of NaCl (Matar et al. 1975, Joshi 1984, Venkatesalu et al. 1994). In the present study, in Zoysia, soluble sugar concentration was not that high when compared to total solute (Fig. 2 and Fig. 3), and no correlation was observed between soluble sugar accumulation and the degree of NaCl tolerance (Fig. 4). Zoysia might retain high ability of osmotic adjustment with inorganic or other organic solutes.

Proline was an important solute accumulated under osmotic and salinity stresses, and was considered as an osmoticum or osmoprotectant (Vann et al. 1993, Naidoo 1994, Naidu 2003). In Fig. 4, it showed that proline content increased with increasing level of NaCl in both shoot and rhizome + root, and the NaCl-sensitive clones showed higher proline accumulation in shoot than those of NaCl-tolerant clones. However, the content of proline was very low as compared to other solutes. Thus, proline might not be adequate for osmotic adjustment in salt-stressed Zoysia. The results were similar to that reported in tomato (Botella et al. 1997), in which proline was considered as a possible salt-drought injury sensor (Pérez-Alfocea et al. 1993).

Marcum et al. (1998) and Qian et al. (2000) pointed out that salt injury of zoysiagrass was associated with their leaf length and texture. Short and fine leaf-textured Zoysiagrasses (Z. matrella and their hybrids) were most salt tolerant, and coarse leaf-textured entries (Z. japonica and their hybrids) were intermediate, while long and fine leaf-textured entries exhibited the least salt tolerance. However, the present study indicated that in both long (LRM, Z. sinica) and short (LCG, Z. matrella) leaves clones showed the same response in salt tolerance. Since both NaCl-sensitive clones are Z. matrella, the salt tolerant Zoysia clones native in Taiwan were not
associated with their leaf length but with rainfall and geology of their habitats (Weng and Chen 2001).

As a result, it was concluded that NaCl-tolerant *Zoysia* clones, both *Z. matrella* (short leaf) and *Z. sinica* (long leaf), maintained a lower Na\(^+\) content in shoot by salt gland secretion in the leaf and Na\(^+\) exclusion in shoots, and might be able to keep membrane integrity under NaCl treatment.

**REFERENCES**


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