Evaluation of low-temperature tolerance of zoysia grass

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Abstract

Low temperature is one of the main factors limiting the growth of zoysia grasses (*Zoysia* spp.). This study was conducted to assess the low-temperature tolerance of zoysia grasses, and especially to explore the low-temperature tolerance of different organs. The leaf LT_{50} (temperature at which 50% of leaves die) of zoysia grasses tested ranged from −1.9°C to −10.4°C (CV 27.8%). The LT_{50} of different species ranked as: *Z. japonica* (−6.68°C) < *Z. sinica* (−5.90°C) < *Z. matrella* (−5.35°C) < *Z. tenuifolia* (−3.5°C) < *Z. sinica* var. *longiflora* (−3.1°C) < *Z. macrostachya* (−2.7°C). The ranges in LT_{50} values were *Z. japonica* (−1.9°C to −10.4°C), *Z. sinica* (−3.0°C to −9.5°C) and *Z. matrella* (−2.8°C to −6.8°C). The average LT_{50} values were leaf (−6.9°C) > stolon (−7.8°C) > rhizome (−8.4°C). There was a significant correlation between LT_{50} values of different organs (P<0.05) suggesting that leaf LT_{50} could be used an indicator of low-temperature tolerance of zoysia grasses.

Introduction

Zoysia grasses (*Zoysia* spp.) are widely used warm season turf-grass species indigenous to the countries of the western Pacific Rim and are also environmentally friendly. They possess excellent tolerance of wear, drought, salinity and low fertility, are disease resistant and have low maintenance requirements. The native distribution of the recognised species in the genus extends from New Zealand to the island of Hokkaido in Japan, and from French Polynesia through Malaysia west to Mauritius (Anderson 2000). In eastern China, their natural distribution covers from 19°03′N to 41°02′N and 109°03′E to124°04′E.

Most accessions are adapted to warm climates and grow well in coastal areas. Low freezing tolerance is the primary limiting factor for distribution of zoysia grasses in the transitional regions and temperate regions. However, Beard (1973) stated that zoysia grass had greater freezing tolerance than other warm season turf grasses and Dunn (1999) demonstrated a wide range of cold tolerance among zoysia grass cultivars. In a study of low-temperature tolerance of 54 representative zoysia grass accessions in China by electrolyte leakage (EL), Li *et al*. (2003) found that the lethal temperature 50 of leaf (LT_{50}: temperature at which 50% of leaves die) of these accessions ranked as: *Z. japonica* < *Z. sinica* < *Z. matrella* < *Z. tenuifolia* < *Z. sinica* var. *longiflora* < *Z. macrostachya*. Photosynthetic enzyme activities of different *Zoysia* spp. and accessions declined when exposed to low temperature stress, with the degree of decline being different among species and populations. However, as the degree of decline was not associated with the climate of origin of the zoysia grasses, it was concluded that freezing tolerance could be determined by inheritance (Hong 1977; Okawara and Kaneko 1995, 1997; Matsuba *et al*. 1997).

Several methods have been used to assess the low-temperature tolerance of grasses. Gusta *et al*. (1980) demonstrated that the EL procedure was a simple and quick method for evaluating the low-temperature tolerance of cool season grasses. This was confirmed by Rajashekar *et al*. (1983) and Anderson *et al*. (1988), although the EL procedure was relatively labour-intensive and

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analysis of the results was difficult. Fry *et al*. (1993) proposed using re-growth after controlled environment freezing as an indicator of low-temperature tolerance. However, this procedure was even more time-consuming than the EL method and, in addition, experimental conditions in the field or greenhouse are not easily controlled.

Since leaves, rhizomes and stolons of warm season grasses experience different temperatures when a given environmental temperature occurs, examining the behaviour of a single vegetative organ might not provide an accurate estimate of the low-temperature tolerance of a grass overall. In the studies of low-temperature tolerance of limited zoysia grass accessions described above (Beard 1973; Dunn 1999; Li *et al*. 2003), only one vegetative organ such as leaf was studied.

In total, 160 native accessions have been collected by the Institute of Botany, Jiangsu Province and the Chinese Academy of Sciences during 1994–2006. However, a comprehensive evaluation of the low-temperature tolerance of the germplasm has not been conducted. The objective of this study was: (1) to assess the low-temperature tolerance of 5 species of zoysia grass including 97 accessions; and (2) to explore the relationships between low-temperature tolerances of different vegetative organs.

Materials and methods

Plant materials and culture

The 97 accessions (5 species and 1 variety) were selected from 160 accessions (Table 1). Most of the accessions were native to China with 9 cultivars introduced from USA and 2 accessions from Japan. Stolons were collected during 1995–2001 and planted in the experimental field of the Institute of Botany, Jiangsu Province and the Chinese Academy of Sciences (32°02′N, 118°28′E; elevation 30m). Plot size was 0.6 m × 0.6 m with plots separated by 50 cm borders to prevent the accessions from growing together. Annual average temperature of the region is 15.4°C, and average temperature and the absolute minimum temperature in January are 2.3°C and −13.0°C, respectively. Standard procedures were conducted including fertilisation and irrigation, with necessary stolon and rhizome clearance. Zoysia grass was mowed at 5 cm about 4 or 5 times per month. Only one accession was available for *Z. macrostachya*, *Z. tenuifolia* and *Z. sinica* var. *longiflora* owing to the limited material available.

Low-temperature tolerance of leaf evaluated by electrolyte leakage

The experiment was conducted in June–July 2004. Fresh healthy leaves (20 gm) were collected from the lawn turf, washed and rinsed with deionised water 3 times and dried on filter paper. The leaves were cut into 1–2 cm fragments, before being placed in glass tubes (25 mm × 200 mm). The glass tubes were placed in a refrigerator (Kelon, BCD-272/HCP, China) at 2°C for 12 h, then cooled in a freezing bath of polyethylene glycol in a programmable freezer (Polyscience 9610, Polysciences, Inc., U.S. Corporate Headquarters, Pennsylvania USA). The temperature was lowered at a rate of 4°C/h and the glass tubes were exposed to 5 low temperature levels (–2, −6, −10, −14 and −18°C) for 1.5 h. After removal from the freezer, the samples were thawed at 2°C for 12 h. The leaves from each glass tube were divided into 5 samples, and each sample was placed into a new tube, to which 20 mL of deionised water

Table1. Accessions tested.

Species	Number of accessions		Latitude[1]	Longitude[1]
	Native	Introduced		
Z. japonica	57	9	28°36′–41°10′	106°33′–123°00′
Z. sinica	21	1	20°05′–37°30′	116°22′–121°24′
Z. matrella	5	1	22°00′–32°06′	106°33′–120°12′
Z. macrostachya	1	0	30°01′	122°30′
Z. tenuifolia	1	0	29°32′	106°33′
Z. sinica var. *longiflora*	1	0	33°48′	120°17′
Total	86	11	20°05′–41°10′	106°33′–123°00′

[1] Refers to the collection site of the native accessions.

was added. The leaf samples were held at room temperature for 24 h and EL_1 was measured with a DOS-307 model conductance resistance meter (Shanghai REX Instruments, Shanghai, China). After EL_1 measurements were completed, all samples were boiled for 10 min and EL_2 measured after the tubes had cooled down to room temperature. Freeze damage for each leaf sample was calculated as relative conductivity (%) = (EL_1/EL_2) × 100. The EL_1 and EL_2 values were the means of the 5 samples.

Low-temperature tolerance of stolons and rhizomes evaluated by survival rate

This test was conducted in September 2004 and repeated in January 2007 according to the procedure of Dunn (1999). Based on data from the testing of leaves for low-temperature tolerance, 15 representative accessions were selected to evaluate the low-temperature tolerance of stolons and rhizomes. Rhizomes and stolons with 3 nodes were used for these tests with 4 replications.

The rhizome and stolon samples were held in a refrigerator at 2°C for 12 h, then cooled in a programmable freezer, with temperature lowered at the rate of 4°C/h. The rhizomes and stolons were exposed to 5 low temperature levels (–2, –6, –10, –14 and –18°C) for 1.5 h. After removal from the freezer, samples were thawed in a refrigerator at 2°C for 12 h. Control samples were held at 2°C during the freezing test. The thawed rhizomes and stolons were planted in plastic pots (diameter 23 cm, height 28 cm) filled with 90% sand and 10% compound fertiliser (15:15:15; N:P:K) with 4 replications. The pots were placed in the field, irrigated as necessary and survival rate was observed after 6 weeks. The exposed temperature resulting in 50% rhizome or stolon survival was regarded as the LT_{50}.

Data analysis

The data were analysed using the methods of Mo (1983) and Zhu and Zhu (1984). The relative conductivity was used to fit a Logistic curve, and LT_{50} was calculated according to the Logistic formula. The SPSS 10.0 (Yu and He 2003) was used to predict LT_{50} and analyse variance.

Results

Low-temperature tolerance of leaves

The LT_{50} values for different *Zoysia* spp. ranged from –1.9°C to –10.4°C with an average LT_{50} of –6.4±1.77°C (Table 2).

The rankings of low-temperature tolerance of different species were: *Z. japonica* > *Z. sinica* > *Z. matrella* > *Z. tenuifolia* > *Z. sinica* var. *longiflora* > *Z. macrostachya*, with the leaf LT_{50} of *Z. japonica*, *Z. sinica* and *Z. matrella* being significantly lower than that of *Z. tenuifolia*, *Z. sinica* var. *longiflora* and *Z. macrostachya*. Moreover, grasses selected from seashore regions, *Z. macrostachya* and *Z. sinica* var. *longiflora*, were less tolerant of low temperature than the other species tested.

Variation within Z. japonica. The LT_{50} of accessions of *Z. japonica* covered the full range for the genus (Table 2). Of the 66 accessions tested, 4 accessions [Z141, Z020, Z108 and Z136 (J-36)] had LT_{50} lower than –9.0°C, while more than half of the accessions had LT_{50} values between –6°C and –8°C. LT_{50} of Z125 (Palisades) and Z104

Table 2. Leaf LT_{50}[1] of *Zoysia* spp.

Species	Range (°C)	Average (°C)	Coefficient of variation (%)
Zoysia spp.	–1.9 to –10.4	–6.47 ±1.77	27.8
Z. japonica	–1.9 to –10.4	–6.9 ±1.69 b[2]	25.3
Z. sinica	–3 to –9.5	–5.9 ±1.70 b	28.9
Z. matrella	–2.8 to –6.8	–5.4 ±1.72 b	32.2
Z. macrostachya	–2.7	–2.7 a	
Z. tenuifolia	–3.5	–3.5 a	
Z. sinica var. *longiflora*	–3.1	–3.1 a	

[1] LT_{50} = lethal temperature 50, the temperature at which 50% of leaves died.
[2] Within columns' values followed by the same letter do not differ significantly (P>0.05).

was −2.2°C and −1.9°C, respectively, which was higher than that of the other accessions.

Variation within Z. sinica. The LT_{50} of accessions of *Z. sinica* varied from −3.0°C to −9.5°C (mean 5.9 ± 1.70; Table 2). Seventeen of the 22 accessions had LT_{50} between −5°C and −8°C (Table 2).

Variation within Z. matrella. The LT_{50} of *Z. matrella* varied from −2.8°C to −6.8°C (mean 5.4 ± 1.72; Table 2). Four accessions fell between −5.8°C and −6.8°C, while Z123 and Z075 exhibited a poorer low-temperature tolerance at −2.8°C and −3.6°C, respectively.

Low-temperature tolerance of rhizomes and stolons

Survival rate of representative accessions. Accessions differed significantly in their survival rates as measured by stolon and rhizome regrowth in 2004. Survival rate of stolons differed significantly from that of rhizomes of the same accession at the same low temperature exposure (Table 3).

The survival rates of stolons and rhizomes of representative groups of accessions following exposure to low temperatures tended to follow the same pattern as leaf tolerance of low temperatures. In general, accessions with low leaf tolerance had low stolon and rhizome survival, while accessions with good leaf tolerance had good stolon and rhizome survival (Table 4).

Table 3. Statistical analysis of the survival rate of stolons and rhizomes of zoysia grass representative accessions in 2004.

Source	df	Mean Square	F
organ	1	2129.773**	22.163
accession	14	3246.995**	33.789
temperature	5	207685.747**	2161.205
organ × accession	14	1113.066**	11.583
organ × temperature	5	530.105**	5.516
accession × temperature	70	1484.928**	15.452
organ × accession × temperature	70	473.389**	4.926
Error	540	96.097	

Relative LT_{50} of stolons and rhizomes in September 2004 and January 2007. LT_{50} of stolons and rhizomes of all accessions declined from September 2004 to January 2007. For accessions with poor leaf low-temperature tolerance, LT_{50} of stolons decreased from (−5.7 to −7.1°C) to (−8.5 to −12.0°C), and for rhizomes from (−5.3 to −7.6°C) to (−7.9 to −11.7°C); for accessions with intermediate leaf low-temperature tolerance, LT_{50} of stolons decreased from (−6.3 to −10.3°C) to (−11.0 to −15.6°C), and for rhizomes from (−7.3 to −12.3°C) to (−11.2 to −16.7°C); comparable values for accessions with good leaf low-

Table 4. The survival rate and LT_{50}[1] of stolons and rhizomes of zoysia grass representative accessions.

LT_{50} of leaf		Temperature (°C)													
		Stolon							Rhizome						
	Accession	Control	−2	−6	−10	−14	−18	LT_{50}	Control	−2	−6	−10	−14	−18	LT_{50}
Poor	Z125 (Palisades)	100	88.9	33.3	0	0	0	−5.7	100	79.0	63.5	0	0	0	−6.3
	Z123 (Diammond)	100	88.9	44.4	38.5	0	0	−7.1	100	100	78.6	7.1	0	0	−7.6
	Z114	100	100	45.5	0.	0	0	−6.5	100	100	14.3	0	0	0	−5.3
	Z160	100	77.8	66.7	22.2	0	0	−7.0	100	92.9	61.5	11.1	0	0	−7.4
	Z075	100	80.0	30.0	10.0	0	0	−5.5	100	76.9	45.5	45.5	0	0	−6.4
Intermediate	Z091	100	100	100	80.0	0	0	−10.3	100	63.6	64.2	42.9	0	0	−7.3
	Z137 (J-37)	100	73.33	66.7	60.0	26.7	0	−9.0	100	71.4	92.3	76.9	46.2	0	−12.3
	Z132	100	62.5	50.0	37.5	0	0	−6.3	100	100	100	58.3	0	0	−9.6
	Z102	100	77.78	55.6	22.2	0	0	−6.7	100	100	84.6	69.2	0	0	−9.5
	Z045	100	62.5	62.5	25.0	0	0	−6.4	100	66.7	100	33.3	0	0	−8.0
Good	Z145 (Meyer)	100	100	76.9	100	0	0	−10.5	100	76.9	83.3	76.9	0	0	−10.6
	Z136 (J-36)	100	100	91.7	66.7	25.0	0	−11.6	100	84.6	100	76.9	30.8	0	−6.9
	Z021	100	85.6	53.8	23.1	7.7	0	−8.3	100	88.2	64.7	41.2	17.7	0	−7.5
	Z020	100	85.7	85.7	50.0	14.3	0	−9.1	100	84.1	76.5	11.8	11.8	0	−7.5
	Z141	100	100	100	66.7	0	0	−9.9	10	100	93.3	86.7	0	0	−10.3

[1] LT_{50} = lethal temperature 50, the temperature at which 50% of stolons or rhizomes died.

temperature tolerance were: stolons decreased from (−8.3 to −11.6°C) to (−15.3 to −16.2°C), and rhizomes from (−6.9 to −10.6°C) to (−14.8 to −17.7°C).

The relationship between LT_{50} of stolons in September 2004 and LT_{50} of stolons in January 2007 had an R^2 value of 0.373; the comparable value for rhizomes was 0.339 ($P \leq 0.05$, Table 5).

Table 5. Relationship between LT_{50}[1] of stolons and rhizomes of zoysia grass in different seasons (January 2007 and September 2004).

Regression equation[2]	R^2 value	$R^2_{0.05}$
$Y_1=-5.01616+1.05539X_1$	0.373*	0.332
$Y_2=-4.25898+1.10978X_2$	0.339*	0.332

[1] LT_{50} = lethal temperature 50, temperature at which 50% of stolons or rhizomes died.
[2] Y_1 = LT_{50} of stolons in Jan 2007; X_1 = LT_{50} of stolons in Sep 2004; Y_2 = LT_{50} of rhizomes in Jan 2007; X_2 = LT_{50} of rhizomes in Sep 2004.

Low-temperature tolerance in different vegetative organs

The average leaf LT_{50} of tested accessions was −6.9°C, which was higher than that of stolons (−7.8°C) and rhizomes (−8.4°C). In addition, the range of LT_{50} for stolons and rhizomes was narrower than for leaf (Table 6).

The R^2 value for the relationship between LT_{50} of stolons and leaves was 0.58, between rhizomes and leaves was 0.67 and between rhizomes and stolons was 0.55 ($P \leq 0.05$, Table 7).

Table 7. Relationship between LT_{50}[1] of different vegetative organs of *Zoysia* spp.

Regression equation	R^2 value	$R^2_{0.05}$
$Y_1^{2}=-5.35556+0.360239X^{3}$	0.58*`	0.514
$Y_2^{4}=-5.354365+0.4500438X$	0.67**	0.514
$Y_2=-3.855405+0.5852675Y_1$	0.55*	0.514

[1] LT_{50} = lethal temperature 50, temperature at which 50% of stolons or rhizomes died.
[2] LT_{50} of stolons.
[3] LT_{50} of leaves.
[4] LT_{50} of rhizomes.

Discussion

The results of this study confirmed earlier findings that there was considerable variation among *Zoysia* spp. in terms of low-temperature tolerance. Li *et al*. (2003) reported that the LT_{50} of *Zoysia* spp. was ranked as follows: *Z. japonica* (LT_{50} = −7.92°C) < *Z. sinica* (−6.75°C) < *Z. matrella* (−5.94°C) < *Z. tenuifolia* (−4.94°C) < *Z. longiflora* (−3.28°C) < *Z. macrostachya* (−2.72°C). While our study showed the same ranking of species for freezing tolerance, the

Table 6. LT_{50}[1] of different vegetative organs.

Low-temperature tolerance of leaf	Accessions	Leaf (°C)	Stolon (°C)	Rhizome (°C)
Poor	Z125 (Palisades)	−2.2	−5.7	−6.3
	Z123 (Diammond)	−2.8	−7.1	−7.6
	Z114	−3.0	−6.5	−5.3
	Z160	−3.5	−7.0	−7.4
	Z075	−3.6	−5.5	−6.4
Intermediate	Z091	−7.0	−10.3	−7.3
	Z137 (J-37)	−8.2	−9.0	−12.3
	Z132	−8.2	−6.3	−9.6
	Z102	−8.3	−6.7	−9.5
	Z045	−8.6	−6.4	−8.0
Good	Z145 (Meyer)	−8.8	−10.5	−10.6
	Z136 (J-36)	−9.1	−11.6	−6.9
	Z021	−9.5	−8.3	−7.5
	Z020	−10.0	−9.1	−7.5
	Z141	−10.4	−9.9	−10.3
Mean		−6.9	−7.8	−8.4
Range		−2.2 to −10.4	−5.5 to −10.6	−5.3 to −12.3

[1] LT_{50} = lethal temperature 50, temperature at which 50% of stolons or rhizomes died.

absolute LT_{50} values for all species were generally higher than those reported by Li *et al*. (2003). This might be a function of testing in a different month and different year.

Freezing tolerance of Diammond, Palisades, J-36 and Meyer in the study of Patton and Reicher (2007) was −8.4°C, −11.0°C, −10.8°C and −11.5°C, respectively, compared with LT_{50} for stolons of −7.1°C, –5.7°C, –11.6°C and –10.5°C, for the same cultivars in our study. This suggests poorer freezing tolerance than that reported by Patton and Reicher (2007), especially for Diammond and Palisades. However, freezing tolerance does not always reflect absolute winter hardiness (Anderson *et al*. 2002), which can be affected by additional environmental factors in the field such as snow cover, soil moisture and temperature fluctuations. In addition, in some cultivars, environmental conditions in the field may induce a greater level of adaptation to cold than that induced in the growth chamber (Anderson *et al*. 2002).

It was significant that LT_{50} of all tested accessions declined from September 2004 to January 2007, *i.e*., plants became more tolerant of cold temperatures with time, a result similar to that reported for Meyer zoysia grass (Rogers *et al*. 1975) and salt grass (Shahba *et al*. 2003). Beard (1966) was the first to report that tolerance of freezing stress by turf grass fluctuated throughout the autumn and winter, and suggested that tolerance of freezing stress by turf grass developed and was lost gradually, with peak hardiness occurring in early winter. The positive linear correlation between LT_{50} of accessions in September 2004 and January 2007 indicated that the LT_{50} of stolons and rhizomes soon after being introduced would be a satisfactory indicator of how plants would perform after growing in the area for some years.

Data on freezing tolerance of various organs were of interest, in that accessions with poor leaf low-temperature tolerance showed better tolerance of cold in stolons and rhizomes. By comparison, accessions with intermediate and good low-temperature tolerance in leaves showed similar cold tolerance in stolons and rhizomes. It was different from the report in Fylking Kentucky bluegrass (Gusta *et al*. 1980) and Bermuda grass (Dunn and Nelson 1974). Gusta *et al*. (1980) reported that leaves (LT_{50} = –40°C) had the greatest freezing stress tolerance during midwinter, followed by crowns (LT_{50} = −28°C) and roots and rhizomes (LT_{50} = −20°C) in Fylking Kentucky, and Dunn and Nelson (1974) found Bermuda grass rhizomes to be less tolerant of freezing stress than stolons. In our study, LT_{50} of stolons of Meyer zoysia grass was −10.5°C, and that of rhizomes was −10.6°C, a result similar to that reported by Rogers *et al*. (1975).

The low-temperature tolerance of species and accessions located in seashore regions was poorer than that of accessions from other regions. This is a reflection of natural selection processes. Since the annual variations in temperature and humidity in these regions are much smaller than from other regions, accessions which developed in these coastal areas did not need to develop characteristics which allowed them to survive very low temperatures.

Several of the commercial cultivars included in our study were less tolerant of low temperatures than some native accessions. In a different study, we found that some native accessions with good tolerance of low temperatures also had good turf quality, suggesting that these native accessions represent an excellent source of breeding material for developing new cultivars.

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