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Suzhi 5: A New Hybrid Zoysiagrass Cultivar with High Shoot Density and Fine Leaf Texture

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Zoysiagrass (*Zoysia* spp. Willd.) is a warm-season, perennial C4 turfgrass native to certain regions of China, Japan, and Korea. There are 11 known species and variations of zoysiagrass (Loch 2015) that are extensively used in the transition and warm climatic regions of the United States (Chandra et al. 2020; Sladek et al. 2011). Zoysiagrass is known for its stiff leaves, deep rhizome system, and bicellular salt glands, which contribute to its wide adaptability (Turgeon 2008). Zoysiagrass exhibits remarkable tolerance to traffic, cold temperatures (Kauffman 2010; Patton and Reicher 2007), drought conditions (White et al. 2001), and salinity (Uddin et al. 2011), and it has low fertility requirements (Briscoe et al. 2012). As a result, zoysiagrass is commonly used on home lawns, athletic fields, golf courses, recreational areas, and ecological restoration projects. However, zoysiagrass also has some limitations. For instance, *Zoysia japonica* and *Zoysia sinica* have relatively fast establishment rates and cold tolerance, but they generally have a grayish-green leaf color, coarse texture, low density, poor elasticity, and a short green period. Conversely, *Zoysia tenuifolia* and *Zoysia matrella* have a fine texture, attractive coloration, and longer green periods; nevertheless, these species have limited cold tolerance, slow establishment rates, thick thatch layers, and poor uniformity, and they are predominantly used for ornamental and recreational lawns.

China is one of the regions with the richest germplasm resources of zoysiagrass worldwide. However, there are only a few newly cultivated cultivars of zoysiagrass in China, such as Qingdao (*Zoysia japonica* Steud.), Lanyin 3 (*Zoysia japonica* Steud), Liaodong (*Zoysia japonica* Steud), Huanan (*Zoysia tenuifolia* Willd.), and Shanghai (*Zoysia japonica* Steud). These cultivars were selected through systematic breeding or introduction. Compared with systematic breeding and introduction, hybrid breeding is more advantageous for integrating the desirable traits of both parents and shortening the time required to breed excellent cultivars. It is a highly effective means of breeding superior hybrid offspring.

Since 1993, we have collected more than 230 germplasm resources of zoysiagrass, including six species and one cultivar. Preliminary evaluations of their genetic diversity (Guo et al. 2008, 2009), morphological variations (Li et al. 2002a; Xuan and Liu 2007), morphological types (Li et al. 2002b), phenological periods (Xuan et al. 2008), seed setting characteristics (Liu et al. 2003), and stress resistance (Hu et al. 2014; Li et al. 2012; Liu et al. 2005; Xuan et al. 2009) have been conducted. The results indicated that an abundance of genetic variation is available in Chinese germplasm. Therefore, cross-breeding of zoysiagrass was conducted to obtain hybrid offspring. Among them, the hybrid zoysiagrass 'Suzhi 1' obtained from the cross-breeding of *Zoysia japonica* and *Zoysia tenuifolia* has been authorized by the Grass Cultivar Registration Committee of China in 2010 (cultivar registration no. 410). 'Suzhi 1' exhibits several excellent characteristics, including a long green period, soft texture, and fast growth. However, the nodes on the shoots are relatively high, resulting in a lack of tolerance to low mowing heights. The objective of the research was to develop a new cultivar with high turf quality (high shoot density, fine and soft texture, dwarf growth habit) with a long green period, tolerance to cold, drought, diseases, and pests, low mowing heights, and low maintenance requirements.

Materials and Methods

Origins. In 2004, pairwise cross-pollination was performed between Suzhi 1 zoysiagrass (♀; no. Z31-3) and Diamond (♂; no. Z123) to combine the long green period, soft leaf texture, and rapid growth of Suzhi 1 with the low mowing height tolerance of Diamond, which is a *Z. matrella* cultivar. A total of 12 seeds were obtained. In May 2005, the hybrid seeds were sown in pots that were assigned numbers Z0413-1 to Z0413-12. Eight hybrid offspring were obtained and transplanted to a nursery field in Apr 2007. Then, the authenticity of the hybrids between two parents were identified through morphological characteristics and molecular marker [such as sequence-related amplified polymorphism (SRAP)] methods. In 2007 and 2008, the cold resistance and green period of 'Suzhi 5' were evaluated and compared with those of its parents and commercial zoysiagrass 'Lanyin 3'. From 2009 to 2011, a cultivar comparison trial was conducted in Nanjing for 'Suzhi 5', its female parent 'Suzhi 1', and commercial zoysiagrass 'Lanyin 3' as the control. From 2015 to 2017, 'Suzhi 5' was entered in the national grass variety regional trial conducted at the following four test stations: Guangzhou, Nanjing, Jianyang, and Danzhou. The results showed that the hybrid zoysiagrass 'Suzhi 5' (no. 13-1) has high turf quality and good stress tolerance, such as high shoot density, deep green color, uniformity, prolonged green period, well-developed stolons, fine and flexible texture, and strong disease resistance. In 2018, the hybrid zoysiagrass 'Suzhi 5' was officially authorized and registered by the Grass Cultivar Registration Committee of China (cultivar registration no. 556).

Morphological comparisons. The authenticity of the hybrids between two parents were identified through morphological characteristics and molecular marker (such as SRAP) methods. The experiment was conducted at the Institute of Botany in Jiangsu Province and Chinese Academy of Sciences in Nanjing (32°05′N, 118°47′E, altitude 30 m). The soil type was sandy loam, with an organic matter content of 2.19%, total nitrogen content of 0.33%, available phosphorus content of 21.20 ppm, available potassium content of 237.40 ppm, and soil pH of 7.03. On 15 Apr 2007, eight hybrid offspring [named 13-1 ('Suzhi 5'), 13-2, 13-3, 13-4, 13-5, 13-6, 13-8, 13-12] and two parents were transplanted to the nursery with a plot area of 50 × 60 cm and randomized complete block with four replications. Plots were established with sprigs planted in a 1:4 ratio, followed by a light covering with topdressing sand; they were frequently watered through establishment. The first mowing was conducted when the turf cover reached 65% to 75%, with a cutting height of 3.0 cm. After the turf was established, routine mowing was performed at a height of 3 to 4 cm. During the vigorous growing season, mowing was performed approximately once per month. After the final mowing (approximately 1 month before the first frost), 15N–6.5P–12.5K fertilizer was applied at a rate of 150 kg/ha. The hybrid identification was determined based on six vegetative characteristics (shoot density, turf height, leaf length, leaf width, stolon length,

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Table 1. Vegetative traits of 'Suzhi 5' compared with those of its parents and other offsprings (planted Apr 2007) at Nanjing.

Genotypes	Shoot density[i] (tillers/cm^2)	Turf ht[ii] (cm)	Leaf length[iii] (cm)	Leaf width[iv] (cm)	Stolon length[v] (cm)	Stolon diam[vi] (cm)
'Suzhi 1'(♀)	4.13 a	11.10 cd	8.20 e	0.32 f	1.90 cd	0.12 d
Diamond (♂)	5.19 ab	3.29 a	3.01 a	0.12 a	1.15 a	0.09 a
'Suzhi 5' (13-1)	9.54 e	8.77 b	5.33 bc	0.14 b	1.85 c	0.10 ab
13-2	7.53 cd	10.64 c	6.24 cd	0.19 de	1.45 b	0.11 cd
13-3	5.87 b	9.50 bc	6.18 cd	0.17 c	2.12 cd	0.10 abc
13-4	6.16 bc	7.90 b	4.85 b	0.18 cd	1.93 cd	0.11 cd
13-5	7.85 d	12.56 d	6.96 de	0.21 e	2.44 e	0.11 cd
13-6	8.14 d	9.50 bc	6.45 cd	0.20 de	1.92 cd	0.09 ab
13-8	7.78 d	12.65 d	7.30 de	0.20 de	2.17 d	0.11 cd
13-12	6.64 bcd	9.31 bc	6.30 cd	0.18 cd	2.05 cd	0.11 bcd
Coefficient of variation (%)	15.99	23.33	24.56	15.87	19.27	11.4

[i] Shoot density refers to the number of tillers per 1 cm^2.
[ii] Turf height was measured using a ruler.
[iii] Leaf length refers to the length of the second fully expanded leaf.
[iv] Leaf width refers to the width at the middle of the second fully expanded leaf.
[v] Stolon length refers to the length of the third internode.
[vi] Stolon diameter refers to the diameter at the midpoint.
The lowercase letters following the data indicate significant differences ($P < 0.05$).

Table 2. Reproductive traits of 'Suzhi 5' and those of parents and other offsprings (planted Apr 2007) at Nanjing.

Genotypes	Seed head ht[i] (cm)	Inflorescence length[ii] (cm)	Inflorescence width[iii] (cm)	Spikelet length[iv] (cm)	Spikelet width[v] (cm)	Spikelets per spike[vi] (number)	Spikelet pedicel length[vii] (cm)
'Suzhi 1'(♀)	9.66 d	2.33 e	0.13 c	0.28 d	0.10 d	26.5 f	0.14 c
Diamond (♂)	1.73 a	0.62 a	0.07 a	0.21 a	0.05 a	7.70 a	0.06 a
'Suzhi 5'	4.75 c	1.62 c	0.13 bc	0.28 d	0.08 bc	16.60 de	0.11 b
13-2	/[viii]	/	/	0.24 b	0.07 b	/	0.10 b
13-3	4.66 d	1.86 d	0.12 bc	0.27 cd	0.07 b	17.70 e	0.13 c
13-4	3.13 b	1.19 b	0.11 b	0.24 b	0.09 d	11.40 b	0.10 b
13-5	5.58 c	1.31 b	0.12 bc	0.25 b	0.07 b	14.20 cd	0.10 b
13-6							
13-8	5.36 c	1.32 b	0.11 b	0.25 bc	0.07 b	12.50 bc	0.10 b
13-12							
Coefficient of variation (%)	30.65	21.56	16.27	9.41	14.72	24.32	19.08

[i] Seed head height refers to the natural distance from the ground to the top of the inflorescence.
[ii] Inflorescence length refers to the length of the floral inflorescence.
[iii] Inflorescence width refers to the central width of the floral inflorescence.
[iv] Spikelet length was measured using a vernier caliper for the length of spikelets in the middle of the inflorescence.
[v] Spikelet width was measured using a vernier caliper for the width at the widest part of the spikelets.
[vi] Spikelets per spike: the measurement of the number of spikelets per spike randomly selected.
[vii] Spikelet pedicel length: the measurement of the length of the pedicel of spikelets in the middle part of the inflorescence using a vernier caliper.
[viii] The symbol / represents a single value for the determination of morphological indicators of the offspring; therefore, it is not included in the analysis of variance because it is nonrepetitive.
No data represent the absence of inflorescence in the offspring; therefore, it is not included in the analysis of variance for reproductive trait indicators.
The lowercase letters following the data indicate the significant differences ($P < 0.05$).

Table 3. Primer sequences of 50 pairs of SRAP markers used in this study

Code	Forward primers	Code	Reverse primers
Me1	5′-TGAGTCCAAACCGGATA-3′	Em1	5′-GACTGCGTACGAATTCAA-3′
Me2	5′-TGAGTCCAAACCGGAGC-3′	Em2	5′-GACTGCGTACGAATTCTG-3′
Me3	5′-TGAGTCCAAACCGGACC-3′	Em3	5′-GACTGCGTACGAATTGAC-3′
Me4	5′-TGAGTCCAAACCGGACA-3′	Em4	5′-GACTGCGTACGAATTTGA-3′
Me5	5′-TGAGTCCAAACCGGTGC-3′	Em5	5′-GACTGCGTACGAATTAAC-3′
		Em6	5′-GACTGCGTACGAATTGCA-3′
		Em7	5′-GACTGCGTACGAATTGAG-3′
		Em8	5′-GACTGCGTACGAATTGCC-3′
		Em9	5′-GACTGCGTACGAATTTCA-3′
		Em10	5′-GACTGCGTACGAATTCAT-3′

SRAP = sequence-related amplified polymorphism.

stolon diameter) and seven reproductive traits (reproductive branch height, inflorescence length, inflorescence width, spikelet length, spikelet width, spikelets per spike, spikelet pedicel length) (Tables 1 and 2). The turf height was measured using a ruler. The shoot density was determined by the number of tillers within every 100 cm^2. This was repeated 10 times. The length of the second fully expanded leaf and the width at its widest point were measured; this was repeated 30 times. Healthy stolons were randomly selected, and the length of the third section and diameter at the midpoint were measured; this was repeated 30 times. After the zoysiagrass bloom,

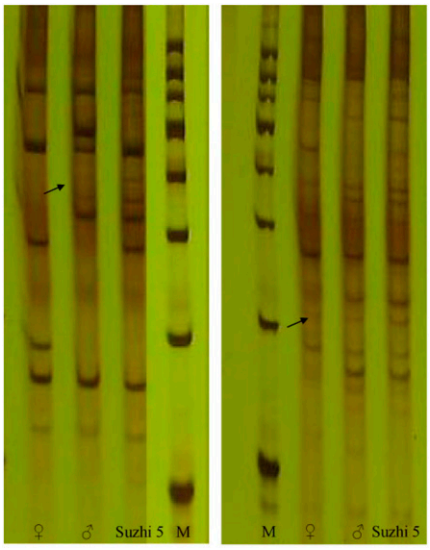

Fig. 1. Amplification map of sequence-related amplified polymorphism (SRAP) primers Me1Em1 and Me3Em1 for hybrid identification of 'Suzhi 5' at Nanjing in 2007. The arrow refers to the specific band possessed by the male parent for identifying true hybrids.

the natural height from the ground to the top of the inflorescence was measured using a ruler, and the basic length and midwidth of the inflorescence were also measured using a ruler; this was repeated 30 times. Upon the maturation of the inflorescence of zoysiagrass, mature inflorescences were randomly selected, and the length of the spikelets at the midsection and the width at the widest point of the spikelets were measured using a vernier caliper. The number of spikelets was counted, and the length of the spikelet pedicels at the midsection of the inflorescence was measured using a vernier caliper, with 30 repetitions. Variance analyses of the characteristics of eight hybrid offspring and two parents were conducted using IBM SPSS Statistics version 13.0 statistical software (Chicago, IL, USA).

Hybrid identification using SRAP molecular markers. In 2007, samples of 'Suzhi 5' and two parents, 'Suzhi 1' and 'Diamond', were collected from the nursery. Total DNA extraction was performed using the sodium dodecyl sulfate method (Rogers and Bendich 1989). Five forward primers and 10 reverse primers were designed for the SRAP analysis (Table 3). A total of 50 pairs of SRAP primers were generated and used to assess the hybrid authenticity of 'Suzhi 5'.

Cold resistance. In Oct 2007 and 2008, 6 g of leaf material of Suzhi 5, Suzhi 1, Diamond, and commercial zoysiagrass cultivar Lanyin 3 were taken from the nursery. The samples were subjected to a low-temperature treatment using a low-temperature circulator (model 9610; Polyscience, Niles, IL, USA) with temperature gradients of −2 °C, −6 °C, −10 °C, −14 °C, and −18 °C for 1.5 h, followed by a uniform cooling rate for 1 h after each treated temperature. After thawing, the treated samples were extracted at room temperature, and the conductivity and boiling conductivity were measured. The electrolyte leakage rate (%) was calculated using the following formula:

$$\text{Electrolyte leakage rate (\%)} = \text{freezing conductivity / boiling conductivity (100\%)} \quad [1]$$

A data analysis was performed using SPSS 13.0 software, and the conductivity was fitted to the following logistic equation:

$$[Y = K/(1 + ae - bx)] \quad [2]$$

to obtain the simulated curve. Finally, the LT_{50} value was calculated as follows:

$$(LT_{50} = \ln[(1/a)]/b) \quad [3]$$

based on the parameters "a" and "b" in the equation to evaluate the cold resistance. Additionally, the duration of the green period was also recorded. The green period referred to the days from the spring green-up stage to the withering stage. The present experiment refers to the natural green period without mowing, where the spring green-up stage refers to the date when the greenness of the early spring turf reaches 50%, and the withering stage refers to the date when 50% of the turfgrass leaves turn yellow (both determined by visual assessment). Variance analyses of the green period of 'Suzhi 5', 'Lanyin 3', and two parents were conducted using IBM SPSS Statistics version 13.0 statistical software.

Field trials. From 2009 to 2011, a cultivar comparison trial was conducted at the Hushu site of Nanjing (32°05′N, 118°47′E, elevation 30 m) for 'Suzhi 5', female parent 'Suzhi 1', and commercial zoysiagrass 'Lanyin 3'. The experiment followed a randomized complete block design with four replications, with each plot measuring 4 m^2 and spacing of 0.5 m between plots. The plots were established using the sprigs planted in a 1:4 ratio and frequently watered for establishment. When the plot coverage reached 80%, the first mowing was conducted at a height of 3.0 cm. Regular mowing at a height of 3 to 4 cm was performed after the plots were fully established. During the peak growing season, mowing should be performed approximately once per month. Approximately 1 month before the first frost, the final mowing occurred, followed by the application of 15N–6.5P–12.5K fertilizer at a rate of 150 kg/ha. The evaluated traits were turf quality (shoot density, genetic color, elasticity, uniformity, leaf width), adaptability and stress resistance (establishment rate, oversummer rate, overwinter rate, green period, drought resistance, heat resistance, cold resistance, disease resistance, insect resistance), and comprehensive turf quality score. The data collection followed the National Turfgrass Evaluation

Table 4. Logistic equation, LT_{50}, and green period of 'Suzhi 5' compared with those of its parents and commercial zoysiagrass 'Lanyin 3' at Nanjing in 2007–08.

Cultivar	2007			2008				Green period (d)
	Logistic equation	Correlation coefficient R^2	LT50 (°C)	Logistic equation	Correlation coefficient R^2	LT50 (°C)	Avg LT50 (°C)	
'Suzhi 5'	$Y = 96.02914 / (1 + 24.18983e^{-0.3138255X})$	0.9820**	−10.15	$Y = 92.44335 / (1 + 6.360636e^{-0.2860668X})$	0.9010*	−6.47	−8.31	263 a
'Suzhi 1' (♀)	$Y = 91.15368 / (1 + 58.01605e^{-0.5576185X})$	0.9813**	−7.28	$Y = 91.61168 / (1 + 4.992813e^{-0.2806135X})$	0.9594**	−5.73	−6.51	243 c
Diamond (♂)	$Y = 92.65169 / (1 + 7.118363e^{-0.4384257X})$	0.9905**	−4.48	$Y = 87.6778 / (1 + 6.925698e^{-0.2676755X})$	0.9766**	−7.23	−5.86	246 c
'Lanyin 3'	$Y = 85.6811 / (1 + 12.93864e^{-0.3821869X})$	0.9436*	−6.7	$Y = 92.04359 / (1 + 36.98621e^{-0.3825578AX})$	0.9828**	−7.21	−6.95	257 b

The lowercase letters following the data indicate the significant differences among cultivars in 2007–08 at Nanjing ($P < 0.05$).

Table 5. Turf quality, adaptability and stress resistance, and comprehensive turf quality score of 'Suzhi 5' compared with those of its female parent and commercial zoysiagrass 'Lanyin 3' at Nanjing in 2009–11.

Cultivar	Shoot density[i] (number/cm^2)	Uniformity[ii] (cm)	Elasticity[ii] (d)	Genetic color[ii] (d)	Leaf width[iii] (%)	Green period[iv] (%)	Establishment rate[v]	SPGC[vi]	WPGC[vii]	Drought resistance[ii]	Heat resistance[ii]	Cold resistance[ii]	Disease resistance[ii]	Insect resistance[ii]	Turf quality[ii]
'Suzhi 5'	9.15 a	8.60 a	8.10 a	7.40 a	0.14 c	262.33 a	100.00 a	100.00 a	100.00 a	8.50 a	9.00 a	9.00 a	8.50 a	9.00 a	8.33 a
'Suzhi 1'	4.13 b	8.50 a	7.73 b	7.13 b	0.32 b	260.33 a	93.00 b	100.00 a	100.00 a	8.47 a	9.00 a	9.00 a	8.40 ab	9.00 a	7.95 b
'Lanyin 3'	2.71 c	7.63 b	7.50 b	6.43 c	0.42 a	254.00 b	93.00 b	100.00 a	100.00 a	8.40 a	9.00 a	9.00 a	8.27 b	9.00 a	7.35 c

[i] Shoot density refers to the number of tillers per 1 cm^2 in 2009–11 at Nanjing.
[ii] Uniformity, elasticity, genetic color, drought resistance, heat resistance, cold resistance, disease resistance, insect resistance, and turf quality rating on scale of 1 to 9 (1 = poor and 9 = excellent) in 2009–11 at Nanjing.
[iii] Leaf width refers to the width of the second fully expanded leaf at the middle in 2009–11 at Nanjing.
[iv] Green period refers to the number of days of the annual green-up period and withering period in 2009–11 at Nanjing.
[v] Establishment rate: the turf was established using a ratio of 1:4 stolon during the growing season with the required number of days from establishment to 85% cover in 2009.
[vi] SPGC: four representative 50-cm × 50-cm plots were selected. The cover of each plot was measured before and after the summer period to calculate the oversummering rate in 2009–11 at Nanjing.
[vii] WPGC: four representative sample plots measuring 50 cm × 50 cm each were selected. By measuring the cover of these plots before and after overwintering, the overwintering rate could be statistically calculated in 2009–11 at Nanjing.
The lowercase letters following the data indicate the significant differences among cultivars in 2009–11 at Nanjing ($P < 0.05$).
SPGC = summer percent green cover; WPGC = winter percent green cover.

Table 6. Turf quality, adaptability and stress resistance, and comprehensive turf quality score of 'Suzhi 5' compared with those of its female parent 'Suzhi 1' and commercial zoysiagrass 'Lanyin 3' at four stations in 2015–17.

Regions	Cultivar	Shoot density[ii] (number/cm^2)	Uniformity[iii] (cm)	Genetic color[iii] (d)	Leaf width[iv] (d)	Green period[v] (%)	Establishment rate[vi] (%)	SPGC[vii]	WPGC[viii]	Disease resistance[iii]	Insect resistance[iii]	Turf quality[iii]
GZ[i]	'Suzhi 5'	5.37 a	6.90 a	7.05 b	0.18 c	365.00 a	101.00 b	99.74 a	100.00 a	8.93 a	8.13 a	7.19 b
	'Suzhi 1'	2.09 b	7.27 a	7.52 ab	0.31 b	365.00 a	113.00 a	98.94 a	100.00 a	8.97 a	8.53 a	7.54 ab
	'Lanyin 3'	2.12 b	7.80 a	7.88 a	0.37 a	365.00 a	92.00 c	98.98 a	100.00 a	8.90 a	8.54 a	7.75 a
NJ[i]	'Suzhi 5'	7.27 a	6.43 a	6.42 a	0.16 c	253.50 a	103.00 a	100.00 a	94.79 a	9.00 a	9.00 a	6.34 a
	'Suzhi 1'	3.30 b	6.77 a	6.45 a	0.28 b	254.50 a	99.00 b	100.00 a	92.50 a	9.00 a	9.00 a	6.24 a
	'Lanyin 3'	2.43 b	6.67 a	6.39 a	0.34 a	241.50 a	99.00 b	100.00 a	90.21 a	9.00 a	9.00 a	6.08 a
JY[i]	'Suzhi 5'	3.01 a	7.72 a	8.28 a	0.17 c	276.00 a	61.00 a	100.00 a	92.50 a	9.00 a	9.00 a	7.62 a
	'Suzhi 1'	1.64 b	7.72 a	7.75 a	0.30 b	264.50 a	52.00 b	100.00 a	97.00 a	9.00 a	9.00 a	7.49 a
	'Lanyin 3'	1.32 b	8.17 a	8.25 a	0.37 a	267.00 a	42.00 c	100.00 a	94.50 a	9.00 a	9.00 a	8.21 a
DZ[i]	'Suzhi 5'	4.80 a	7.85 a	8.43 a	0.20 b	365.00 a	55.00 b	100.00 a	100.00 a	9.00 a	9.00 a	7.98 a
	'Suzhi 1'	2.26 b	8.26 a	8.43 a	0.30 a	365.00 a	65.00 a	100.00 a	100.00 a	9.00 a	9.00 a	8.16 a
	'Lanyin 3'	1.78 b	8.18 a	8.51 a	0.35 a	365.00 a	45.00 c	100.00 a	100.00 a	9.00 a	9.00 a	8.27 a

[i] GZ refers to Guangzhou (23°13′N, 113°39′E), NJ refers to Nanjing (32°05′N, 118°47′E), JY refers to Jianyang (27°19′N, 118°8′E), and DZ refers to Danzhou (19°30′N, 109°30′E).
[ii] Shoot density refers to the number of tillers per 1 cm^2 at GZ, NJ, JY, and DZ in 2015–17.
[iii] Uniformity, elasticity, genetic color, disease resistance, insect resistance, and turf quality rating on scale of 1 to 9 (1 = poor and 9 = excellent) at GZ, NJ, JY, and DZ in 2015–17.
[iv] Leaf width refers to the width of the second fully expanded leaf at the middle at GZ, NJ, JY, and DZ in 2015–17.
[v] Green period refers to the number of days for the annual green-up period and withering period at GZ, NJ, JY, and DZ in 2015–17.
[vi] Establishment rate: the turf was established using a ratio of 1:4 stolon during the growing season with the required number of days from establishment to 85% cover at GZ, NJ, JY, and DZ in 2015.
[vii] SPGC: four representative 50-cm × 50-cm plots were selected. The cover of each plot was measured before and after the summer period to calculate the oversummering rate at GZ, NJ, JY, and DZ in 2015–17.
[viii] WPGC: four representative sample plots measuring 50 cm × 50 cm each were selected. By measuring the cover of these plots before and after overwintering, the overwintering rate can be statistically calculated at GZ, NJ, JY, and DZ in 2015–17.
The lowercase letters following the data indicate the significant differences among cultivars separately at GZ, NJ, JY, and DZ in 2015–17 ($P < 0.05$).
SPGC = summer percent green cover; WPGC = winter percent green cover.

Program guidelines (Morris and Shearman 1998) with slight modifications. The measurement methods for shoot density and leaf width were the same as "hybrid verification," and the green period was the same as "cold resistance." Uniformity, elasticity, genetic color, drought resistance, heat resistance, cold resistance, disease resistance (including cercospora leaf spot, powdery mildew, Fusarium wilt disease, dollar spot), insect resistance (including *spodoptera depravata*, fine breasted, oriental mole cricket, beet webworm), and turf quality were rated using the National Turfgrass Evaluation Program scale of 1 to 9. Four representative 50-cm × 50-cm plots were selected. The cover of each plot was measured before and after the summer period to calculate the summer percent green cover, and before and after overwintering to calculate the winter percent green cover.

From 2015 to 2017, the national grass variety regional test was conducted at Guangzhou (23°13′N, 113°39′E, elevation 41 m; loam), Nanjing (32°05′N, 118°47′E, elevation 30 m; loam), Jianyang (27°19′N, 118°8′E, elevation 160 m; red soil), and Danzhou (19°30′N, 109°30′E, elevation 149 m; latosol) experimental stations using commercial zoysiagrass Lanyin 3 and Suzhi 1 as control cultivars. A randomized complete block design was conducted with each 4-m^2 (2 m × 2 m) plot replicated four times. For planting, sprigs were harvested as 3.0 to 5.0 segments and sown on the surface of the seedbed with row spacing of 10 cm and covered with a 1-cm deep layer of soil. Weed control was performed using manual weeding, and timely irrigation was applied to ensure that the seedbed remained moist (sprigging rate, 0.3 kg–0.5 kg/m^2 of the stem). After establishment, appropriate irrigation was provided. During the peak growing season, the turf was mowed to a height of 3 to 4 cm based on the climatic characteristics of the experimental site and the turf growth rate. During the first year of planting, 15N–6.5P–12.5K fertilizer was applied as a base fertilizer at a rate of 15 g/m^2. During the spring green-up stage and autumn of each subsequent year, the 15N–6.5P–12.5K fertilizer was applied at a rate of 10 g/m^2. Additionally, urea was applied monthly during the growing season at a rate of 2.5 g/m^2. The evaluated traits included turf quality (shoot density, genetic color, uniformity, leaf width), adaptability and stress resistance (establishment rate, summer percent green cover, winter percent green cover, green period, disease resistance, insect resistance), and comprehensive turf quality score. The measurement methods of these traits was the same as that of the cultivar comparison trial. Variance analyses of the characteristics of 'Suzhi 5', 'Suzhi 1', and 'Lanyin 3' were conducted using IBM SPSS Statistics version 13.0 statistical software.

Results

Morphological comparisons. The results showed that morphological traits of the offspring 'Suzhi 5', 13-2, 13-5, and 13-8 were significantly different compared with those of the female parent 'Suzhi 1' and male parent 'Diamond', except for stolon length, stolon diameter, inflorescence width, and spikelet length of 'Suzhi 5', the turf height and stolon diameter of 13-2, the turf height, leaf length, stolon diameter, and inflorescence width of 13–5, and the turf height, leaf length, stolon length, and stolon diameter of 13-8 (Tables 1 and 2). Except for shoot density, turf height, stolon length, stolon diameter, seed head height, inflorescence width, spikelet length, and spikelet pedicel length, 13-3 showed significant differences in all other traits compared with those of the parents. Except for shoot density, stolon length, stolon diameter, and spikelet width, 13-4 was also significantly different from both parents. Additionally, 13-6 and 13-12 did not bloom and bear fruit; therefore, no reproductive traits were collected. The shoot density, leaf length, and leaf width of 13-6 and the leaf length and leaf width of 13-12 were significantly different from those of the parents. The number of shoots per cm^2 of Suzhi 5 was significantly higher than that of the parent cultivars Suzhi 1 and Diamond, as well as that of the other offsprings. The leaf width of 'Suzhi 5' was 0.14 cm, which was intermediate between the male and female parents but finer than that of the other offspring. A comparison of the characteristics of 'Suzhi 5' and parents showed that most of the characteristics of 'Suzhi 5' were between those of the two parents (Tables 1 and 2).

Hybrid identification using SRAP molecular markers. The results showed that there were two pairs of primers, Me1Em1 and Me3Em1, that could successfully identify 'Suzhi 5' as a true hybrid. The amplification profiles of these two pairs of primers showed specific bands in 'Suzhi 5' from the male parent 'Diamond' as compared with those from the female parent 'Suzhi 1' (Fig. 1).

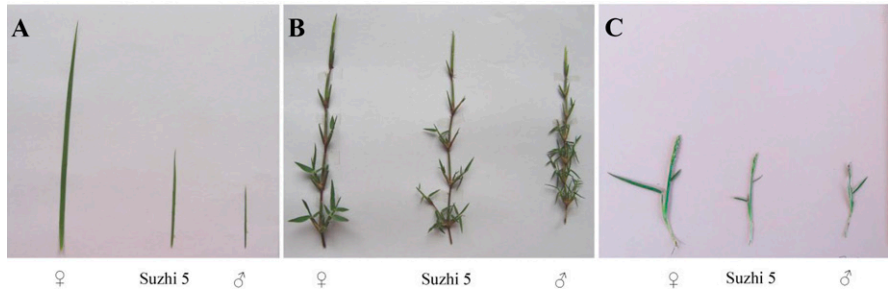

Fig. 2 Leaf (**A**), stolon (**B**), and inflorescence (**C**) of 'Suzhi 5' and its parents.

Cold resistance. The results revealed that the cold resistance (LT_{50}) of 'Suzhi 5' was −8.31 °C, which was significantly better than that of both parents and commercial zoysiagrass 'Lanyin 3'. Additionally, the green period of 'Suzhi 5' lasted 263 d, which was significantly longer than that of 'Suzhi 1', 'Diamond', and 'Lanyin 3' (Table 4).

Field trials. In the varietal comparison trial (Table 5), the shoot density of 'Suzhi 5' was 9.15 tillers per cm^2, which was significantly higher than the 4.13 tillers per cm^2 of the female parent 'Suzhi 1' and the 2.71 tillers per cm^2 of the commercial zoysiagrass 'Lanyin 3'. The leaf width of 'Suzhi 5' was 0.14 cm, which was significantly narrower than that of the female parent 'Suzhi 1' (0.32 cm) and the commercial zoysiagrass 'Lanyin 3' (0.42 cm).

In the national grass variety regional trial, 'Suzhi 5' exhibited excellent performance that was comparable to that of 'Suzhi 1' and 'Lanyin 3' across all testing locations. However, 'Suzhi 5' had greater shoot density and finer leaves than 'Lanyin 3' and 'Suzhi 1' at all locations (Table 6).

Conclusion

'Suzhi 5' is an interspecific hybrid of *Zoysia japonica* × *Zoysia tenuifolia* hybrid zoysiagrass 'Suzhi 1' (♀) and *Z. matrella* 'Diamond' (♂). The hybrid was verified by SRAP molecular markers. Morphological measurements from several trials indicate that 'Suzhi 5' has a fine leaf texture with high shoot density and many morphological traits that are intermediate between its parents such as turf height, leaf length, leaf width, stolon length, stolon diameter, seed head height, inflorescence length, spikelet width, spikelets per spike, and spikelet

Fig. 3 Whole plant (**A**), sward (**B**), and sod roll (**C**) of 'Suzhi 5'.

pedicel length (Figs. 2 and 3). The results showed that 'Suzhi 5' had excellent cold resistance, a long green period, and is comparable to other commercial cultivars in terms of resistance to drought, diseases, and insects. 'Suzhi 5' produces a high-quality turf surface and should be more tolerant to low mowing heights than 'Suzhi 1'. It is suitable for use on athletic fields, golf courses, and home lawns.

Availability

'Suzhi 5' is protected as a new cultivar (no. CNA20161921.2). During the protection period, the cultivar can only be used through the transfer of its rights. For specific transfer procedures and methods, please contact Dr. Hailin Guo, Institute of Botany, Jiangsu Province and Chinese Academy of Sciences (Nanjing Botanical Garden Memorial Sun Yat-Sen).

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