Kor. Turfgrass Sci., 11(4), 321~326, 1997

Characteristics Evaluation of a Zoysiagrass Line '232' in the Tissue Culture and Field

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종자 다수확계통 '232'의 조직배양 및 포장에서의 특성 평가**

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ABSTRACT

A high seed yield '232' was evaluated for morphological characteristics and tissue culture response. The effect of mowing and urea application on coverage in '232' was also studied and the results were as follows;

1. '232' produced high seed yield than any other lines, but its other characteristics such as coverage, establishment rate, stolon length, stolon number and colour change were not good.
2. Callus induction of '232'(52.9%) was relatively high, and higher than that of *Z. sinica*(49.0%). However, embryogenic callus formation of '232' was not as good as that of *Z. japonica* and *Z. sinica*.
3. Coverage of '232' was very much enhanced by mowing on July 18, but not at all by mowing on May 29 and September 4.
4. Urea application did not increase the coverage in '232'. Therefore, it can be used as a breeding material rather than direct use.

Key words: Zoysiagrass Line '232', Tissue culture, *Zoysia sinica*, *Zoysia japonica*.

INTRODUCTION

Zoysiagrasses are very well adapted across the country in Korea, Japan and China, and have many desirable traits such as tolerance to drought, heavy traffic, disease, insect and

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This paper was supported by the Academy of Life Science at the Kon-Kuk University(1996-1997).

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low maintenance requirement, etc. Although they have a good potential as the trufgrass for playgrounds, golf courses, parks, and sea coasts in the areas with extremely high and low temperature like the transition belt in USA (Beard, 1973; Turgeon 1991), their low seed yield limited their popularity due to the high construction cost. Therefore, one of the breeding targets for zoysiagrass is to increase the seed yield and to develop the seed propagated cultivars(Joo *et al.*, 1997). Recently, some zoysiagrass cultivars with seed propagation were put on the entry list of the National Evaluation Turfgrass Program (NETP) and tested in many locations of USA (National Turfgrass Evaluation Program, 1994).

It is important that zoysiagrass should have a large variation for breeding. Yu *et al.* (1974) and Hong *et al.*(1985) indicated that there was a large range of variation in zoysiagrass due to mutation, natural adaptation and natural interspecific hybridization. Kim *et al.*(1996) also showed that zoysiagrasses had a large variance of morphological characteristics. For the trait of seed yield, there were three lines with the high seed yield introduced from the Seoul National University. Among them, '232' showed the highest seed yield but its establishment and coverage was slow, and its quality was also poor. Mo (1997) reported that '232' was grouped with the lines of *Z. sinica* according to RAPD markers, and suggested that '232' was produced by the hybridization between *Zoysia sinica* and *Z. japonica*. In spite of its poor characteristics, '232' can be used very effectively for developing the cultivars with high seed yield.

The objectives of this study were to evaluate 1) morphological characteristics, 2) callus induction and embryogenic callus formation, and 3) effect of mowing and nitrogen fertilizer on coverage in '232'.

MATERIALS AND METHODS

1. Evaluation of morphological characteristics in '232'

Five zoysiagrass lines(*Zoysia japonica, Zoysia matrella, Zoysia koreana, Zoysia sinica* and a high seed yield line '232') introduced from the Seoul National University were planted in a field at the Kon-Kuk University in 1996.

Plant height, leaf width, first leaf sheath height, coverage, establishment rate, stolon length, stolon number, shoot density, color change, genetic leaf color and quality were measured on September 27, and color change was on October 26. Visual evaluation was done on a 1~9 scoring system with 9 being a perfect expression of coverage, establishment rate, stolon length and number, shoot density, color change, genetic leaf color, quality and seed yield.

2. Callus induction and embryogenic callus formation in '232'

Stolons of five zoysiagrass lines including '232' were collected from field and then washed in tap water. They were soaked in 70% ethanol for 3 min and then 0.1% $HgCl_2$ for

15 min with agitation. The stolons were then rinsed in five changes of sterile distilled water and cut into segments each containing one node. The disinfected stolon segments were placed in a petridish(60×15mm) containing MS medium with 2mg/l 2,4-D and 3% sucrose. The pH was adjusted to 5.8 with 1N NaOH and 1N HCl. Callus induction took place in dark at 27℃ five weeks later. Embryogenic callus formation took place under fluorescent light.

3. Effect of mowing frequency on coverage of '232'

This study was conducted to study the effect of mowing frequency on coverage of '232' from May 3 to October 15 1996. Sods of '232' were vegetatively propagated in 1 m×1 m plots from 10 cm diameter plugs. Plots were arranged in a randomized complete block design with three replications.

Mowing with 3 cm height was done on May 29, June 18 and September 4 at the frequency of none, once, twice or all three times. All plots received the equal fertilizer applications of 8 g · N/m² during growing season. Irrigation was non-limiting. Data was analysed by ANOVA and significant difference of means was determined at the 95% confidence level using Duncan's Multiple Range Test(DMRT).

4. Effect of urea on coverage of '232'

This experiment was conducted to study the effect of applying frequency and rate of urea fertilizer on coverage of '232'. Sods of '232' with 10 cm plug were vegetatively propagated in 1 m×1 m plots on May 3, 1996. Plots were arranged in a randomized complete block design with three replications.

Urea treatments were applied at the rate of none, 10 g/m² or 20 g/m² on August 7, or twice of 10 g/m² on August 7 and September 2. Data analysis was done the same as the above experiment.

RESULTS AND DISCUSSION

1. Evaluation of morphological characteristics in '232'

A high seed yield line '232' was compared with the other four zoysiagrass species for the characteristics such as plant height, leaf width, first leaf sheath height, coverage, seed yield, etc. Plant height of '232'(18 cm) was the lowest and leaf width(4 mm) was relatively low(Table 1). Mo(1997) grouped '232' with the sinica lines(S2, S3) from the RAPD marker analysis, and suggested that it is a hybridization between *Z. japonica* and *Z. sinica*. '232' produced the highest seed yield but it had several undesirable characteristics such as low quality and coverage. Therefore, the trait of high seed yield for '232' can be combined with the other desirable traits from the other lines in order to develop the seed propagated cultivars in zoysiagrass.

Table 1. Comparison of morphological characteristics of '232' with the other *Zoysia* species

Lines	Species	Characteristics*											
		①	②	③	④	⑤	⑥	⑦	⑧	⑨	⑩	⑪	⑫
232	–	18	4.0	1.7	1	5	6	6	8	2	6	7	9
Japonica	*Z. japonica*	35	4.8	2.8	5	5	8	7	6	2	8	5	5
Jeju	*Z. matrella*	26	3.7	3.7	3	6	7	6	6	7	8	8	4
Koreana	*Z. koreana*	33	4.0	5.9	5	7	7	8	8	7	8	8	5
S_5	*Z. sinica*	24	5.0	3.6	6	8	8	9	8	6	6	7	4

* ① Plant height(cm) ② Leaf width(mm)
③ First leaf sheath height(cm) ④ Coverage 1-9 scale, 9 = highest
⑤ Establishment rate 1-9 scale, 9 = fastest ⑥ Stolon length 1-9 scale, 9 = longest
⑦ Stolon number 1-9 scale, 9 = highest ⑧ Shoot density 1-9 scale, 9 = highest
⑨ Color change 1-9 scale, 9 = no change ⑩ Genetic leaf color 1-9 scale, 9 = dark green
⑪ Quality 1-9 scale, 9 = best ⑫ Seed yield 1-9 scale, 9 = many

2. Callus induction and embryogenic callus formation in '232'

Callus from the stolons was induced about five days after incubation without much difference among the five lines. The callus induction percentage of '232' (52.9%) was as high as that of the other lines. Callus growth and embryogenic callus formation of '232' was rated as good and normal, respectively, but not as good as *Z. japonica* and *Z. sinica* (Table 2). Kim(1998) found that the callus growth of *Z. japonica* and *Z. sinica* was generally better than that of *Z. matrella*.

Table 2. Callus induction and embryogenic callus(E.C.) formation of five zoysiagrass lines

Cell lines	Species	Callus induction(%)	Callus growth	E. C. formation
232	–	52.9%	+++*	++
Japonica	*Z. japonica*	59.2%	++++	++++
Jeju	*Z. matrella*	57.1%	+++	+++
Koreana	*Z. koreana*	46.7%	+++	++
S_5	*Z. sinica*	49.0%	++++	++++

* ++++ : very good, +++ : good, ++ : normal, + : poor

3. Effect of mowing frequency on coverage

The coverage '232' is very slow and this expeiment was conducted to find the effect of mowing on the coverage of '232'. Mowing on July 18 considerably enhanced the coverage of '232' while mowing on May 29 and September 4 did not seem to increase the coverage (Table 3). Madison (1960, 1962) also showed that mowing significantly increased the recuperative potential and vigor in bentgrass. Plant response to the increased frequency of mowing are different, but in most cases mowing resulted in prostrating growth habit (Leukel *et al.*, 1934). Mowing on September 4, the late growth stage of '232', gave poor influence on coverage of '232'. Excessive frequency of mowing broke leaf area balance and reduced shoot and root growth, chlorophyll content, and recuperative potential(Beard, 1973).

Table 3. Coverage(%) affected by mowing frequency in '232'

Mowing		Evaluation date					
Frequency	Date	June 8	June 21	July 11	Aug. 1	Aug. 23	Sept. 12
0	–	5.0 a	10.0 a	18.3 a	21.7 a	38.3 a	53.3 b
1	May 29	8.3 a	11.7 a	20.0 a	30.0 a	51.6 a	68.3 ab
2	May 29, July 18	5.0 a	8.3 a	16.7 a	35.0 a	58.3 a	80.0 a
3	May 29, July 18, Sept. 4	6.7 a	11.7 a	21.7 a	38.3 a	56.6 a	65.0 ab
	DMRT(0.05)	NS	NS	NS	NS	NS	*

* = Significantly different at 0.05 level
NS = Not significantly different.

Table 4. Coverage(%) affected by urea fertilizer in '232'

Fertilization application			Evaluation date				
Frequency	Date	Rate(g /m²)	Aug. 15	Aug. 23	Aug. 31	Sept. 12	Sept. 30
0		0	11.5 b	19.7 a	25.4 a	35.9 a	44.6 a
1	Aug. 7	10	16.0 a	23.3 a	29.0 a	40.1 a	46.1 a
1	Aug. 7	20	10.8 b	18.3 a	23.7 a	31.7 a	37.2 a
2	Aug. 7, Sept. 2	10+10	14.7 ab	22.0 a	26.7 a	34.6 a	42.4 a
	DMRT(0.05)	–	*	NS	NS	NS	NS

* = Significantly different at 0.05 level
NS = Not significantly different

4. Effect of nitrogen fertilizer on coverage

To the turfgrasses nitrogen fertilizer showed the effect on several aspects such as color, density, shoot and root growth, and recuperative ability (Hummel, 1980; Beard, 1973). This trial was conducted to evaluate the effect of applying frequency and fertilization rate on the coverage of '232'.

Application of urea fertilizer did not increase the coverage of '232'. This result was different from the report that urea application increased zoysiagrass coverage (Juska, 1959; Hubbell *et al.*, 1985). It seems that the establishment and propagation of '232' can not be enhanced by applying nitrogen fertilizer. Therefore, '232' should be genetically improved by breeding to be used for turfgrass.

적 요

한국잔디류중 종자 다수확 계통인 '232'를 품종 또는 육종 소재로 사용하기 위하여 형태적 특성을 조사하였으며, gene gun, agrobacterium, electroporation 등에 의한 형질전환을 위하여 '232'의 캘러스 유도 및 배발생 캘러스를 형성하였다. 또한 예취 및 질소시비가 '232'의 피복율에 미치는 영향을 분석하였으며 그 결과를 요약하면 다음과 같다.

1. '232'는 종자생산량이 많은 장점외에는 피복율, 활착율, 포복경 길이, 포복경수 등 대부분의 형질이 나쁘게 나타났다.

2. '232'의 캘러스 유기율은 52.9%로 zoysiagrass중 중간 정도로 양호하였으나 배발생 캘러스 형성율은 *Z. japonica*와 *Z. sinica* 보다 낮았다.
3. '232'의 피복속도는 매우 낮으나 7월 18일에 실시한 예취가 피복속도를 상당히 증가시켰으며, 반면에 5월 29일과 9월 4일에 실시한 예취는 큰 영향을 주지 않았다.
4. 질소시비는 '232'의 피복율 증가에 영향이 없는 것으로 나타났다. 그러므로 '232'는 직접 잔디로 이용되기 보다는 종자번식 품종을 위한 육종재료로 이용될 수 있을 것이다.

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