Development of Simple Sequence Repeat (SSR) Markers and Their Use to Assess Genetic Diversity in Apomictic Guineagrass (*Panicum Maximum* Jacq.)

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Introduction Guineagrass is an important and widely grown tropical forage grass. Despite its importance and increasing popularity, only little is known about its genetic diversity (Ebina *et al*., 2001). Such information is useful for the selection of diverse parents in breeding programmes. Moreover, no simple sequence repeat (SSR) markers have been reported in any apomixis species. In this study SSR markers were developed and used to investigate genetic diversity in germplasm of apomictic guineagrass.

Materials and methods For the development of SSR markers, genomic DNA was isolated from the cultivar 'Natukaze'. An enriched SSR library was developed according to Yamamoto *et al.* (2003). EST-derived SSR markers were also used in this study. The ESTs were derived from a cDNA library of immature flowers of guineagrass. The SSR amplification method was according to the protocol of the Maize Genome Database (http://www.maizegdb.org). SSR banding patterns were detected by an ABI 310 (Applied Biosystems). The phylogenetic analysis was conducted by Diversity Database software provided by Bio-RAD laboratories.

Results 14 primers from the enriched library and 21 primers from the EST-derived SSR primers were designed successfully. Among these, 20 primers indicated polymorphic banding patterns in the first 12 guineagrass accessions. Results for 13 out of the 20 SSR primers are summarized in Table 1. Allelic differences among 96 accessions for each of the SSR markers ranged from 6 to 39. Genotype variations ranged from 6 to 54 among the 96 accessions. Therefore, the PD (Power of Discrimination) values were also high ranging from 0.485 to 0.943. The phylogenetic analysis was carried out using data obtained from the same experiment as Table 1 (data not shown).

Table 1 Summaries of SSR primers, allelic divergences, genotype variations, Ho, He and PD

Primer name	sample no.	allele no.	genotype no.	size range	Ho	He	PD
GNK01-3	94	11	36	125-138	0.693	0.833	0.943
GNK01-4	96	6	12	144-173	0.142	0.418	0.576
GNK01-5	96	17	43	208-261	0.572	0.851	0.944
GNK02-2	92	39	54	140-228	0.710	0.942	0.947
GNK02-3	96	19	54	174-221	0.655	0.899	0.943
GNK02-4	95	20	49	166-193	0.685	0.934	0.939
GNK04-2	96	8	10	94-162	0.336	0.478	0.740
GNK05-1	96	15	35	99-140	0.387	0.738	0.888
GNK03-e2	96	9	21	153-178	0.718	0.739	0.769
GNK03-e4	96	13	29	88-110	0.731	0.780	0.903
GNK03-e19	96	17	53	135-171	0.657	0.905	0.905
GNK03-e23	96	13	27	135-171	0.395	0.830	0.924
GNK03-e47	96	7	6	145-159	0.185	0.289	0.485

[#] Ho; Observed Heterozygosity, He; Expected Heterozygosity, PD; Power of Discrimination.
[#] e contained in Primer name indicates EST-derived SSR marker.

Conclusions Guineagrass is a facultive apomictic species, however, a large amount of allelic and genotypic variation was observed in this study. The accessions, which were collected from all over native Guineagrass areas in east and south Africa, have wide genetic diversity. This indicates that sexual propagation has occurred and has resulted in considerable diversity in apomictic guineagrass in nature.

References

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