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Isolation of SSR markers from Zoysiagrass

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Introduction The genus *Zoysia* consists of 16 species that are naturally distributed on sea coasts and grasslands around the Pacific. In Japan, five species of natural zoysiagrasses have been identified from southern Hokkaido to the southwest islands. Of these, *Z. japonica* Steud. and *Z. matrella* Merr. have been utilized extensively as turf in Japan and other countries in East Asia. Linkage maps based on RFLP and AFLP markers have been reported in *Zoysia* (Yaneshita *et al*., 1999, Cai *et al*., 2004). Simple sequence repeat (SSR) markers have the advantages of being PCR-based and multiallelic. They are highly polymorphic compared to other types of markers such as RFLPs and AFLPs, and are widely used in linkage map construction, gene tagging and QTL mapping. However, only few SSR markers from zoysiagrass have been reported. The objectives of this study were to develop zoysiagrass SSRs in larger numbers and to map them on to an AFLP-based linkage map.

Materials and methods An individual zoysiagrass plant, F08 (progeny of *Z. japonica* X *Z. matrella*), was used to construct an SSR-enriched genomic library. The repeat motifs used were $(CA)_n$, $(GA)_n$, $(AAG)_n$, $(AAT)_n$. The methods of sequencing and primer design etc. were the same as those described by Cai *et al*. (2003). To screen the working primer pairs, a panel consisting of 8 varieties including both *Z. japonica* and *Z. matrella* species was used.

Results A total of 4,000 clones (1,000 clones from each of four libraries) were sequenced. Of these, 768 unique SSR clones which could be used to design primers were identified and about half of the unique SSR clones were from the B library (GA/CT motif) (Table 1). All of the four libraries contained perfect clones with high frequencies, ranging from 67.9 % to 96.0 % and the A and C libraries contained 27.9% and 11.6% compound clones, respectively (data not shown). So far it has been found that out of the 144 primer pairs tested using the screening panel, 132 primer pairs (91.7%) could amplify polymorphic SSR products and two primer pairs amplified PCR products but gave no polymorphism in the eight varieties used (Figure 1). The rest of the 10 primer pairs were considered to be unsuitable for use, because they amplified multi-copy products or amplified no bands.

Table 1 Efficacy of SSR isolation from *Zoysia* libraries

Library	Motif	Unique SSR	Perfect SSR(%)
A	CA/GT	140	95(67.9%)
B	GA/CT	408	371(90.9%)
C	AAG/TTC	125	120(96.0%)
D	AAT/TTA	95	74(77.9%)
Total		768	

Figure 1 PCR products amplified by the ZSSR17 locus in a screening panel including 8 *Zoysia* varieties

Future work After screening the working primers, we will map the markers in a segregating population derived from the selfed progenies of a *Z. japonica* clone, 'F02' (see Cai *et al*., 2004).

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