GENIC MICROSATELLITE MARKERS IN Spartina species (Poaceae)

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ABSTRACT

The genus Spartina represents a monophyletic lineage of subfamily Chloridoideae, where no member is known to be diploid and polyploidy has almost no role in its speciation. Simple sequence repeat (SSR) markers have been developed from a set of expressed sequence tags (ESTs) generated from salt-stressed Spartina alterniflora. Di- and tri-nucleotide repeats were most abundant. Out of 100 SSRs with 5 or more repeats, successful amplification was obtained in 81 cases in an initial screening with 8 S. alterniflora genomes and 18 SSRs were polymorphic. Fifteen SSRs were studied in detail to study the polymorphism and cross-transferability among 6 different species of Spartina: S. alterniflora, S. cynosuroides, S. patens, S. foliosa, S. pectinata, S. patens. Twelve primers showed amplification across all the species, while 100% cross-transferability was observed with at least one species. This suggested a high level of conservation of sequences in the transcribed region. Sequence analysis of a SSR locus coding for reversibly glycosylated polypeptide showed stretches of conserved sequence across different species of Spartina. The genic SSRs could be valuable to understand the pattern of evolution in Spartina. The genic SSRs were more useful for molecular breeding applications and diversity analysis of germplasm. 

RESULTS & DISCUSSION

A total of 100 SSR primers were designed from the SSR-ESTs with 5 or more repeats. An initial screening of the primers with 8 genotypes of Spartina alterniflora resulted in amplification by 81 eSSRs. Of these, 18 showed polymorphism (Fig. 1). The high level of polymorphism could be due to the multilocus nature of the loci in this polyploid grass. Twelve elite genotypes of Spartina alterniflora including CP9 (Vermilion), which is a released cultivar, were grouped into 3 distinct clusters (Fig. 2).

INTRODUCTION

Microsatellites or simple sequence repeats (SSRs) are considered as robust markers for construction of high density linkage maps, gene mapping, and marker-assisted selection in plants because of their simple and cost-effective deployment, reliability, co-dominance, multilocus nature, chromosome specificity, and amenability to high-throughput genotyping. However, SSR motifs may evolve too rapidly to be valuable as the sole assay for interspecific diversity analysis. In addition, markers shown to have tight genetic linkage to target genes in interspecies mapping populations may lose their selective power when used in backcross programs based on interspecific derivatives. Hence, the development and utilization of genic microsatellites (eSSR) would be more useful for molecular breeding applications and diversity analysis of germplasm.

Spartina alterniflora (smooth cordgrass), which grows along the Gulf and Atlantic coasts has unusually high level of salt tolerance. Its introduction and subsequent hybridization with indigenous species are of ecological and evolutionary importance. Further, because of high biomass yield and ability to grow in marginal soil, cordgrasses are now being considered as an alternative biomass yield and ability to grow in marginal soil, cordgrasses are now being considered as an alternative

MATERIALS & METHODS

Generation of EST, Identification of SSR-EST and primer design

A total of 495 unigenes genes from 1,227 ESTs of the expression libraries from root and leaf tissues of Spartina alterniflora were screened for SSR motifs. The SSR-ESTs with at least penta-nucleotide repeats or above were used for designing primers with Primer3@ software.

PCR amplification, sequencing, and data analysis

PCR Amplified products were resolved in 1 X TBE- 4 % SFR agarose gel. Cloning of the SSR products were done using pEASY-easy system (Promega Corp). sequenced and analyzed using ClustalX. The gel score data matrix was analyzed using NTSYS-PC v2.1.

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Fig. 1. A representative gel picture showing polymorphism within Spartina alterniflora among different genotypes (# 1 to 32) with eSSR112, M = 1 kb Plus molecular marker.

Fig. 2. Dendrogram of 12 genotypes of smooth cordgrass with 18 eSSR primers using UPGMA clustering method.

Fig. 3. A representative gel showing polymorphism among different species of Spartina using eSSR23, eSSR24 & eSSR25.

Fig. 4. A dendrogram showing the phylogenetic relationship among different genotypes of Spartina with 56 loci using UPGMA clustering method.

Fig. 5. Multiple alignment of the sequences showing sequence divergence at the repeat region of the gene Rgp1 using ClustalX.

Fig. 6. Cladogram showing the phylogenetic relationship among different Spartina species based on sequence similarity in Rgp1 locus.

Sequence analysis of eSSR24-generated fragments of a SSR locus (Rgp1) coding for reversibly glycosylated polypeptide showed stretches of conserved sequence across different species of Spartina. The polymorphism observed among different species was mainly due to the number of tandem repeats within the coding regions (Fig. 5). However, single nucleotide polymorphisms (SNPs) with indels were also observed in the coding regions of different species outside the repeat region. This single locus sequence-based phylogeny grouped S. alterniflora and S. foliosa in one group (Fig. 6), which showed cross-transferability for all six species (Fig. 3). This is indicative of the high level of sequence conservation in the transcribed region of the different cordgrasses. Cluster analysis with 10 eSSR primers (56 loci) resulted in 2 major groups (Fig. 4), where S. alterniflora (CP9) was clustered with S. foliosa along with S. pectinata and S. patens.

CONCLUSION

The genic SSRs could be valuable to understand the pattern of evolution in Spartina. Because of their origin from the transcribed region and cross-species transferability, the evolutionary significance of the number of repeats and SNPs could be useful in comparative genomics studies among the related species for genes of a particular biosynthetic pathway.

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