GENIC MICROSATELLITE MARKERS IN *Spartina species (Poaceae)*

NIRANJAN BAISAKH, ARNOLD PARCO, PRASANTA SUBUDHI

School of Plant, Environmental and Soil Sciences

Louisiana State University Agricultural Center, Baton Rouge, LA 70803, U.S.A.

ABSTRACT

The genus *Spartina* represents a monophyletic lineage of subfamily Chloridoideae, where no member is known to be diploid and polyploidy has played an important role in 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* genotypes 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. spartinae, 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* and length polymorphism among different species was mainly due to the number of tandem repeats within the coding region. However, single nucleotide polymorphisms (SNPs) were also observed in the sequences in the coding region of different species outside the repeat region. A single locus sequence-based phylogeny grouped *S. alterniflora* and *S. foliosa* in one group. The same was the result with the tree formed with 10 SSRs spanning 56 loci where *S. alterniflora* clustered with *S. foliosa* in one clad that also included *S. spartinae* and *S. pectinata*. The second clade consisted of *S. cynosuroides* and *S. patens*. Our study showed that the EST-derived SSR markers might be more useful in genetic diversity, evolutionary and comparative genomics studies in *Spartina* as opposed to genomic SSRs.

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, multiallelic 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 interspecific 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, cord grasses are now being considered as an alternative source of feedstock for bioenergy production. We used the eSSRs to study the genetic divergence within *S. alterniflora* and among different species of *Spartina.*

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 pGEMT- easy system (Promega Corp), sequenced and analyzed using ClustalX. The gel score data matrix was analyzed using NTSYS-PC v2.1.

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 alternflora* resulted in amplification by 81 eSSRs. Of these, 18 showed polymorphism (Fig. 1). The high level of polymorphism could be due to the multiallelic nature of the loci in this polyploid grass. Twelve elite genotypes of *Spartina alterniflora* including CP8 (Vermilion), which is a released cultivar, were grouped into 3 distinct clusters (Fig. 2).

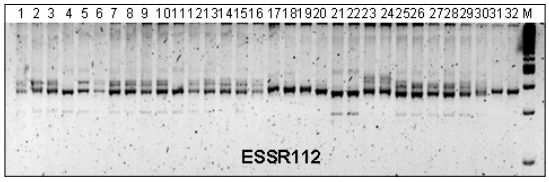

Fig. 1. A representative gel picture showing polymorphism within *Spartina alterniflora* among different genotypes (# 1 to 32) with eSSR112, M = 1 kbPlus molecular marker.

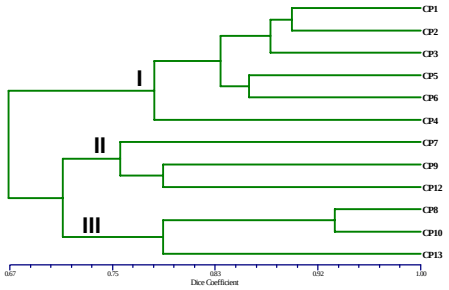

Fig. 2. Dendrogram of 12 genotypes of smooth cordgrass with 18 eSSR primers using UPGMA clustering method.

Cross-species transferability

At least one of the six species of *Spartina* such as *S. alterniflora* (smooth cordgrass), *S. cynosuroides* (big cordgrass), *S. spartinae* (gulf cordgrass), *S. foliosa (California cordgrass)*, *S. pectinata* (prairie cordgrass), *S. patens* (saltmeadow cordgrass) was amplified using the selected 15 primers. Twelve primers (80 %) 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* (CP8) was clustered with *S. foliosa* along with *S. pectinata* and *S. spartinae* .

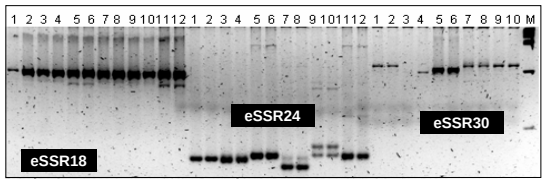

Fig. 3. A representative gel showing polymorphism among different species of *Spartina* using eSSR23, eSSR24 & eSSR25.
 # 1, 2 = *S. cynosuroides*; 3, 4 = *S. spartinae*; 5, 6 = *S. foliosa*; 7, 8 = *S. pectinata*; 9, 10 = *S. patens*; 11, 12 = *S. alterniflora*; M = 1 kbPlus molecular marker

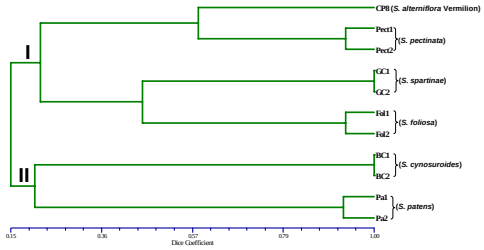

Fig. 4. A dendrogram showing the phylogenetic relationship based on 56 loci using UPGMA clustering method.

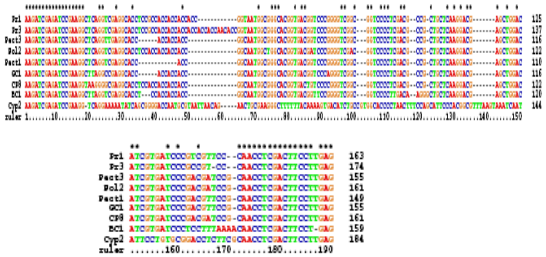

Fig. 5. Multiple alignment of the sequences showing sequence divergence at the repeat region of the gene *Rgp1* using ClustalX.

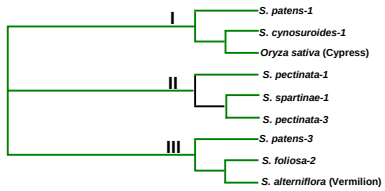

Fig. 6. Cladogram showing the phylogenetic relationship among different *Spartina* species based on sequence similarity in *Rgp1* locus.

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) as observed in Fig. 4. This indicates that the species that hybridize (*S. alterniflora* with *S. foliosa*) belong to the same lineage, but display different levels of genetic divergence. Further, polymorphism at the genotypic level may be different than at the genic level, which could be easily detected by the use of genic SSRs. Such inconsistencies between the gene trees indicated that reticulate events might be more frequent in *Spartina* species.

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.

ACKNOWLEDGMENT

This work was supported by a grant from the United States Department of Agriculture - Cooperative State Research, Education, and Extension Service (USDA-CSREES).

LSU AgCenter Research & Extension