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ORIGINAL PAPER

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An AFLP-based survey of genetic diversity among accessions of sea oats (*Uniola paniculata*, Poaceae) from the southeastern Atlantic and Gulf coast states of the United States

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Abstract *Uniola paniculata*, commonly known as sea oats, is a C_4 perennial grass capable of stabilizing sand dunes. It is most abundant along the Gulf of Mexico and southeastern Atlantic coastal regions of the United States. The species exhibits low seed set and low rates of germination and seedling emergence, and so extensive clonal reproduction is achieved through production of rhizomes, which may contribute to a decline in genetic diversity. To date, there has been no systematic assessment of genetic variability and population structure in naturally occurring stands in the USA. This study was conducted to assess the genetic relationship and diversity among nineteen *U. paniculata* accessions representing eight states: Texas, Louisiana, Mississippi, Alabama, Florida, South Carolina, North Carolina, and Virginia, using amplified fragment length polymorphism (AFLP). Twelve AFLP *Eco*RI + *Mse*I primer combinations generated a wide range of polymorphisms (42–81%) with a mean of 59%. Overall, the sea oats plants exhibited a low range of genetic similarity. Florida accessions, FL-33 and FL-39, were most genetically diverse and the accessions from both Carolinas and Virginia (NC-1, NC-11, SC-15, and VA-53) harbored less genetic variability. Cluster analysis using the UPGMA approach separated *U. paniculata* plants into four major clusters which were also confirmed by principal coordinate analysis (PCO). Further examination of the different components of genetic variation by analysis of molecular variance (AMOVA) indicated the largest proportion of variability at the state level (47.8%) followed by the variation due to the differences among the genotypes within an accession (34.4%), and the differences among the accessions within a state (17.8%). The relationship between genetic diversity and geographic source of sea oats populations of the United States as revealed through this comprehensive study will be helpful to resource managers and commercial nurseries in identifying suitable plant materials for restoration of new areas without compromising the adaptation and genetic diversity.

Keywords Amplified fragment length polymorphism · Dune stabilizing grass · Gene flow · Genetic variation · Population structure · Sea oats · *Uniola paniculata*

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Introduction

Sea oats (*Uniola paniculata* L.) is a semi-tropical, perennial dune grass capable of thriving in the unstable and nutrient deficient environments of barrier islands and beaches that are exposed to regular salt spray. It is one of the most effective native sand-binding grasses and is an excellent dune builder (Wagner 1964; Dahl and Woodard 1977), being found in native states along the southeast Atlantic and Gulf coast states where it is increasingly utilized for the restoration of coastal areas damaged by tropical storms and erosion (Bachman and Whitwell 1995). It produces a well-developed root and rhizome system that spreads locally through vegetative reproduction. This extensive rhizome system helps stabilize sand dune formation while the stem acts as a windbreak (Wagner 1964; Westra and Loomis 1966; Harper and Seneca 1974). Nevertheless, the majority of new environments are colonized through the dispersal of seeds (Hester and Mendelssohn 1987).

Sea oats is a cross-pollinating species and cross pollination is effected by wind, but the seeds are dispersed by both wind and water (Harper and Seneca 1974; Hester and Mendelssohn 1987). Existence of distinct differences for many morphological attributes in native sea oats populations of the United States has been documented. A study on flowering by Harper and Seneca (1974) revealed early flowering in populations from more southerly Atlantic and Gulf coast regions compared to those from North Carolina coast. Seed production in sea oats tends to decrease with decreasing latitude (Hester and Mendelssohn 1987). Sea oats from Bogue Bank, North Carolina produced an average of 2.24 seeds per spikelet, whereas only 0.6 seeds per spikelet were produced by sea oats in southern Florida. Sea oats populations found on the Louisiana coast had very low seed set (0–9.53 seeds per culm). There is difference in germination and seedling response among sea oats populations (Seneca 1972). It was noted that sea oats populations from Virginia and North Carolina required cold treatment for germination and seedlings showed homogenous potential for vegetative growth but the Florida populations have low potential for vegetative growth with requirement of cold treatment. Gulf coast populations exhibited intermediate germination response and produced more biomass. These observations suggest clear differences among sea oats populations from different regions in its native range.

Recently, the amplified fragment length polymorphism (AFLP) technique (Vos et al. 1995) has been routinely used to analyze genetic diversity and population structure in a wide range of plant species (Ude et al. 2003; Chen et al. 2004). It eliminates the limitations and drawbacks associated with several DNA fingerprinting techniques, such as restriction fragment length polymorphism, random amplified polymorphic DNA, sequence-tagged sites, etc. This technique is a high resolution PCR-based molecular marker assay with high reproducibility with no requirement for prior sequence information of the genome being studied. It is capable of generating a large number of reproducible loci with genome wide distribution (Zhu et al. 1998). To date, no DNA-based marker has been used to assess the genetic diversity among the native sea oats populations of the United States. One recent study (Franks et al. 2004) used only 27 allozyme loci to examine the geographic and fine scale population structure in this species. Their results revealed a weak correlation between genetic distance and geographic distance, and genetic diversity was uniform across its range but lower in populations from the lower Gulf of Mexico.

The information on the distribution of genetic variation over the scale of large geographical regions as well as local populations is crucial to our understanding of the ecological adaptation of any particular species. While sea oats present many challenges: poor seed set, low rate of germination, poor emergence, and slow establishment, it remains the plant of choice for dune restoration. A thorough understanding of the relationship between genetic diversity and geographic source of sea oats populations may be useful to the resource managers and commercial nurseries, aiding in their selection of suitable plant materials for restoration of damaged dunes as well as new areas without compromising the levels of adaptation and genetic diversity. The objective of this study was to conduct a survey of genetic diversity and genetic relatedness among native *U. paniculata* accessions collected from throughout its geographic range in the United States to aid in devising successful conservation and restoration strategies.

Materials and methods

Accessions and collection sites

Eight states (Texas, Louisiana, Mississippi, Alabama, Florida, South Carolina, North Carolina, and Virginia) in the southeastern Atlantic and Gulf coasts of the United States were selected as source sites for *U. paniculata* accessions (Fig. 1). All 19 collection sites are well separated physically and 15–20 panicles of *Uniola paniculata* were harvested from an area of 50–100 m radius in each location during September–December 2001 (Table 1). Panicles harvested from each of the collection sites within a state were designated as an accession. The harvested panicles were kept in paper bags, and transported to the Department of Agronomy and Environmental Management, Louisiana State University, Baton Rouge, Louisiana. Seeds were mechanically threshed, counted, and kept dry in small plastic vials.

Germination tests were performed during March 2003 for all the accessions. Since seed supply of *U. paniculata* was limited, all available good quality seeds belonging to all accessions were used for germination. Brown germination paper was cut into rectangular shapes and placed into rectangular petri dishes (9.5×9.5 mm; Falcon®). A total of 25 seeds per petri dish were placed and laid on the germination paper. A 0.05% solution of the fungicide Vitavax 200® (Gustafson Company; www.agsco-agdepot.com/infosheets) was used to moisten the germination paper to limit fungal growth. All petri dishes containing the seeds were placed in a germinator set at alternating day and night temperatures of 34°C and 23°C.

Germinated seeds were immediately transplanted into Speeding® trays (10×6 holes) and labeled. The Speeding® trays were filled with Jiffy-Mix Plus® (Jiffy Products of America), soil, and sand (1:1:2). All transplanted seedlings in the trays were placed in the greenhouse maintained at a temperature of approximately 20°C under natural light conditions and photoperiod.

Leaf tissues were collected from four-month old, greenhouse grown *U. paniculata* seedlings from nineteen accessions representing eight Gulf coast and south Atlantic states. From each accession, ten seedlings were

Fig. 1 Locations of the 19 collection sites for *Uniola paniculata* on the Atlantic and Gulf Coasts of the United States. Eight states, Texas (TX), Louisiana (LA), Mississippi (MS), Alabama (AL), Florida (FL), South Carolina (SC), North Carolina (NC), and Virginia (VA) are represented in this study. Location details are in Table 1

sampled randomly with their leaf tissues handled separately for AFLP analysis.

AFLP analysis

Leaf tissue was ground to a fine powder in liquid nitrogen using a mortar and pestle. Up to 100 mg of the powder was transferred to a microcentrifuge tube and kept in the freezer at −80°C. Genomic DNA was extracted using the GenElute™ Plant Genomic DNA kit (Sigma-Aldrich®) from each of the 190 individuals. A small aliquot of DNA was run on a 1% agarose gel to inspect DNA quality and concentration. DNA concentration of each sample was adjusted to 50 ng/µl.

AFLP protocol was performed as described by Vos et al. (1995). Twelve *Eco*RI + *Mse*I primer combinations were used for this study (Table 2). Five hundred nanograms of DNA from each of the 190 samples was digested in a total volume of 30 µl with 0.4 µl of *Eco*RI (12 units per µl) (Invitrogen) and 0.5 µl of *Mse*I (10 units per µl) (New England BioLab) for 2 h at 37°C in a PTC-100 Programmable Thermal Cycler (MJResearch, Inc., Waltham, Mass.) and then heated to 70°C for 10 min to inactivate the enzymes. Twenty microliters of digested DNA was then added to 10 µl ligation mixture containing *Eco*RI adapter (5 pMol) and *Mse*I adapter (50 pMol), 1 U T4 DNA ligase, and 6 µl of 5x ligation buffer with ATP and incubated at 20°C for 3 h. Adapter sequences are as follows:

*Eco*RI adapter 5′-CTCGTAGACTGCGTACC-3″
3′-CTGACGCATGGTTAA-5′
*Mse*I adapter 5′-GACGATGAGTCCTGAG-3″
3′-TACTCAGGACTCAT-5′

Table 1 Accessions of *Uniola paniculata* collected from 19 different geographic locations on the southeastern Atlantic and Gulf coasts of the United States

Sl. No.	Accession name	Collection site	State	Coast
1	TX-02	Hwy 87 Bolivar Peninsula, Flake	Texas	Gulf
2	TX-05	Hwy 87 Follets Island, SW Toll Bridge	Texas	Gulf
3	TX-09	Hwy 53 NE Newport Pass	Texas	Gulf
4	TX-17	North Padre Island	Texas	Gulf
5	LA-15	Fourchon Beach	Louisiana	Gulf
6	LA-16	Fourchon Beach	Louisiana	Gulf
7	VA-53	Assateague Island	Virginia	Atlantic
8	MS-41	West Ship Island	Mississippi	Gulf
9	MS-47	Petit Bois Island	Mississippi	Gulf
10	AL-19	Dauphin Island, West	Alabama	Gulf
11	AL-21	Gulf Shores	Alabama	Gulf
12	FL-29	Eglin Air Force Base, Sta. Rosa Island	Florida	Gulf
13	FL-33	Henderson Beach	Florida	Gulf
14	FL-35	Tyndall Air Force Base, West Crooked Island	Florida	Gulf
15	FL-39	Perdido Key	Florida	Gulf
16	NC-1	Atlantic Beach	North Carolina	Atlantic
17	NC-11	Sunset Beach	North Carolina	Atlantic
18	SC-15	Debordieu Beach	South Carolina	Atlantic
19	SC-19	Prince George	South Carolina	Atlantic

Table 2 Polymorphism in *Uniola paniculata as* revealed by 12 AFLP primer combinations

Primer combination	Total bands	Polymorphic bands	Polymorphism rate (%)	PIC
*Eco*RI-AGG + *Mse*I-CAC	68	29	43	0.196
*Eco*RI-AGG + *Mse*I-CCT	60	30	50	0.195
*Eco*RI-AGG + *Mse*I-CGT	64	36	56	0.207
*Eco*RI-AGG + *Mse*I-CGA	58	33	57	0.150
*Eco*RI-ACT + *Mse*I-CTC	97	73	75	0.217
*Eco*RI-ACT + *Mse*I-GAC	48	28	58	0.303
*Eco*RI-ACT + *Mse*I-CTG	58	33	57	0.264
*Eco*RI-CAG + *Mse*I-ACG	55	40	73	0.339
*Eco*RI-CAG + *Mse*I-CCT	40	17	43	0.247
*Eco*RI-CAG + *Mse*I-CGA	69	56	81	0.186
*Eco*RI-CAA + *Mse*I-CCT	52	22	42	0.292
*Eco*RI-CAA + *Mse*I-CGT	34	20	59	0.308
Mean	-	-	59	0.234
Total	703	417	-	-

Ligated DNA products were diluted ten-fold with sterile nanopure water for preselective-amplification.

Preselective amplification was performed in the thermal cycler using 5 µl of the ten-fold diluted ligation mixture in a 30 µl reaction. Other components of the preamplification reaction mix were 50 ng of each of the preamplification primers of *Eco*RI + 0 (5′-GAC-TGCGTACCAATTC-3′) and *Mse*I + 0 (5′′-GAT-GAGTCCTGAGTAA-3′), 200 µm dNTPs, 1 U Taq Polymerase, 1×PCR buffer, and 1.5 mM MgCl2. The thermal profile was set to thirty cycles of 94°C for 30 s, 56°C for 60 s, and 72°C for 60 s. Pre-amplification product DNA was diluted five-fold with sterile nanopure water, and was then used as template for the selective amplification.

The selective amplification PCR reaction was performed in a final volume of 12-µl containing 3 µl of preamplified sample DNA, 25 ng of each *Eco*RI + 3 and *Mse*I + 3 primers, 1× PCR buffer (10 mM Tris–HCL pH 8.3, 50 mM KCl), 2.5 mM MgCl2, 200 µm dNTPs, 0.4 U Taq polymerase. The thermal cycler was programmed for a touchdown cycle profile as follows: cycle 1, 94°C, 30 s; 65°C, 60 s; 72°C, 60 s; cycles 2–13: annealing temperature was reduced by 0.7°C in every successive cycle; cycles 14–36: annealing at 56°C, and a final extension for 5 min at 72°C. Amplified DNA products were stored at 4°C.

Amplified fragment length polymorphism reaction products (12 µl) were added with an equal volume of loading dye (98% formamide, 10 mM EDTA, 0.005% each of bromophenol blue and xylene cyanol), then denatured at 95°C for 4 min and placed immediately on ice. Four microliters of the mix was loaded onto a 6% denaturing polyacrylamide gel. A BioRad Sequi-Gen® (38×50 cm) unit was used for performing the electrophoresis with 0.5× TBE as running buffer. The gels were pre-run at 110 W for 1 h and then run for another 100 min after loading the samples. The same running condition was used for all the gels. Because more than one gel was run to accommodate all 190 samples, a 10 bp ladder was used on both sides of each gel to facilitate standardization and alignment. The gels were silver-stained using the silver sequence DNA staining kit (Promega cat. #Q4132, Madison, WI, USA) following the manufacturer's instructions. A sheet of 3 mm Whatman white paper was placed on the bottom of the developed gel to visualize the AFLP bands. As an alternative, the gel was soaked in 2% NaOH solution, lifted from the plate with the help of a sheet of Whatman paper; the scanned images were saved in JPEG format for documentation.

Collection and analyses of AFLP data

The silver stained AFLP bands in each gel were scored as present (1) or absent (0) and only distinct polymorphic bands were scored. AFLP fragments with the same electrophoretic mobility were assumed to be allelic and those with different mobility as nonallelic. Binary matrices were prepared for analysis using numerical taxonomy system (NTSYSpc) version 2.10t (Rohlf 1997). Jaccard similarity coefficient (Jaccard 1908) was used to estimate similarity among plants within accessions. This coefficient is considered appropriate since it ignores negative matches. Using the formula, Jaccard coefficient = $a/(a+b+c)$ where; a = the number of matching present bands (''1'' and ''1''), b = the number of unmatched bands (''1'' and ''0''), and c = the number of unmatched band (''0'' and ''1''), the similarity coefficients were computed. Similarity matrices were subjected to cluster analysis using the unweighted pair group method with the arithmetic averages (UPGMA) (Sneath and Sokal 1973) clustering approach and principal coordinate analysis (PCO). Using the matrix comparison (MXCOMP) module a cophenetic correlation was computed to test the goodness of fit or the correlation of the two matrices (cophenetic and tree matrix). Bootstrap analysis was performed to determine the strength of the clusters using software WINBOOT (Yap and Nelson 1996).

The polymorphic information content (PIC) value (Botstein et al. 1980), a commonly used measure to describe the ability to generate polymorphism, was calculated as PICi = 2 fi (1-fi); where PICi is polymorphic information content of marker 'I', fi is frequency of the

amplified alleles (bands present), and (1-f*i*) is the frequency of the null alleles (no amplification).

Analysis of molecular variance (AMOVA) was performed using ARLEQUIN version 2.0 (Schneider et al. 2000) to partition the genetic variation among individual plants, among accessions and among states.

Results

AFLP polymorphism and Polymorphic information content (PIC)

Twelve primer combinations with high polymorphism rate and distinctness of the bands were selected to genotype the entire population of sea oats accessions. The number of bands that were amplified by each primer combination ranged from 34 to 97 (Table 2). These polymorphic bands ranged from 75 to 450 bp with a majority within the 100- to 350-bp range. A total of 703 AFLP bands were generated across all plant samples. Among these, 417 bands were polymorphic with a mean polymorphism rate of 59% ranging from 42 to 81%. The highest polymorphism rate (81%) was observed with primer combination *Eco*RI-CAG + *Mse*I-CGA followed by *Eco*RI-ACT + *Mse*I-CTC and *Eco*RI-CAG + *Mse*I-ACG at 76% and 73%, respectively. Three primer combinations (*Eco*RI-AGG + *Mse*I-CAC, *Eco*RI-CAG + *Mse*I-CCT and *Eco*RI-CAA + *Mse*I-CCT) produced only 42–43% polymorphic bands.

The usefulness of a particular marker in a set of genotypes can be judged from its PIC values which range from 0 in the case of monomorphic markers to 0.5 for markers with 50% in each amplified and null allele group. The PIC values for all 12 primer combinations ranged from 0.15 (E-AGG/M-CGA) to 0.34 (E-CAG/M-ACG) with an average value of 0.23 (Table 2). In general, the primer combination of *Eco*RI selective nucleotides AGG was less informative compared to others. The average PIC value over all AFLP markers was 0.23. Despite small and different sample sizes in each accession, there are some indications from the PIC values about the genetic variance in sea oats belonging to different states. The AFLP markers on an average are more polymorphic in thegene pools of populations derived from Florida, Texas, Mississippi, Louisiana, and Alabama than in those obtained from South Carolina, North Carolina, and Virginia (data not shown).

Analysis of molecular variance (AMOVA)

The AMOVA analysis from the AFLP binary matrix profile for the individual *U. paniculata* AFLP fingerprints permitted partitioning of the genetic variability in sea oats accessions (Table 3). Of the total genetic variation, the largest proportion (47.83%) was attributable to differences among the states. Individual genetic differences within accessions accounted to 34.36% whereas only 17.81% of the total genetic variation was attributable to differences among accessions within states. The variance components for each of the sources of variation were highly significant.

Genetic variation within and between accessions

Jaccard similarity coefficients were calculated between pairs of plants. Pair-wise similarity values ranged from 0.69 to 0.99. To examine the degree of differentiation among the accessions, average Jaccard similarities for plants belonging to the same and/or to different accessions were calculated (data between accessions not shown). Higher values for the Jaccard coefficients indicated that the plants belonging to the accessions were more genetically similar than the plants belonging to accessions with lower coefficients. This suggested some degree of differentiation among the accessions. From the means and ranges for all 19 accessions (Fig. 2), more variability was observed in the FL-33 and FL-39 accessions. Overall, Florida accessions were more variable genetically. The lowest range of Jaccard values was noted in accessions TX-2, VA-53, and NC-11. The accessions from both Carolinas and Virginia were more homogenous with higher Jaccard similarity values but when compared to other accessions, interaccession similarity values were relatively lower than intraaccession values. A similar trend was noticed when the accessions from other states were compared among themselves. Comparison of mean similarity values of all possible pairs of accessions revealed VA-53 to be significantly different from all accessions except the accessions from the Carolinas and TX-5. SC-19 differed from only accession FL-33. Similarly, both NC-1 and SC-15 were different from TX-17, LA-15, LA-16, AL-19, MS-41, MS-47, FL-33, and FL-39, whereas NC-11 differed from all accessions from Louisiana, Alabama, Mississippi, and two accessions of Texas (TX-9 and TX-17).

Table 3 Analysis of molecular variance (AMOVA) for 190 *Uniola paniculata* individuals belonging to 19 accessions from 8 southeastern Atlantic and Gulf coast states of the United States

Source of variation	Degrees of freedom (df)	Sum of squares (SS)	Variance components	Percentage of variation	*P* values
Among states	7	4929.67	25.51	47.83	< 0.00
Among accessions within states	11	1247.10	9.50	17.81	< 0.00
Among genotypes	171	3134.60	18.33	34.36	< 0.00
Total	189	9311.37	53.35		

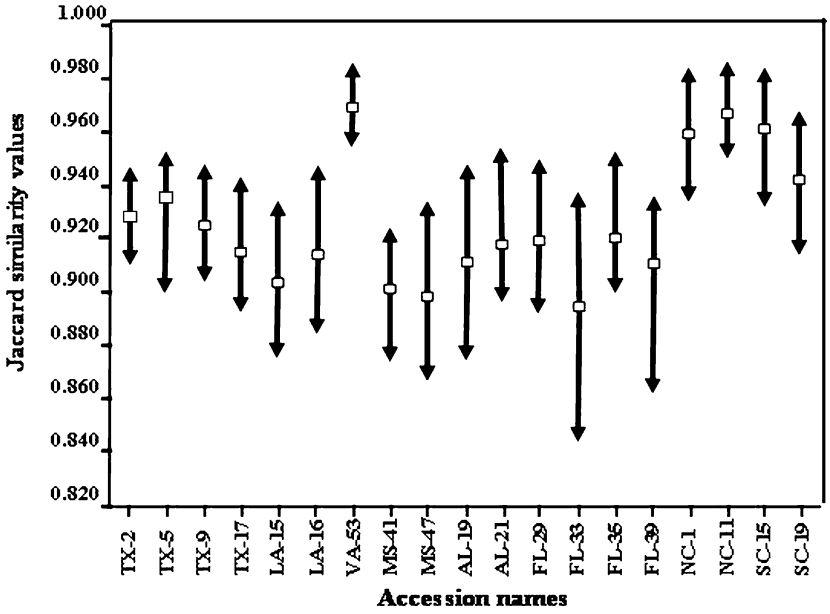

Fig. 2 Distribution of the "within accession" pair-wise Jaccard similarity coefficients. In each case *open square* represents mean values and the range is indicated by *arrows*. Significance of the differences in mean Jaccard similarity values between any two accessions was determined by *t*-test at $P = 0.05$. VA-53 was significantly different from all accessions except TX-5, NC-1, NC-11, SC-15, and SC-19. NC-1 was significantly different from accessions: TX-17, LA-15, LA-16, MS-41, MS-47, AL-19, FL-33, and.FL-39. SC-15 showed similar pattern as NC-11 with an additional accession AL-21 showing significant difference. NC-11 showed significant difference from TX-9, TX-17, LA-15, LA-16, AL-19, AL-21, MS-41, MS-47, FL-29, Fl-33, FL-35, and FL-39. The accession, SC-19, was significantly different from FL-33

Genetic variation within and between different states

To obtain a more detailed view of the distribution of genetic variation within and between different states, mean Jaccard similarities between pairs of plants belonging to the same or to different states were estimated (Table 4). As expected, the individuals within a state were more similar compared to those from other states. But the degree of similarity varied from state to state. Examination of the range and mean similarity values indicated that Florida was the most diverse state followed by Mississippi, Louisiana, Alabama, and Texas. The sea oats accessions from Virginia, North Carolina, and South Carolina were more homogenous as indicated from high but narrow range of Jaccard similarity values. The sea oats from Texas and Louisiana were more genetically related compared with those from other states. The sea oats from the state of Virginia were clearly different from other states. Sea oats accessions from Mississippi and Alabama were more similar to one another, as were also observed for plants from the two Carolina states. Florida accessions were more related to Mississippi and Alabama than those from other states.

Cluster analysis

The UPGMA cluster analysis based on Jaccard similarity coefficients resolved the genetic relationship among the sea oats individuals of 19 accessions from eight states of the United States. In general, the major clusters of sea oats genotypes were supported with high bootstrap values (Fig 3), indicating the reliability and stability of the inferred relationships as well as the robustness of AFLP dataset. The goodness-of-fit of the AFLP-generated dataset for the cluster analysis was also supported by high cophenetic correlation coefficient (0.939) (Rohlf 1992). The UPGMA-derived dendrogram assigned all the sea oats individuals into four major clusters (Fig. 3) designated as I, II, III, and IV at the 0.82 similarity level. All individual genotypes were distinctly separated from each other. All four clusters were clearly separated from each other. Cluster I represented all accessions from Texas, Louisiana, and Virginia. Cluster II consisted of all four accessions from Mississippi and Alabama and two accessions FL-29 and FL-33 from Florida. The accessions from Florida, FL-35 and FL-39, formed Cluster III and the accessions from North Carolina and South Carolina were represented in Cluster IV. Cluster I and Cluster II were separated from each other at 79.6% genetic similarity level. Cluster III was separated from Cluster I and II at 79% similarity level where as Cluster IV was separated at 76% similarity level. In Cluster I, the accessions from Louisiana and Texas were separated from the only Virginia accession VA53 forming two subclusters (IA and IB). Similarly, the accessions FL-29 and FL-33 from Florida formed one subcluster (IIB) and the accessions from Mississippi and Alabama together were represented in subcluster IIA but supported with lower bootstrap values. The accessions FL-35 and FL-39 were also separated from each other in Cluster III at a genetic

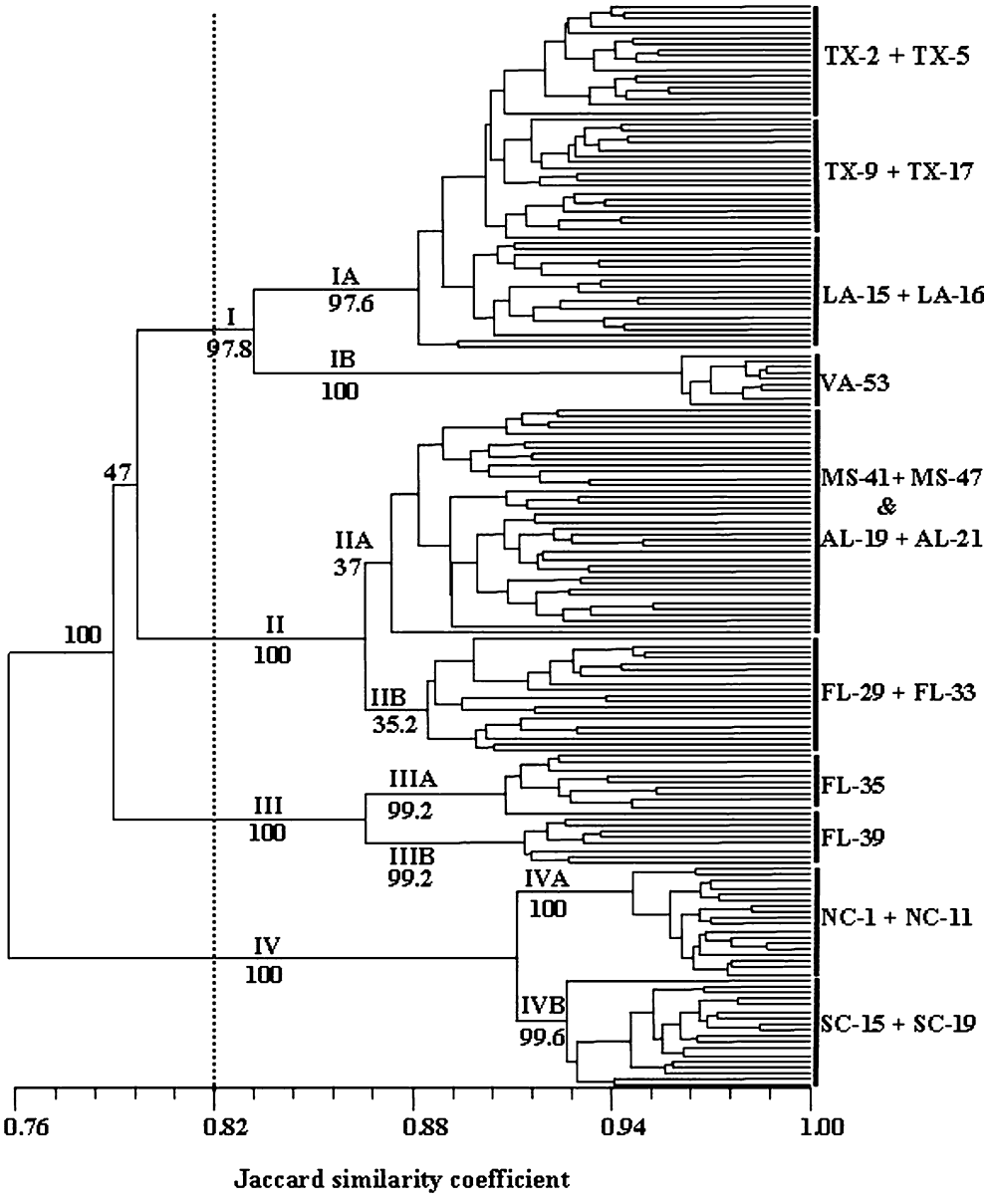

Fig. 3 Dendrogram of sea oats (*Uniola paniculata*) individuals belonging to 19 accessions collected from 8 different Gulf coast and South Atlantic States (Texas, Louisiana, Virginia, Mississippi, Alabama, Florida, North Carolina, and South Carolina) produced by UPGMA clustering method based on the Jaccard similarity coefficient matrix derived from 703 AFLP markers. The numbers shown at different nodes represent the bootstrap values based on 500 replications

similarity level of 86.5%. The accessions from South Carolina accessions (subcluster IVA) were also resolved from those from North Carolina (subcluster IVB) at the 91.2% similarity level.

Principal coordinate analysis

Principal coordinate analysis further validated the results of cluster analysis (Fig. 4). In PCO analysis, the first two components explained more than 39% of the variation in the estimates of genetic similarity. Altogether, five distinct groups were revealed by the first two principal coordinates (Fig. 4). Group 1 included all accessions from Texas and Louisiana. The Virginia accession VA-53 was clearly differentiated from those from Texas and Louisiana and formed group 2. Group 4 included Florida accessions FL-35 and FL-39. Two of the remaining Florida accessions, FL-29 and FL-33, were grouped together with the accessions from Mississippi and Alabama (Group 3). Accessions from North Carolina and South Carolina were included in Group 5. Within the Group 5, accessions from South Carolina seemed to be more heterogeneous like FL-35 and FL-39 accessions belonging to group 3 as indicated by the larger extent of spread of the accession cluster.

Discussion

AFLP Polymorphism

The present study demonstrated the potential of the AFLP technique for the generation of extensive genetic

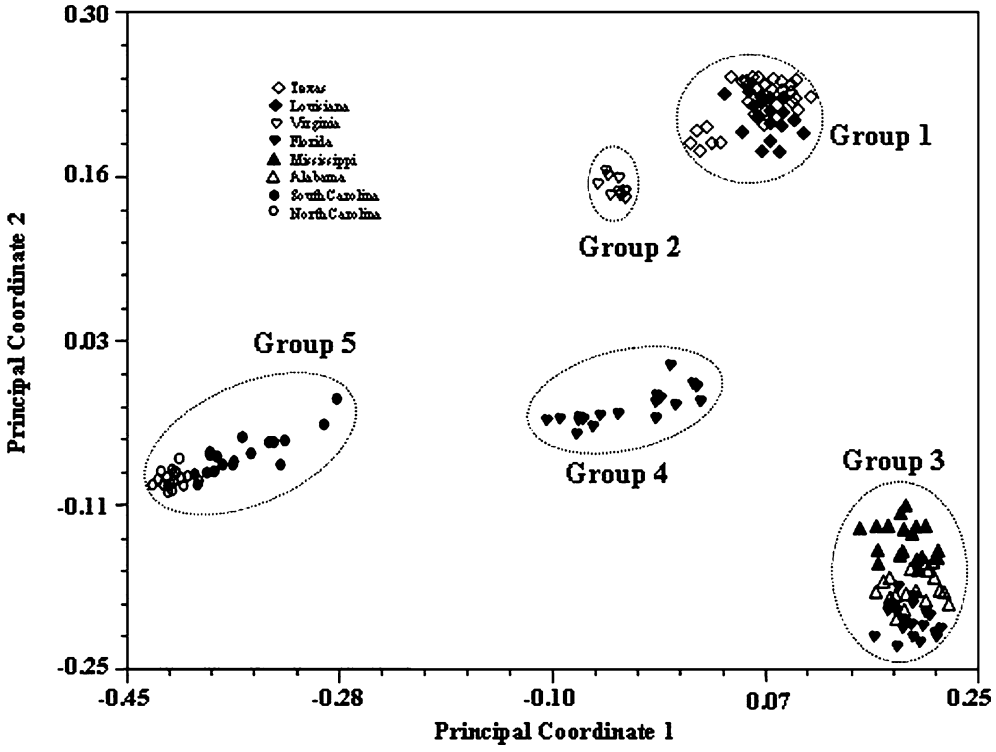

Fig. 4 Principal coordinate analysis of sea oats plants from eight different states with the first and second principal coordinates derived from the Jaccard similarity coefficient matrix computed from 703 AFLP markers. Sea oats individuals of all 19 accessions from North Carolina, South Carolina, Texas, Louisiana, Virginia, Florida, Mississippi, and Alabama are represented by *open circles*, *closed circles*, *open diamond*, *closed diamond*, *open heart*, *closed heart*, *closed triangle*, and *open triangles*, respectively. The first two coordinates explain more than 39% of the genetic diversity

polymorphism data appropriate to the analysis of individual DNA profile, population structure, and genetic diversity. The moderate level of polymorphism observed in our study was comparable to a study in dioeceous Texas bluegrass (*Poa arachnifera*) (Renganayaki et al. 2001) but much lower than studies in other cross pollinating grass species like ryegrasses (*Lolium* spp.) and smooth bromegrasses (*Bromus inermis* Leyss.), for which very high polymorphism rates (83–90%) have been reported (Roldan-Ruiz et al. 2000; Ferdinandez and Coulman 2004). Ranamukhaarachchi et al. (2000) used a modified AFLP technique with single restriction enzyme in sea oats and amplified 95 bands with 8 AFLP primers of which 52 were polymorphic. Compared to their study, the present study detected a higher rate of polymorphism and provided higher resolution due to generation of large number of loci. Sea oats genotypes were discriminated from each other by their distinctive DNA fingerprints. The primer combinations used in this study provide sufficient discriminatory ability to allow efficient varietal identification based on the AFLP fingerprints in *U. paniculata*.

Population structure

Due to their independence from environmental influence, DNA markers provide a desirable means of determining population structure and genetic diversity. This is the first comprehensive survey of genetic diversity and population structure in sea oats accessions of the United States using AFLP markers. The principal coordinate analysis showed differentiation into five groups following a clear geographic pattern of genetic structure (Fig 4). This could be due to limited gene flow among populations due to the linear and fragmented distribution of this species (Berg and Hamrick 1997). Our observation is in sharp contrast to the report of Franks et al. (2004) who found a lack of regional differences in this species. The reason for this discrepancy could be due to analysis of a few isozyme loci by Franks et al. (2004) which might not have been sufficient to sample the genetic variability in sea oats compared to the high resolution DNA fingerprints generated by a large number of AFLP loci in this study.

Accessions from states in geographic proximity shared a common gene pool. Along the Gulf coast, Texas and Louisiana formed a group clearly separated from the group representing Alabama and Mississippi. Similarly, along the southeastern Atlantic coast, North Carolina and South Carolina accessions were similar, while Virginia formed a group on its own. Despite the physical proximity of the collection sites of the four Florida accessions to Mississippi and Alabama sites, only accessions FL-29 and FL-33 shared the same gene pool as Mississippi and Alabama. The notable deviation to geographic population structure in sea oats is the cluster containing FL-35 and FL-39 accessions (Figs. 3, 4). These two sites were in close proximity to the FL-29 and FL-33 accessions collection sites in the Florida Panhandle. Since this species is used in restoration projects and we have no knowledge of the restoration history of the collection sites of these two Florida accessions, it is possible that these two accessions are not

Table 4 Average Jaccard similarity coefficients between pairs of *Uniola paniculata* plants belonging to the same state (diagonal values in bold) or different state

	Texas	Louisiana	Virginia	Mississippi	Alabama	Florida	North Carolina	South Carolina
Texas	**0.9098 ± 0.0006** (**0.8681–0.9574**)							
Louisiana	0.8883 ± 0.0005 (0.8433–0.9318)	**0.9014 ± 0.0012** (**0.8509–0.9481**)						
Virginia	0.8332 ± 0.0007 (0.8065–0.8636)	0.8293 ± 0.0009 (0.8000–0.8569)	**0.9699 ± 0.0015** (**0.9556–0.9871**)					
Mississippi	0.8133 ± 0.0004 (0.7780–0.8466)	0.8071 ± 0.0007 (0.7698–0.8424)	0.7811 ± 0.0010 (0.7519–0.8121)	**0.8910 ± 0.0014** (**0.8519–0.9335**)				
Alabama	0.8086 ± 0.0004 (0.7673–0.8383)	0.8010 ± 0.0007 (0.7698–0.8424)	0.7673 ± 0.0006 (0.7452–0.7894)	0.8829 ± .0007 (0.8496–0.9192)	**0.9055 ± 0.0011** (**0.8722–0.9527**)			
Florida	0.7959 ± 0.0003 (0.7495–0.8310)	0.7909 ± 0.0005 (0.7510–0.8301)	0.7577 ± 0.0005 (0.7292–0.7824)	0.8177 ± 0.0016 (0.7429–0.8971)	0.8305 ± 0.0018 (0.7587–0.9108)	**0.8399 ± 0.0021** (**0.7562–0.9536**)		
North Carolina	0.7599 ± 0.0005 (0.7321–0.7966)	0.7487 ± 0.0005 (0.7291–0.7763)	0.7765 ± 0.0006 (0.7519–0.7953)	0.7274 ± 0.0006 (0.7004–0.7539)	0.7297 ± 0.0005 (0.7062–0.7578)	0.7450 ± 0.0013 (0.6940–0.8160)	**0.9589 ± 0.0008** (**0.9283–0.9870**)	
South Carolina	0.7829 ± 0.0007 (0.7444–0.8288)	0.7720 ± 0.0007 (0.7412–0.8168)	0.7943 ± 0.0006 (0.7738–0.8159)	0.7510 ± 0.0006 (0.7223–0.7795)	0.7518 ± 0.0006 (0.7262–0.7797)	0.7654 ± 0.0012 (0.7108–0.8373)	0.9113 ± 0.0009 (0.8629–0.9511)	**0.9464 ± 0.0013** (**0.9022–0.9848**)

Range of Jaccard coefficients is indicated in parentheses. Results are based on all 703 AFLP markers generated with 12 primer combinations. Pair-wise comparison of eight states were significantly different from each other revealed in *t*-test at $P = 0.001$

the native accessions and might have been planted with materials introduced from outside. The two accessions were distinct from each other forming separate groups in both UPGMA cluster and PCO analyses suggesting that sea oats materials were likely to be from two different places. If they were native accessions, due to the close proximity of these collection sites along the Gulf coast, we would have expected them to be sharing the same gene pool as Mississippi and Alabama. The geographic pattern of population structure revealed in this study suggests that AFLP markers could provide a useful tool to predict the geographic origin of sea oats ecotypes of the United States.

Pattern of genetic diversity

The pattern of genetic diversity in sea oats germplasm in the United States was investigated in a number of ways to ascertain if any pattern was evident between different states, or between accessions within a state, or between individuals within an accession. The Jaccard similarity values were around 90% among accessions. As expected, individuals within an accession were genetically more similar compared to individuals belonging to other accessions. A similar pattern was observed in sea oats within and between states (Table 4). In sharp contrast to our results, Franks et al. (2004) reported a low level of genetic diversity in all populations along the Gulf Coast west of the Florida panhandle and more diversity in populations from North Carolina and South Carolina. The Atlantic accessions (VA-53, NC-1, NC-11, SC-15, and SC-19) analyzed in this study were genetically more homogeneous compared to accessions from Gulf Coast states. The accessions from Florida were the most diverse. This overall genetic pattern revealed a strong Gulf coast-Atlantic coast or south-north division for *Uniola* with the exception of Virginia from which only one accession was analyzed. The highest genetic variation detected at the state-level, which implies isolation by distance confirms the results from the UPGMA cluster and the PCO analysis where the accessions were grouped accordingly by its geographical origin with the exception of the two accessions from Florida. Variation among the accessions within a state was statistically significant but was not enough to separate the accessions of a state either in PCO or UPGMA analysis.

In a cross pollinating species such as sea oats, reduced genetic differentiation among accessions was observed at the local scale. There was no clear genetic differentiation between accessions within a state with the exception of FL-35 and FL-39. Regional genetic clustering of accessions indicates some degree of gene flow in states of physical contiguity. Though it is clonally propagated, the *Uniola* samples in each accession harbored genetic variability likely due to sexual propagation (Franks et al. 2004).

Implications for selection and conservation of sea oats germplasm for dune restoration

Uniola paniculata, the most abundant dune-building perennial grass along the Gulf and southeast Atlantic coastal areas (Dahl and Woodard 1977), helps to establish foredunes by intercepting and stabilizing drifting sand with its roots, rhizomes, and tillers. Foredunes are frequently damaged by storm surge and wave attack caused by powerful hurricanes necessitating planting and establishment of beach areas. The knowledge of the distribution of genetic diversity is essential for survival, ecology, management, conservation, and development of suitable germplasm for restoration efforts. Phenotypic variability for some notable attributes that are important for propagation and establishment in sea oats materials of the United States, have been documented. In this study, for the first time, DNA fingerprints of sea oats germplasm from most of its ecological range in the United States provided evidence of the existence of a regional population structure, which is the primary consideration in the development of conservation strategies. But the relationship between the geographic sources of sea oats plant materials and successful dune restoration is yet to be understood. Field performance data coupled with the molecular profile information will delineate important differences between strains of sea oats in a given area and given population, leading to selection of better *U. paniculata* strain for a specific environment. The new insights regarding the extent of diversity in sea oats populations of the US will be helpful to guide introduction and mixing of suitable *U. paniculata* genotypes to restore dune ecosystems while ensuring adequate genetic diversity at the same time. Furthermore, results from this study will be useful for developing most effective management and conservation strategies for this plant genetic resource.

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