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Genetic diversity of selected sea oats (Uniola paniculata L.) lines using Amplified Fragment Length Polymorphism (AFLP)

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Genetic diversity of selected sea oats (Uniola paniculata L.) lines using Amplified Fragment Length Polymorphism (AFLP)

by

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Abstract

Sea oats (*Uniola paniculata* L.) is a dunegrass native to the Gulf and Atlantic coasts of the United States. It is often used in beach restoration because it produces an extensive below-ground rhizome system to stabilize sand and above ground biomass to catch eroding sand. To increase the genetic diversity of sea oats plants used in coastal restoration projects in the northern Gulf of Mexico Basin, the Louisiana State University Agricultural Center's Coastal Plants Breeding Program initiated a sea oats breeding program in 2001, which has evaluated and selected sea oats lines that perform well in the northern Gulf of Mexico Basin. The objective of this study was to identify the genetic diversity of 156 selected sea oats lines collected from five states. Ten AFLP *Eco*RI + *Mse*I primer combinations identified 658 loci, including 284 polymorphic loci. The Jaccard similarity ranged from 73% to 98% in selected lines. Cluster analysis produced 2 major clusters at the 90% similarity threshold, one having 90.2% similarity among 147 lines, and the other having 92.8% similarity among 8 lines. No strong clustering by state was found. In selected lines, polymorphism rates, genetic variation, and number of major clusters all decreased slightly from unselected lines as expected. However, enough diversity exists to develop multiple, genetically diverse sea oats lines for the northwestern Gulf of Mexico coast using traditional plant breeding methods.

Introduction

Beaches along the Atlantic and Gulf of Mexico coasts are socially, economically, and ecologically critical. They function mainly as a first line of defense against storms that threaten residents and infrastructure of coastal communities (Thom et al.2005). They are also very important as protection for marshlands which are vital for fisheries (Boesch and Turner 1984), oil and gas production, and ecological habitats for coastal wildlife and migratory birds (Boesch et al. 1994). Unfortunately, coastal land loss is threatening the health of these vital areas. Over the last 2 centuries, the United States has lost 50% of its wetland areas (Dahl 1990), with 90% of the loss being concentrated in Louisiana which contains 30% of all US wetlands (Finkl and Khalik 2005). Current estimates state that 1,760 km^2 of additional land Loss from Louisiana could be endured over the next 50 years if prevention measures are not taken. This would potentially erode Louisiana's coastline inward up to 50 km in some areas (Hanchey 2005), converting sandy beaches and marshland to open gulf waters (Dahl and Johnson 1991; Boesch et al. 1994). Land loss of this magnitude would have wide-ranging, negative anthropogenic, ecological, and economic impacts because of the many varied functions of wetland areas. Major factors contributing to this land loss include storm destruction (Finkl and Kahlik 2005), subsidence of wetland soils (Boesch et al. 1994; Finkl and Khalik 2005), increased relative sea level rise (Penland and Ramsey 1990; Roberts and Coleman 1996), changes in the course of the Mississippi river over time, levee systems which prevent river flooding required for sediment deposition, reduced sediment load in the Mississippi River (Kesel 1988; Kesel 1989; Boesch et

al.1994; Finkl and Khalik 2005), and creation of channels that facilitate saltwater intrusion (Bosch et al. 1994).

Many different efforts have been made to address the problem of coastal land loss such as environmental regulations, engineered structures and diversions, and vegetation. One approach, human engineered structures such as seawalls, breakwaters, and groins, have had little success and require constant maintenance (Finkl and Khalik 2005). A more successful solution to coastal land loss is the use of plant material to stabilize existing coastal lands and rebuild land that has been lost. Planting vegetation along beaches stabilizes existing sand and increases sand deposits over time. This builds sand dunes while effectively reducing the wind speed of storms and storm surge and increasing the aesthetic appearance of beaches. One excellent plant used extensively in Gulf of Mexico beach restoration projects is sea oats (*Uniola paniculata* L.).

Sea oats is a dunegrass that is often chosen for beach restoration along the Gulf of Mexico because it produces an extensive rhizome system below the sand which stabilizes beach sand to decrease erosion (Wagner 1964; Dahl and Woodard 1977). The aboveground tissue of sea oats acts as a windbreak to collect windblown sand and build sand dunes (Wagner 1964; Woodhouse et al. 1977). Sea oats is well adapted to low nutrient, sandy soils as well as regular salt sprays, and it is resilient after storm events, making it a low-maintenance, sustainable plant for these environments. Sea oats is also ideal for beach restoration along the struggling northwestern Gulf of Mexico coastline because it is native to the Gulf and Atlantic coasts of the United States (Wagner 1964) and is aesthetically pleasing along beaches.

Clonal reproduction appears to be the most successful form of reproduction in sea oats, because sea oats spread rapidly through rhizomes. Unfortunately, this can reduce genetic diversity which is necessary for cross-pollination and the production of genetically unique seed (Crewz 1987). Dispersal of seeds produces new, genetically diverse populations and colonizes the majority of new environments (Hester and Mendelssohn 1987; Subudhi et al. 2005). Sexual reproduction is important for constant adaptation especially in the northwestern Gulf of Mexico where beach conditions are continuously changing. Unfortunately, a decrease in seed production with decreasing latitude has been observed in sea oats (Hester and Mendelssohn 1987) as well as low genetic diversity (Franks et al.2004; Subudhi et al. 2005) and germination rates in this area. In the northwestern Gulf of Mexico, native sea oats populations have declined significantly because of changes in beach conditions, low genetic diversity, and low seed production (Hester and Mendelssohn 1987). Beaches in these areas generally endure extremely high rates of erosion and subsidence and are highly saturated due to sand deficiency and low profile dunes (Mendelssohn et al. 1983; Mendelssohn et al. 1991). These conditions have decreased the genetic diversity of sea oats in these areas, which may have contributed to the low seed production that has been observed (Subudhi et al. 2005). Previous studies have documented that sand-deficient dunes have been attributed to decline and death of sea oats populations (Wagner 1964).

Other factors that contribute to decreasing genetic diversity of sea oats in Louisiana include plant production and restoration practices. Labor intensive vegetative propagation is used to maintain these lines by splitting plants as they grow to create multiple, smaller clones. This practice is especially problematic for genetic diversity because it means all plants purchased from a particular grower for restoration projects will be genetically identical unless seed is germinated to grow new genotypes. This is a problem for sustainability of restoration efforts, because multiple, genetically diverse genotypes are required for populations to cross-pollinate and adapt to changing environmental conditions (Huenneke 1991; Kutner and Morse 1996; Ledig 1996). Currently, the only genotype that is commercially available for restoration projects on the Louisiana coastline is Caminada, and it is therefore the genotype that is most often planted.

In 2001, the Louisiana State University Agricultural Center (LSU AgCenter) expanded its Coastal Plants Breeding Program to develop multiple, genetically diverse sea oats lines that are adapted to the unique conditions of beaches in the northern Gulf of Mexico. Previous research has found genetic diversity in sea oats collected from a wide geographical range. In a 2005 study, the genetic diversity of unselected sea oats lines collected from 8 states was examined using Amplified Fragment Length Polymorphism (AFLP), and 76% similarity between all lines was reported (Subudhi et al. 2005). Lines collected from the same state were more genetically similar than lines collected from different states, and a high amount of genetic diversity existed among the samples (Subudhi et al. 2005). Another study examined genetic diversity of unselected sea oats lines using allozyme loci and starch gel electrophoresis. The results of this study found a weak positive correlation between genetic and geographic distance as well as uniform genetic diversity throughout the geographic range of sea oats, except in the northwestern Gulf of Mexico where genetic diversity was low (Franks et al. 2004). Both of these studies show that genetic diversity exists within natural populations of sea oats in the United States; therefore, the potential exists for a successful breeding program that would select and develop genetically unique sea oats lines for use in beach restoration projects.

The objective of this study is to identify the genetic diversity of 156 selected sea oats lines collected from five states (NC, FL, AL, LA, TX).

Materials and Methods

Accessions

Sea oats seeds collected in 2001 from eight states (TX, LA, MS, AL, FL, SC, NC, VA) were germinated in 2003 (Subudhi et al., 2005). In 2004, 1125 sea oats plants, hereafter referred to as lines, were transplanted to Holly Beach, LA and 904 lines were transplanted to Long Beach, MS. In the summer of 2005, 101 lines from five states (TX, LA, AL, FL, NC; Table 1) were selected based upon their performance in these natural beach environments. Rhizome and stem materials were harvested from the selected lines and transported to the Louisiana State University Agricultural Center's Aquaculture Research Station, Baton Rouge, where they were established in an artificial breeding nursery to increase in size. In the fall of 2005 and spring of 2006, 32 and 19 lines (Table 1) were selected based upon survival at Holly Beach, LA after Hurricane Rita significantly damaged this beach on September 23, 2005. Rhizome and stem material were collected as described above. Four 'Caminada' plants were also included (Table 1). Caminada is the only released sea oats line. Seed were collected by the National Resource Conservation Service (NRCS) near Caminada, LA and used to produce Caminada plants; therefore it is possible that genetic variation exists within Caminada plants obtained from NRCS and used in this study.

DNA Extraction

In May of 2009, two young leaves (approximately 15 ml after grinding) from each line were collected from Aquaculture Research Station and placed immediately on ice. Each sample was ground in liquid nitrogen with a mortar and pestle and stored at -80^{o}C in 50 ml centrifuge tubes. Genomic DNA was extracted using the Potassium Acetate-SDS miniprep method described by Dellaporta et al. (1983) with modifications.

Fifteen ml of extraction buffer (100 mM Tris, 500 mM NaCl, 50 mM EDTA [mw=372.2], 40 mM SDS; pH 8) were added to each sample. Samples were incubated in extraction buffer at 65^{o}C for 20-30 minutes, inverting periodically. Five ml of 5 M potassium acetate was added to each sample. Samples were incubated on ice for 10 min; centrifuged at 3,500 rpm for 30 min; and filtered through sterile Miracloth (Calbiochem®, La Jolla, CA, USA) into 10 ml cold 100% isopropanol. Samples were incubated at -20°C for 30 min, inverted periodically, and centrifuged at 3,500 rpm for 30 min. Supernatant was discarded and DNA pellets were allowed to air dry for 10 min.

Dry pellets were re-dissolved in 700 µl of TE + RNAse mixture (10 µl RNAse ml^{-1} TE) and incubated at 37°C for 30 min. The entire solution was transferred to a 1.5 ml microcentrifuge tube and 500 µl of chloroform:isoamyl alcohol (24:1) was added. Tubes were centrifuged at 1300 rpm for 10 min and the supernatant was transferred to new 1.5 ml tubes containing 500 µl cold 100% isopropanol and 75 µl 3 M sodium acetate. Samples were incubated at -20°C for 30 min, inverted, and all contents except the DNA pellet were discarded. Approximately 100 µl of 70% ethanol was added to wash pellets and tubes were centrifuged for 30 s. Ethanol was discarded and DNA pellets were dried. The DNA pellets were re-dissolved in 100 µl TE Buffer (10 mM Tris, 1 mM EDTA [mw=372.2]; pH 8). Samples were stored at -20°C.

DNA concentrations were measured using a Thermo Scientific nanodrop spectrophotometer (Nanodrop Technologies, Oxford, UK) and DNA quality was verified using a 1% agarose gel. Genomic DNA was diluted to 500 ng using sterile distilled water.

AFLP Analysis

Amplified Fragment Length Polymorphism was performed as described by Vos et al. (1995) and Subudhi et al. (2005) with slight modifications. Ten *Eco*RI + *Mse*I primer combinations were used (Tables 2 and 3). Five hundred ng of genomic DNA was digested in a 30 µl reaction volume with 12 U *Eco*RI (New England Biolabs® Inc.); 8U *Mse*I (New England Biolabs® Inc.); 3 µl 10X BSA (New England Biolabs® Inc.); and 3µl 10X *Eco*RI and *Mse*I Buffers (New England Biolabs® Inc.) in a BioRad MyCycler™ programmable thermal cycler (BioRad, Hercules, CA) at 37°C for 2 h and then heated to 70°C for 10 min to inactivate the enzymes. Five ml of digestion product and 3 µl of 2x bromophenol blue dye were loaded to a 1.5% agarose gel to confirm successful digestion.

Ten µl of digested DNA was added to 20 µl of ligation mixture containing 5 pMol *Eco*RI adapter; 50 pMol *Mse*I adapter; 1U T4 DNA ligase (Promega, Madison, WI); 10x restriction ligation buffer (50mM Tris-HCL, 50mM Mg Acetate, 250mM K Acetate); and 10mM ATP (Invitrogen™, Carlsbad, CA). The reaction was incubated at 20°C for 4 h in the thermal cycler. Ligated DNA products were diluted ten-fold with sterile distilled water for preselective-amplification.

Five µl of diluted ligation product were preselectively amplified in a total reaction volume of 30 µl which included 30x PCR buffer (Promega, Madison, WI); 50 ng (8.3 µM) *Eco*RI and *Mse*I preamplification primers (Table 1); 60 mM $MgCl_2$ (Promega, Madison, WI); 6.5 mM dNTP (Fermentas Inc., Glen Burie, MD); and 2 U Taq polymerase (Promega, Madison, WI). Preamplification was performed with the following thermal profile: 29 cycles of 94°C for 30 s; 56°C for 60 s; and 72°C for 60 s. Preamplification product DNA was diluted five-fold with sterile distilled water and used for selective amplification.

Selective amplification volume was 10 µl and included 2 µl diluted preamplification product DNA; 6 ng (1 µM) *Eco*RI primer 700 spectrum and *Eco*RI primer 800 spectrum (Table 1); 50ng (8.3 µM) *Mse*I primer (Table 1); 2.5 mM dNTP; 10x PCR Buffer; 30 mM $MgCl_2$; and 1 U Taq polymerase. Selective amplification product was obtained with the following program: 94°C for 2 min; 13 cycles of 94°C for 30 s, 65°C for 30 s, and 72°C for 1 min with the annealing temperature decreasing from 65°C to 56°C by 0.7°C per cycle; 21 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s; and 1 cycle of 72°C for 5 min.

Five µl blue stop solution (Li-Cor® Biosciences) was added to each sample. Selective amplification products were denatured at 95°C for 5 min in a thermal cycler and immediately placed on ice prior to loading in a 6.5% acrylamide gel (20 ml of 6.5% gel matrix [Li-Cor® Biosciences], 150 µl 10% ammonium persulfate, and 15 µl Temed). The gel was placed in a Li-Cor 4300 DNA Analyzer and prerun for 25 min to calibrate the laser and ensure that the thickness of the gel was equal throughout. Gel wells were cleaned by forcing 1x TBE buffer (89 mM Tris Base, 89 mM Boric Acid, 2 mM Na_2EDTA) into each well with a needle syringe. Selective amplification products (0.2 µl) were loaded into a single well. Two 10 bp ladders were loaded per gel (IRDye 700 and IRDye800) and gels were run for 3.5 hours at 1500V and 45°C. AFLP bands were visualized using NEN® Model 4300 DNA Analyzer version 2.0.20-1 realtime viewing software at 1028 pixels.

Collection and analyses of AFLP data

Distinct AFLP bands were scored as present (1) or absent (0). Bands with the same electrophoretic mobility were considered allelic, and bands with different mobility were considered nonallelic. Binary matrices were prepared for analysis with numerical taxonomy system (NTSYSpc) version 2.10t (Rohlf 1997). The Jaccard similarity coefficient (Jaccard 1908) was determined to approximate the genetic similarity of the lines. Similarity matrices were used for cluster analysis with the Unweighted Pair Group Method with the Arithmetic Averages (UPGMA; Sneath and Sokal 1973) clustering approach and principal coordinate analysis (PCO). Bootstrap analysis was performed using WINBOOT (Yap and Nelson 1996) to determine the strength of the clusters. The Polymorphic Information Content (PIC; Botstein et al. 1980) was calculated as PICi=2 fi (1-fi), where PICi is the polymorphic information content of marker 'i'; fi is the frequency of the amplified alleles (bands present); and 1-fi is the frequency of the null alleles (bands absent).

Results/Discussion

AFLP Polymorphism and Polymorphic Information Content

Ten primer combinations (Table 3) were chosen to genotype 156 sea oats lines that have been selected for superior performance in natural beach environments. A total of 658 scoreable loci were examined within the 50 to 300 base pair range. The number of scoreable loci generated by each primer combination ranged from 47 to 87 (Table 3). Of the 658 loci scored, 284 were polymorphic (Table 3). The number of polymorphic loci generated by each primer combination ranged from 12 to 39 (Table 3), and the percent polymorphism per primer combination ranged from 25% in *Eco*RI-CAG + *Mse*I-AGC to 56% in *Eco*RI-CAG + *Mse*I-CAA with an average polymorphism rate of 43% (Table 3). The Polymorphic Information Content (PIC), a measure of the usefulness of a particular primer combination, ranged from 0.04 in *Eco*RI-AGG + *Mse*I-CAA to 0.09 in *Eco*RI-AGG + *Mse*I-CAA (Table 3) which is very low considering a perfect PIC value is 0.5 and represents markers with 50% in each amplified and null allele group.

Previous work performing AFLP with 12 primer combinations on unselected sea oats lines from a wide geographic range identified 703 scoreable loci, including 417 polymorphic loci (Subudhi et al. 2005). The average polymorphism rate was found to be 59% with a range of 42% to 81% which is higher than the average 43% and range 25% to 56% polymorphism rate found in this study of selected lines. The decrease in polymorphism was expected, because selection often reduces the genetic diversity, and can therefore reduce the presence of polymorphic alleles in a population. The lower polymorphism rate may also be partly attributed to slight differences in procedure and primer combinations used. The previous work was performed using silver-stain AFLP technique which is more labor intensive but can produce scoreable bands at a slightly higher base pair range (50-300) than the Li-Cor 4300 DNA Analyzer AFLP technique that was

used in this study. The PIC in the previous study was much higher, 0.15 to 0.34, as compared to 0.04 and 0.09 for this study which may indicate that the primer combinations used in the previous research were more useful for sea oats genotypes.

Jaccard Similarity

Jaccard similarity coefficients calculated between pairs of lines ranged from 73% to 98% among lines which were very similar, but slightly higher, than unselected lines whose Jaccard similarity coefficients ranged from 69% to 99%. This suggests that selections have not made a significant negative impact on the genetic diversity of the lines in the LSU AgCenter's Coastal Plants Breeding Program.

Cluster Analysis

Using the Jaccard similarity coefficients, Unweighted Pair Group Method with Arithmatic Mean (UPGMA) cluster analysis produced two major clusters at the 90% similarity threshold (Figure 1) in addition to one line with an unknown state of origin that showed only 73% genetic similarity to all other lines. This unknown line's genetic diversity and survival after a major hurricane has made it a plant of interest for future breeding efforts. A bootstrap value of 73 between the 2 major clusters supported the robustness of the dataset as well as the genetic distance findings that were calculated. Cluster I contained 147 lines with approximately 90.2% similarity, and cluster II contained 8 lines with approximately 92.8% similarity. Distinct clustering by state was not found. Determination of clustering by state was made complicated by 51 lines whose state of origin is unknown. These lines were collected at Holly Beach, LA after surviving Hurricane Rita, a major storm event that destroyed the order and majority of research plantings. Due to the disturbance, identification of these lines was impossible, but they were selected as superior lines because of their resilience after a major storm event. These lines, as expected, are scattered throughout the dendrogram because they are expected to have multiple states of origin.

In order to determine the relationship between only the plants with known states of origin, we produced a dendrogram using only the 105 lines with a known state of origin (data not shown). All lines were 87% similar which suggested that the unknown origin lines increase the population's genetic diversity. Two major clusters were again produced with Cluster I containing 101 lines and Cluster II containing four lines. In this analysis, clustering by state was slightly more apparent. A total of 41 lines collected from Louisiana were examined in this study, and 36 of these lines clustered with at least one other Louisiana-derived line in a total of 11 separate areas throughout the dendrogram (data not shown). Also, of the 40 Florida-derived lines, 36 lines are grouped with other Florida lines in a total of 11 separate areas throughout the dendrogram. Cluster II was composed of only North Carolina lines (Data not shown). In both dendrograms, 2 of the 4 Caminada lines grouped together in a subcluster of lines collected from Florida, and the other 2 grouped near Florida and Louisiana lines. This information is especially

important because the genetic relatedness of the released Caminada line has not been previously studied. This line comes from collections made in Caminada, LA that were assumed to be natural populations; however, the actual origin of the plant tissue is unknown.

Principle Coordinate Analysis

Principle Coordinate Analysis (PCOA) explained 64% of the genetic variation among the 156 selected lines (Figure 3) which further validates the marker robustness and genetic similarity found in cluster analysis. Two distinct groups were found in PCOA (Figure 3). Lines from Cluster I seemed to be concentrated in Group 1, and Group II included lines from cluster II. PCOA of 105 lines of known origin (Figure 4) contained three groups, with lines from Cluster I in Groups 1 and 2, and lines from Cluster II in Group 3.

Conclusion

This study showed that genetic diversity can be retained in sea oats lines selected for vegetative performance in natural beach conditions, as well as in an applied breeding program for sea oats. While less genetic variation exists among selected lines than among unselected lines, there is still enough genetic variation within the breeding population to perform controlled crosses and create genetically distinct lines. These lines can be further evaluated, and well-adapted lines will eventually be released for beach restoration along the Gulf of Mexico coast.

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Appendix

Table 1: Selection environment, collection site, and state for 156 sea oats lines evaluated for genetic diversity.

		Selection Environment	Collection	
Line	**Alias**	**(Location; Year)**	**Site**	**State**
UP01NC-19-GP-3457	1054	Long Beach, MS; Summer 2005	Prince George	SC
UP01NC-19-GP-2229	1062	Long Beach, MS; Summer 2005	Prince George	SC
UP01NC-19-GP-2118	1079	Long Beach, MS; Summer 2005	Prince George	SC
UP01NC-19-GP-1312	1147	Long Beach, MS; Summer 2005	Prince George	SC
UP01NC-18-HB-1251	1149	Holly Beach, LA; Summer 2005	Unknown	SC
UP01NC-17-HB-1249	1091	Holly Beach, LA; Summer 2005	Unknown	SC
UP01NC-16-HB-1061	1020	Holly Beach, LA; Summer 2005	Unknown	SC
UP01NC-09-HB-1080	1123	Holly Beach, LA; Summer 2005	Unknown	NC
UP01NC-04-HB-3374	1101	Holly Beach, LA; Summer 2005	Unknown	NC
UP01NC-03-GP-1248	1014	Long Beach, MS; Summer 2005	Unknown	NC
UP01NC-02-GP-3113	1003	Long Beach, MS; Summer 2005	Unknown	NC
UP01LA-52-GP-1420	1023	Long Beach, MS; Summer 2005	Unknown	FL
UP01LA-39-HB-2306	1141	Holly Beach, LA; Summer 2005	Gulf Islands National Seashore	FL
UP01LA-39-GP-3269	1132	Long Beach, MS; Summer 2005	Gulf Islands National Seashore	FL
UP01LA-39-GP-2148	1099	Long Beach, MS; Summer 2005	Gulf Islands National Seashore	FL
UP01LA-38-HB-3125	1148	Holly Beach, LA; Summer 2005	Gulf Islands National Seashore	FL
UP01LA-38-GP-3348	1057	Long Beach, MS; Summer 2005	Gulf Islands National Seashore	FL
UP01LA-38-GP-1144	1134	Long Beach, MS; Summer 2005	Gulf Islands National Seashore	FL
UP01LA-37-HB-3252	1124	Holly Beach, LA; Summer 2005	Gulf Islands National Seashore	FL
UP01LA-37-HB-3113	1104	Holly Beach, LA; Summer 2005	Gulf Islands National Seashore	FL
UP01LA-37-GP-3119	1107	Long Beach, MS; Summer 2005	Gulf Islands National Seashore	FL
UP01LA-37-GP-1107	1161	Long Beach, MS; Summer 2005	Gulf Islands National Seashore	FL
UP01LA-34-HB-2351	1021	Holly Beach, LA; Summer 2005	St. Vincent National Wildlife Refuge	FL
UP01LA-34-GP-2439	1067	Long Beach, MS; Summer 2005	St. Vincent National Wildlife Refuge	FL
UP01LA-34-GP-1477	1118	Long Beach, MS; Summer 2005	St. Vincent National Wildlife Refuge	FL
UP01LA-33-HB-3028	1168	Holly Beach, LA; Summer 2005	Henderson Beach	FL
UP01LA-33-HB-1333	1150	Holly Beach, LA; Summer 2005	Henderson Beach	FL
UP01LA-33-GP-3149	1066	Long Beach, MS; Summer 2005	Henderson Beach	FL
UP01LA-33-GP-1305	1159	Long Beach, MS; Summer 2005	Henderson Beach	FL
UP01LA-33-GP-1303	1151	Long Beach, MS; Summer 2005	Henderson Beach	FL
UP01LA-31-HB-2171	1133	Holly Beach, LA; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-31-GP-3322	1075	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL

Table 1 (continued): Selection environment, collection site, and state for 156 sea oats lines evaluated for genetic diversity.

		Selection Environment	Collection	
Line	**Alias**	**(Location; Year)**	**Site**	**State**
UP01LA-31-GP-3138	1053	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-31-GP-3103	1001	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-31-GP-2271	1025	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-31-GP-1326	1145	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-30-GP-2466	1016	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-30-GP-2432	1139	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-30-GP-1349	1146	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-28-GP-3338	1013	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-28-GP-3272	1166	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-27-GP-3407	1084	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-27-GP-3403	1162	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-27-GP-3401	1012	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-27-GP-3208	1129	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-27-GP-3201	1024	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-26-HB-1101	1114	Holly Beach, LA; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-26-GP-3323	1031	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-26-GP-2370	1058	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-26-GP-2357	1097	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-26-GP-1444	1135	Long Beach, MS; Summer 2005	Santa Rosa Island, Eglin Air Force Base	FL
UP01LA-19-HB-2341	1126	Holly Beach, LA; Summer 2005	West end of Dauphin Island	AL
UP01LA-19-GP-3456	1094	Long Beach, MS; Summer 2005	West end of Dauphin Island	AL
UP01LA-19-GP-2246	1050	Long Beach, MS; Summer 2005	West end of Dauphin Island	AL
UP01LA-19-GP-2215	1137	Long Beach, MS; Summer 2005	West end of Dauphin Island	AL
UP01LA-17-HB-2320	1088	Holly Beach, LA; Summer 2005	Port Arthur	TX
UP01LA-16T-HB-3032	1055	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16S-GP-3138	1049	Long Beach, MS; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16S-GP-2418	1122	Long Beach, MS; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16S-GP-1154	1005	Long Beach, MS; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16Q-HB-1060	1095	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16P-HB-1100	1130	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16O-HB-3224	1051	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16O-HB-3072	1019	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL

Table 1 (continued): Selection environment, collection site, and state for 156 sea oats lines evaluated for genetic diversity.

		Selection Environment	Collection	
Line	**Alias**	**(Location; Year)**	**Site**	**State**
UP01LA-16N-HB-2269	1128	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16N-GP-1231	1008	Long Beach, MS; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16K-HB-2271	1004	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16K-HB-1292	1163	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16J-HB-2327	1086	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16I-HB-2307	1048	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16I-HB-1329	1158	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16H-HB-3005	1102	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16H-HB-2329	1120	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16H-HB-2268	1165	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16H-GP-3132	1160	Long Beach, MS; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16-HB-2365	1074	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16-HB-2288	1092	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16-HB-2147	1090	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16-GP-2352	1077	Long Beach, MS; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16-GP-1355	1027	Long Beach, MS; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16F-GP-1473	1081	Long Beach, MS; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16E-HB-1041	1164	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16E-GP-3314	1096	Long Beach, MS; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16E-GP-2424	1022	Long Beach, MS; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16E-GP-1259	1119	Long Beach, MS; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16D-HB-1353	1117	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16D-GP-2201	1127	Long Beach, MS; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16B-HB-2129	1142	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16A-HB-3052	1125	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-16A-HB-2287	1152	Holly Beach, LA; Summer 2005	Fourchon Beach-FL Populations	FL
UP01LA-15S-HB-2032	1111	Holly Beach, LA; Summer 2005	Fourchon Beach-LA Populations	LA
UP01LA-15P-HB-2354	1093	Holly Beach, LA; Summer 2005	Fourchon Beach-LA Populations	LA
UP01LA-15K-HB-3092	1045	Holly Beach, LA; Summer 2005	Fourchon Beach-LA Populations	LA
UP01LA-15I-HB-2230	1143	Holly Beach, LA; Summer 2005	Fourchon Beach-LA Populations	LA
UP01LA-15F-HB-3340	1155	Holly Beach, LA; Summer 2005	Fourchon Beach-LA Populations	LA
UP01LA-15B-HB-3173	1006	Holly Beach, LA; Summer 2005	Fourchon Beach-LA Populations	LA

Table 1 (continued): Selection environment, collection site, and state for 156 sea oats lines evaluated for genetic diversity.

		Selection Environment	Collection	
Line	**Alias**	**(Location; Year)**	**Site**	**State**
UP01LA-15B-HB-1090	1083	Holly Beach, LA; Summer 2005	Fourchon Beach-LA Populations	LA
UP01LA-09-GP-2435	1015	Long Beach, MS; Summer 2005	Hwy 53 NE Newport Pass	TX
UP01LA-01-HB-2248	1140	Holly Beach, LA; Summer 2005	Hwy 87, Bolivar Peninsula, Flake	TX
UP01LA-01-HB-2153	1052	Holly Beach, LA; Summer 2005	Hwy 87, Bolivar Peninsula, Flake	TX
UP01LA-01-HB-1171	1100	Holly Beach, LA; Summer 2005	Hwy 87, Bolivar Peninsula, Flake	TX
1	1034	Holly Beach, LA; Fall 2005	Unknown	Unknown
2	1056	Holly Beach, LA; Fall 2005	Unknown	Unknown
3	1029	Holly Beach, LA; Fall 2005	Unknown	Unknown
4	1040	Holly Beach, LA; Fall 2005	Unknown	Unknown
6	1047	Holly Beach, LA; Fall 2005	Unknown	Unknown
8	1032	Holly Beach, LA; Fall 2005	Unknown	Unknown
9	1033	Holly Beach, LA; Fall 2005	Unknown	Unknown
10	1026	Holly Beach, LA; Fall 2005	Unknown	Unknown
11	1028	Holly Beach, LA; Fall 2005	Unknown	Unknown
12	1063	Holly Beach, LA; Fall 2005	Unknown	Unknown
14	1064	Holly Beach, LA; Fall 2005	Unknown	Unknown
15	1042	Holly Beach, LA; Fall 2005	Unknown	Unknown
16	1078	Holly Beach, LA; Fall 2005	Unknown	Unknown
17	1044	Holly Beach, LA; Fall 2005	Unknown	Unknown
18	1038	Holly Beach, LA; Fall 2005	Unknown	Unknown
19	1036	Holly Beach, LA; Fall 2005	Unknown	Unknown
20	1070	Holly Beach, LA; Fall 2005	Unknown	Unknown
21	1060	Holly Beach, LA; Fall 2005	Unknown	Unknown
22	1071	Holly Beach, LA; Fall 2005	Unknown	Unknown
23	1041	Holly Beach, LA; Fall 2005	Unknown	Unknown
24	1059	Holly Beach, LA; Fall 2005	Unknown	Unknown
25	1072	Holly Beach, LA; Fall 2005	Unknown	Unknown
27	1035	Holly Beach, LA; Fall 2005	Unknown	Unknown
28	1017	Holly Beach, LA; Fall 2005	Unknown	Unknown
29	1154	Holly Beach, LA; Fall 2005	Unknown	Unknown
31	1061	Holly Beach, LA; Fall 2005	Unknown	Unknown
32	1112	Holly Beach, LA; Fall 2005	Unknown	Unknown

Table 1 (continued): Selection environment, collection site, and state for 156 sea oats lines evaluated for genetic diversity.

		Selection Environment	Collection	
Line	**Alias**	**(Location; Year)**	**Site**	**State**
33	1030	Holly Beach, LA; Fall 2005	Unknown	Unknown
34	1116	Holly Beach, LA; Fall 2005	Unknown	Unknown
35	1076	Holly Beach, LA; Fall 2005	Unknown	Unknown
36	1068	Holly Beach, LA; Fall 2005	Unknown	Unknown
37	1073	Holly Beach, LA; Fall 2005	Unknown	Unknown
UNK-9 (UNKNOWN-9)	1109	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-8 (UNKNOWN-8)	1103	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-6 (UNKNOWN-6)	1089	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-5 (Unknown-5)	1087	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-4 (UNKNOWN-4)	1037	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-3 (UNKNOWN-3)	1018	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-21 (UNKNOWN-21)	1177	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-20 (UNKNOWN-20)	1176	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-2 (UKNOWN-2)	1009	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-19 (UNKNOWN-19)	1175	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-18 (UNKNOWN-18)	1174	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-17 (UNKNOWN-17)	1173	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-16 (UNKNOWN-16)	1172	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-15 (UNKNOWN-15)	1171	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-14 (UNKNOWN-14)	1170	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-13 (UNKNOWN-13)	1169	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-12 (UNKNOWN-12)	1157	Holly Beach, LA; Spring 2006	Unknown	Unknown
UNK-11 (UNKNOWN-11)	1156	Holly Beach, LA; Spring 2006	Unknown	Unknown
UKN-1 (UKNOWN-1)	1002	Holly Beach, LA; Spring 2006	Unknown	Unknown
Caminada-HB-2267	1039	None	Caminada	LA
Caminada	1178	None	Caminada	LA
Caminada	1179	None	Caminada	LA
Caminada	1180	None	Caminada	LA

Table 2: Amplified Fragment Length Polymorphism (AFLP) primer sequences used to determine the genetic diversity of 156 sea oats lines.

AFLP Primer	Sequence (5' to 3')	Function
EcoRI Adapter 1	CTCGTAGACTGCGTACC	EcoRI Adapter for DNA digestion
EcoRI Adapter 2	AATTGGTACGCACTC	EcoRI Adapter for DNA digestion
MseI Adapter 1	GACGATGAGTCCTGAG	MseI adapter for DNA digestion
MseI Adapter 2	TACTCAGGACTCAT	MseI adapter for DNA digestion
EcoRI Pre	GACTGCGTACCAATTC	EcoRI Preamplification primer
MseI Pre	GATGAGTCCTGAGTAA	MseI Preamplification Primer
MseI AgC	GATGAGTCCTGAGTAAAGC	Selective amplification primer
MseI CAA	GATGAGTCCTGAGTAACAA	Selective amplification primer
MseI CCA	GATGAGTCCTGAGTAACCA	Selective amplification primer
EcoRI CAA 700*	GACTGGTACCAATTCCAA	Selective amplification primer
EcoRI AGG 700*	GACTGGTACCAATTCAGG	Selective amplification primer
EcoRI CAG 800*	GACTGGTACCAATTCCAG	Selective amplification primer
EcoRI AAG 800*	GACTGGTACCAATTCAAG	Selective amplification primer

*IRDye flourescent label at 5'-end

Table 3. Polymorphism in sea oats lines using 10 AFLP primer combinations.

Primer Combination	Total Loci	Polymorphic Loci	Polymorphism (%)	PIC
*Eco*RI-AGG + *Mse*I-CAA	73	29	40	0.09
*Eco*RI-CAG + *Mse*I-CAA	68	38	56	0.07
*Eco*RI-AGG + *Mse*I-CAA	47	14	30	0.04
*Eco*RI-AAG+ *Mse*I-CAA	55	25	45	0.08
*Eco*RI-CAA + *Mse*I-CAA	77	42	55	0.08
*Eco*RI-CAG + *Mse*I-CAA	52	18	35	0.08
*Eco*RI-AGG + *Mse*I-CCA	87	41	47	0.05
*Eco*RI-AAG + *Mse*I-CCA	65	26	40	0.06
*Eco*RI-CAA + *Mse*I-AGC	86	39	45	0.07
*Eco*RI-CAG + *Mse*I-AGC	48	12	25	0.06
Total	**658**	**284**	**43**	**0.07**

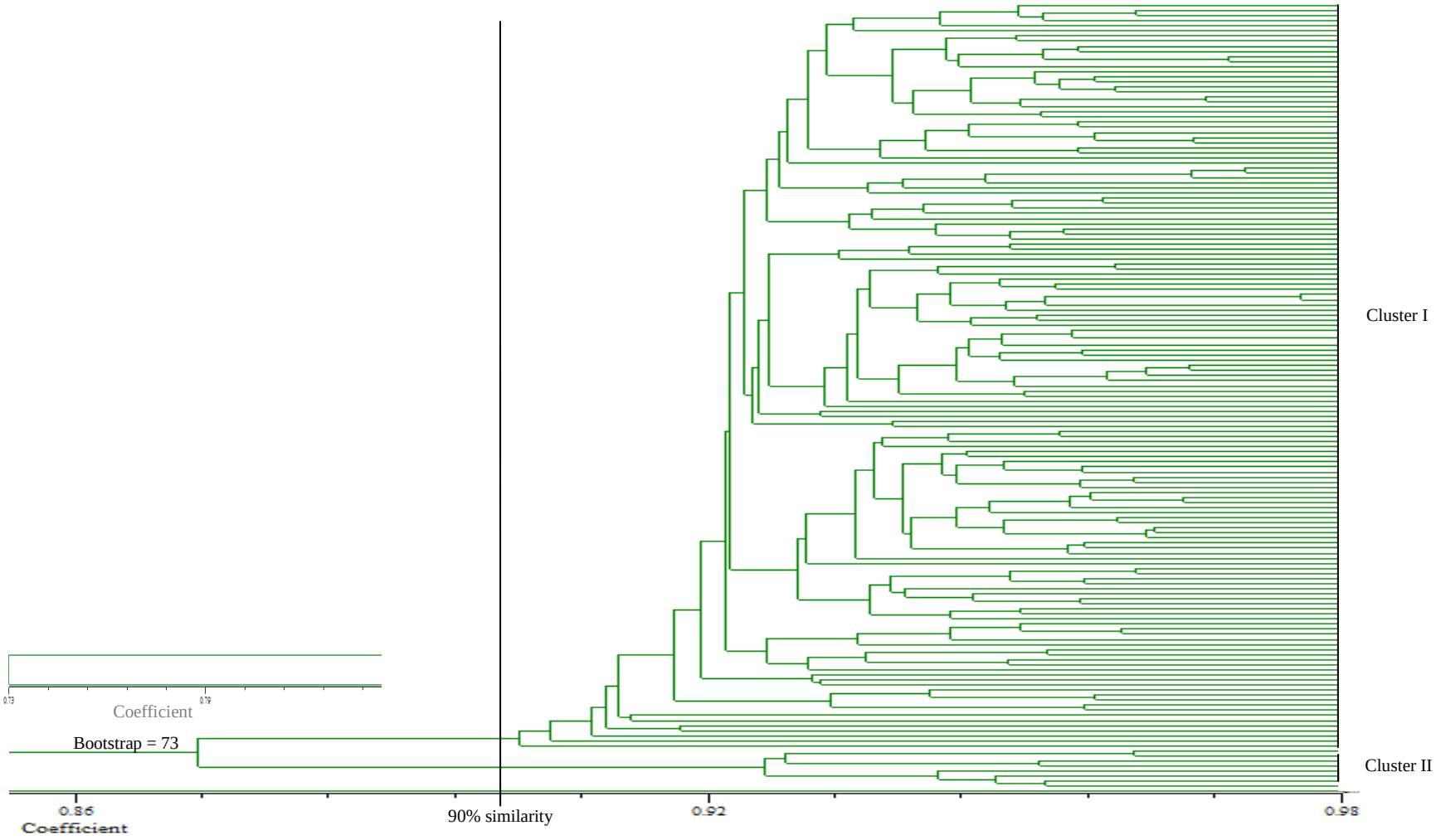

Figure 1. Dendrogram of UPGMA Cluster Analysis of 156 selected sea oats lines based on the Jaccard similarity coefficient matrix from 10 Amplified Fragment Length Polymorphism (AFLP) primer combinations with 658 total loci.

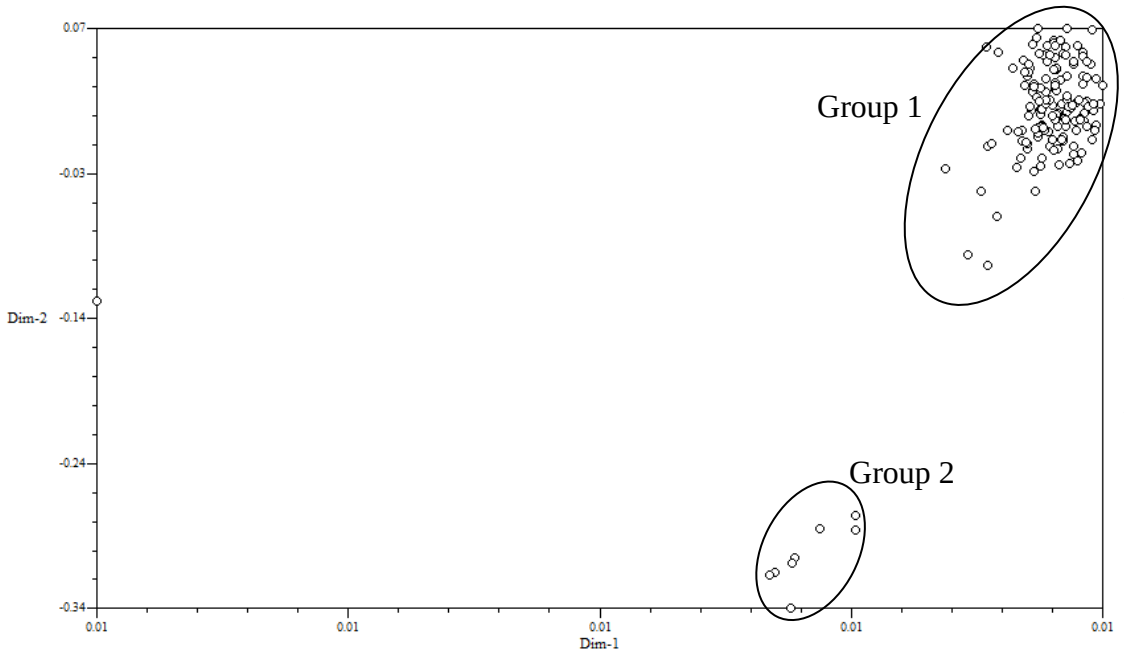

Figure 3. Principle Coordinate Analysis of 156 sea oats lines based on the Dice similarity coefficient.

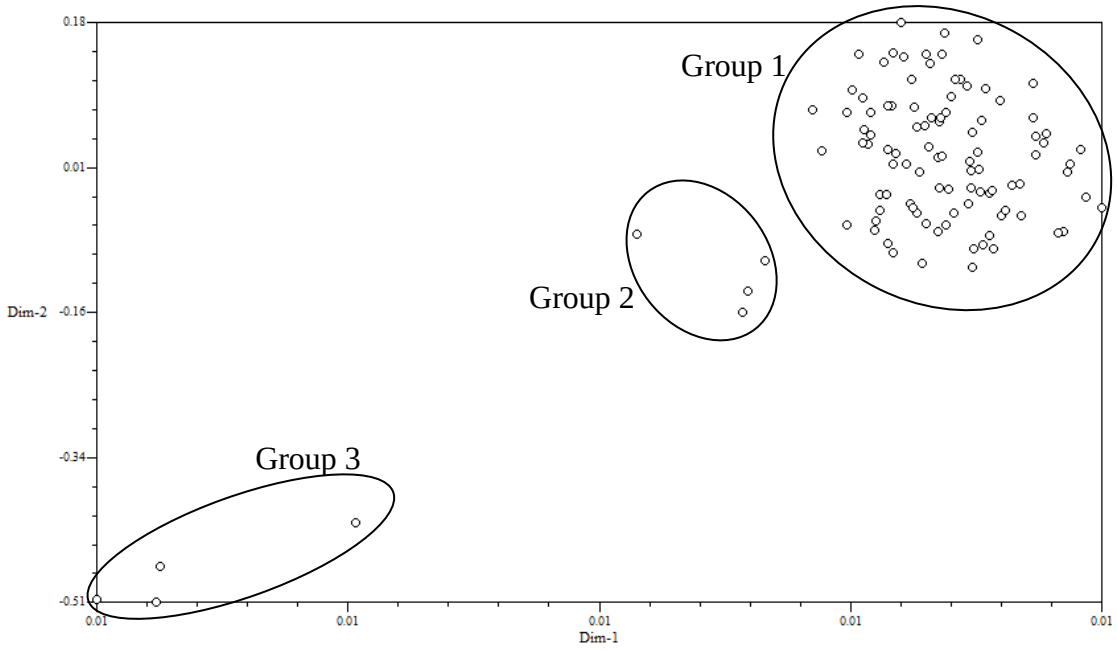

Figure 4. Principle Coordinate Analysis of 105 sea oats lines based on the Dice similarity coefficient.