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Identification and Genetic Characterization of Smooth Cordgrass for Coastal Wetland Restoration

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ABSTRACT

Spartina alterniflora (Loisel.), smooth cordgrass, is a dominant perennial salt marshgrass native to tidal wetland plant communities along the Atlantic and Gulf coasts of North America. It is an important plant species for coastal reclamation and restoration efforts. It spreads quickly by rhizomes and tolerates a wide range of saline, anoxic, and sulfidic soils. However, because of poor seed production this species is propagated vegetatively for reclamation or restoration projects. Current practices reduce genetic diversity of the species by utilizing a single vegetatively propagated genotype. The objectives of this study were; 1) identify multiple genotypes of S. *alterniflora* for use in restoration efforts and 2) assess genetic variability of these genotypes at the molecular level. Identification of desirable plants was based on plant growth characteristics and reproductive traits. Growth characters were plant height, spread, rust reaction, and vigor. Reproductive traits were seed set, germination, kernel weight, seed weight, and total seed per plant. This process resulted in seven plants, descended from seven different original source populations, which demonstrated superior performance for vegetative and reproductive traits. Molecular marker analysis revealed that genetic diversity, essential for success in the restoration projects, was maintained in selected plants. These plants offer an enhanced germplasm base for current restora don efforts. Research is continuing on the feasibility of developing seed based populations that would add even greater genetic diversity to the current accepted restoration practices.

Key words: AFLP, Genetic diversity; *Spartina alterniflora•,* marshland restoration.

INTRODUCTION

Smooth cordgrass, is a dominant perennial salt marshgrass found in tidal wetland plant communities along the Atlantic and Gulf coasts of North America (Valiela et al. 1978). It is an important native plant species for coastal reclamation and restoration efforts. It spreads quickly by rhizome, is tolerant of a wide range of salinity (Nestler 1977, Hester et al. 1998) and tolerates anoxic (Mendelssohn et al. 1981) and sulfidic soils (Chambers et al. 1998). Because of poor seed production (Hubbard 1970, Broome et al. 1974, Bertness et al. 1987, Sayce 1988, Callaway and Josselyn 1992) this species is propagated vegetatively for reclamation or stabilization projects

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(Broome et al. 1988). The cultivar Vermilion', a vegetatively propagated clone representing a single genotype was selected for superior establishment and growth characteristics. This single genotype has been widely disseminated by the Natural Resource Conservation Service (NRCS) for coastal reclamation and restoration projects along the Gulf coast.

While a large volume of data is now available on the succession dynamics of *Spartina alterniflora* in restored coastal marshes (Matthews and Minello 1994), less emphasis has been placed on evaluating genetic diversity of *S. alterniflora.* In periods of rapid environmental change, genetic diversity is essential for natural selection and maintenance of population viability (Hamrick et al. 1991). Such variability may be particularly important in S. *alterniflora,* a primarily out-crossing polyploid species (Somers and Grant 1981) that spreads readily by rhizomes and covers large areas with a few aggressive genotypes (Daehler et al. 1999). Moreover, genetic variability helps maintain high fitness potential among individuals with distinct genotypes for out-crossing.

The high labor cost of vegetative establishment (Oliver 1925, Broome et al. 1988) and the greatly reducedgenetic variability associated with current vegetative propagation of a single genotype support the need to identify multiple "genotypes from diverse populations and explore alternative strategies, such as seed based establishment, for reclamation and restoration projects. Genetically diverse seed-based populations would provide economic and environmental dividends. Variation for seed production does exist (Seneca 1974, Daehler and Strong 1994, Fang et al. 2004) and selection for superior seed producing populations should be possible. Although selection for seed production is critical, growth characteristics necessary for rapid establishment must not be excluded from the selection process. Similarly, genetic diversity must be maintained.

The objectives of this study were; 1) identify multiple genotypes of *S. alterniffora* for use in restoration efforts and 2) assess genetic variability of these genotypes at the molecular level.

MATERIALS AND METHODS

Source Materials and Selection Cycles

One hundred and twenty-six. S. *alterniflora* accessions were assembled beginning in October of 1998 (Fang et al. 2004). A group of plants with a similar phenotype from a small geographic area, approximately 100 m^2, was considered a clonal group and collected as a single accession. This collection was designated as a source population. Techniques used for panicle collection, seed storage and processing, and subsequent germination tests were reported by Fang et al. (2004). Based on the results of germination tests, evaluation of seedling survival, and seedling vigor, 20 seedlings from each of 20 accessions were selected for field evaluation. This group of 400 plants was designated as the cycle I population (Table 2).

After approximately 8 weeks of growth in the greenhouse, cycle 1 seedlings were transplanted on 5-6 October, 1999, to a field site at the Ben Hur Research Farm, Louisiana State University Agricultural Center, Baton Rouge, LA. Experimental design was a ;randomized complete block with four replications of five plants for each accession. Plants were transplanted to the field (Sharkey clay, very-fine, smectitic, thermic Chromic Epiaquerts) with a spacing of 2.4 x 2.4 m. The field was maintained in a flooded condition with occasional drainage for weed control and plot maintenance. Roundup® herbicide (Monsanto) was spot sprayed as needed to control weeds.

Plant height, spread, rust reaction *(Puccinia* sp.), tiller density, and plant vigor data were collected every 2 weeks during the spring and early summer growing season (23 February to 28 June, 2000). Plant height was measured from the soil surface to the uppermost leaf tip of the plant. To determine spread, plants were measured in two directions. The first measurement was parallel to the row and the second measurement was perpendicular to the first measurement. These two measurements were then used to estimate the soil surface area covered by a plant on a particular date (values reported were for late March). Rust reaction, tiller density, and plant vigor were rated on a 1-10 scale (where a '1' indicates no rust or excellent tiller density or vigor, and a `10' indicates severe rust infection or poor tiller density or vigor). Based on analysis of this data, 40 vegetative clones from 9 different accessions were selected and designated as cycle 2 plants (Table 4). These selected clones were from plants demonstrating the most rapid growth and spread and the best combination of low rust rating and high plant vigor and tiller density.

Cycle 2 plants were evaluated at two locations, the Ben Hur Farm, Baton Rouge, LA, and Grand Terre Island, Grand Terre, I.A. Grand Terre Island is an irregularly shaped island in Jefferson Parish located between. Barataria Bay and the Gulf of Mexico. This site represented a portion of the island restored with soil sediment from the Barataria Bay. Soil types were a Sharkey clay at Ben Hur and Scatlake (Very-fine, smectitic, nonacid, hyperthermic Sodic Hydraquents) at Grand Terre. The experimental design was a randomized complete block at both locations with four replications at Ben Hur and three replications at Grand Terre. Each replicadon contained five clones, spaced 2.4 m on center, from each of the 40 selected plants for a total of 200 plants per replication. Plants were established by transplanting on 21 July 2000 at Ben Hur and on 16 February 2001 at Grand Terre. Plants at Ben Hur were maintained in an artificial pond as previously described.

Plant height, spread, disease reaction, and plant vigor data were determined during the growing season as previously described. Trait variables were recorded from 8 March to 24 July, 2001, at the Ben Hur location and 29 May to 19 July, 2001, at the Grand Terre location. Seed set, kernel weight, and percent germination were evaluated at both locations using the methods for characterization previously described by Fang et al. (2004).

AFLP Analysis

Young leaf samples were collected from the 40 cycle 2 genotypes (Table 4). Leaf tissues were ground to fine powder using liquid nitrogen and were stored at -80°C until DNA isolation. Genomic DNA was isolated from frozen ground leaves using a modified potassium-acetate method (Tai and Tanksley 1990). DNA quality and quantity was determined by running a small aliquot of DNA on a 1% agarose gel. The

concentration of genomic DNA was estimated by comparing the size and intensity of each sample band with that of the DNA mass ladder standard (GIBCO).

The AFLP analysis was performed using the AFLP kit from LiCOR (Lincoln, Nebraska, USA). AFLP adapter and primers for both restriction enzymes, *Eco* RI and *Mse I* were the same as Vos et al. (1995). Sixty-four primer combinations were screened by selective amplification using parental template DNA. Twenty-six of these gave reliable and reproducible polymorphisms and were used to generate AFLP data (Table 7). A single PCR reaction generated AFLP products for two primer combinations using three AFLP primers of which two were labeled with IR700 and IR800 dyes. This duplex amplification was conducted by using the AFLP protocol developed for LiCOR selective amplification kits. The AFLP reaction products were electrophoresed in 6.5% KBPlus polyacrylamide gel (LiCOR) using a LiCOR 4300 DNA analysis system. The gels were pre-run for 25 minutes before loading the samples. The AFLP reaction products were denatured by heating the resulting mixtures for 3 min at 90°C and then quickly cooled on ice before loading the gel. Approximately 0.8 pL of each denatured sample and 0.8 pL of the molecular sizing standard (50-700 bp) were loaded after every 8 DNA samples to determine fragment size, to adjust for uneven band migration on the gel (necessary for Genelmage IR software), and to facilitate different gel comparisons. Samples were electrophoresed for 3 hours to resolve fragments up to 700 bp. The lRDye labeled AFLP data (real-time TIF images) were automatically collected and recorded during electrophoresis. A composite image of the gel was later inspected to delete artifacts and to score bands not detected by the Gene Image IR software. Bands were then scored for presence (1) and absence (0) of bands on an Excel® spreadsheet program.

Data Analysis

Univariate and multivariate techniques (Johnson and Wichern 2002) were used to analyze the data. Principal component, biplot (Gabriel 1971) and cluster analyses of accessions and locations were done with SAS v6.2 and 8.2 (SAS Institute 1999). Analysis of variance (ANOVA) was used to reveal the main effects and interactions of independent variables on an interval dependent variable. Multiple analysis of variance (MANOVA) was used to determine the main effects and interactions of variables on multiple dependent interval variables. Correlation coefficients were used to evaluate the relationship among the different variables in the experiment (Freund and Wilson 1997). The biplot method used was aJK (principal component ($\alpha = 1$)), and the cluster method was Ward (Ward 1963) using Euclidean distance to compute dissimilarity. The cluster method and distance were selected to be consistent with the method for biplot analysis (Johnson and Wichern 2002).

Genetic Diversity Analysis

AFLP data were analyzed as follows; 1) genetic similarity was calculated using Jaccard's coefficient (Jaccard 1908), 2) Similarity matrices were subjected to cluster analysis by the Unweighted Paired Group Methods of Arithmetic Average (UPGMA) (Sneath and Sokal, 1973), and (3) dendrograms were constructed with the Tree command.

RESULTS

Source Population Evaluation

Evaluation of reproductive traits for the native S. *alterniflora* accessions revealed considerable variation among the 126 accessions (Table 1). Mean germination among accessions was significantly different and ranged from 0 to 57%, and seed weight per accession (100 seed) ranged from 90 to 279 mg. Data from the collections across South Louisiana showed that 65% of the accessions had less than 10% germination. Mean 100-seed weight also varied among the 126 accessions and ranged from 90 to 279 mg with an average of 162.9 mg.

There was no significant correlation between date of collection and seed weight or percent germination, but a positive correlation was noted between seed weight and percent germination ($r = 0.257$; $P < 0.01$). To determine effect of collection site on trait diversity; cluster analysis by location (Figure 1) was performed using information from all three traits. Four groups were described, with a main differentiation observed between St. Tammany and the other three groups. This analysis also helped to identify the level of geographic diversity, based on collection site of parental accession, being maintained among cycle 1 selections. Genotypes from the other three groups, including accessions from eight parishes, with the exception of St. Tammany, were subsequently represented in the cycle 2 selections.

From the distribution of seed weight and percent germination, multivariate ANOVA, using average values, was used to compare accessions. Significant differences for seed weight and germination rate were detected among accessions from different collection dates. Differences among collection sites were significant for percent germination but not for seed weight. Therefore, accessions were characterized solely on percent germination and plants were identified for the cycle 1 population.

TABLE 1. MEAN, STANDARD DEVIATION, AND RANGE FOR DATE OF COLLECTION, SEED WEIGHT AND PERCENT OF GERMINATION AMONG 126 NATIVE *SPARTINA ALTERNIFLORA* ACCESSIONS COLLECTED IN SOUTH LOUISIANA DURING 1998.

Variable	N	Mean	Std. Dev.	Min.	Max.
Collection date (days after 31 Oct.)	126	28.6	9.4	0	47
Seed weight (mg/100 seed)	126	163	33	90	279
Germination (percent)	126	9.5	11.6	0	57

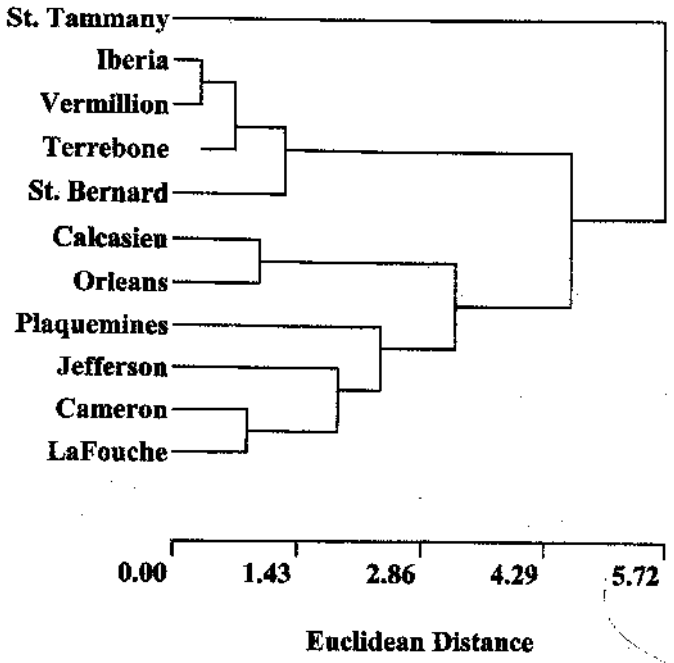

Figure 1. Cluster analysis of original *Spartina alterniflora* accessions, by Parish of origin, for seed weight, percent germination, and collection date (Ward's method).

Cycle 1 Evaluation

Based on results of the source population evaluation, 20 plants from each of 20 accessions were evaluated (Table 2). The results of this analysis, based on parental accession collection site and analysis by parental accession, demonstrated a similar pattern for the relationship between plant height and several other traits. For example, as plant height increased, plant vigor and tiller density rating also tended to increase. However, the interpretation of observations for these traits among accessions was further investigated using biplot analyses.

In Figure 2, adjacent vectors corresponding to height, spread, tiller density, and vigor all point in the same direction, indicating a positive correlation. The vector corresponding to flowering date is almost perpendicular to height, spread, tiller density and vigor, indicating no correlation with them. On the other hand, the vector for rust reaction is pointing in an opposite direction indicating a negative correlation between rust reaction and height, spread, vigor, and tiller density. Similarity among accessions and magnitude of trait variability are indicated from this biplot (Figure 2). For example, plants from accessions 98NR70 and 98NR61 were much more similar in their response to rust infection than were plants from accessions 98NR82 and 98NR86. The plants from accessions 98NR70 and 98NR61 were more susceptible to rust than the plants from accessions 98NR19 and 98NR47.

Biplot and cluster analyses, for the selected plants within accession collection sites (Figure 3) revealed that plants from Iberia, Orleans and Calcasieu accessions were grouped on the right side (Figure 3), which indicated reduced vegetative growth and increased rust reaction for this group. On the other hand, plants from the Vermilion, Lafourche, Terrebonne, and Cameron accessions demonstrated larger values for vegetative growth, and reduced rust reaction. It was possible to identify relationships between traits and collection sites using this approach. For example, plants from the Vermilion and Cameron accessions were much more similar in their mean vigor than plants from the Lafourche and Jefferson accessions. Among plant variation for spread of plants from within Lafourche was less than the among plant variation for spread of plants from the Vermilion and Cameron accessions. Similar comparisons for other traits were readily observable. The magnitude of the trait also increased with distance from the vector origin. Cluster analysis (data not shown) further confirmed the grouping of plants from Iberia, Orleans and Calcasieu accessions indicating maximum differentiation compared to other sites. This was again supported by low mean values for the evaluated traits for the plants of the Calcasieu-Iberia-Orleans group. Similarly, plants from the Vermilion and Cameron accessions were clustered together within Terrebonne, Jefferson, and Lafourche.

As in the previous evaluation of the source population, this type of analysis is important because it distinguishes between collection site and parental accession and improves selection efficiency. It was possible, based on the cluster and biplot analysis, to determine that selected plants from these accessions were a representative sample of three of the four main groups previously described by cluster analysis (Figure 1). The sole exception was the St. Tammany group, which was not represented.

Multivariate ANOVA was conducted to examine the relationship among the different traits included in this analysis. Highly significant ($P < 0.0001$) differences occurred among accessions for traits measured during the vegetative stage (plant height, spread, vigor, tiller density, rust reaction). A similar trend was also noted for days to first panicle emergence. Significant positive correlations were observed among all vegetative traits representing plant growth ($P < 0.01$), whereas negative correlation was observed between those traits and disease reaction ($P< 0.05$) (Table 3). Positive correlations between height, spread, and tiller density vs. vigor were anticipated since these traits contribute significantly to expression of vigor. Flowering was positively correlated with spread ($P < 0.05$; $r = 0.32$), and negatively correlated with tiller density ($P < 0.05$; $r = -0.17$), but no correlation was found for flowering with plant height, disease reaction or vigor. Late flowering accessions were associated with increased spread and tiller density, but not with the other traits.

Forty plants were selected from the 400 cycle 1 plants, across accessions, based on plant height, vigor, spread, and rust reaction of individual plants. The selected plants represented nine parental accessions; five plants each from seven accessions (98NR7, 98NR8, 98NR26, 98NR27, 98NR47, 98NR82, and 98NR99), four plants from accession 98NR38, and one plant from accession 98NR109.

Cycle 2 Evaluation

Mean plant height, spread, .disease reaction, and vigor were different between the Ben Hur and Grand Terre locations (Table 4). Mean height and mean spread were both

TABLE 2. MEAN VALUES FOR PLANT HEIGHT, SPREAD, RUST REACTION, TILLER DENSITY, VIGOR, AND DAYS TO FIRST FLOWER BASED ON 400 CYCLE 1 *SPARTINA ALTERNIFLORA* PLANTS. FROM EACH OF 20 PARENTAL ACCESSIONS, 20 PLANTS WERE DERIVED.

Origin	Parental accession	Height (cm[a])	Spread (m^2)[b]	Rust reaction[c]	Tiller density[c]	Vigor[c]	Days to first flower[d]
Lafourche	98NR7	142	1.67	2.8	7.0	7.7	73
Vermilion	98NR38	134	1.15	3.1	6.2	7.1	86
Cameron	98NR86	133	1.15	2.2	5.9	7.8	82
Lafourche	98NR30	128	1.31	3.7	5.7	6.1	74
Cameron	98NR82	127	0.91	2.4	6.0	7.6	81
Calcasieu	98NR72	124	0.80	3.8	4.7	5.4	88
Terrebonne	98NR27	120	1.46	3.1	6.2	6.8	79
Lafourche	98NR26	118	1.41	3.6	6.4	6.4	73
Lafourche	98NR8	117	1.67	2.3	5.6	7.0	73
Cameron	98NR47	116	0.58	3.3	5.3	6.5	63
Calcasieu	98NR61	113	0.74	5.8	4.6	3.2	73
Terrebonne	98NR98	112	1.24	3.8	5.2	6.4	76
Jefferson	98NR99	110	1.49	2.8	6.1	6.4	70
Terrebonne	98NR109	109	1.35	3.5	5.5	6.4	82
Calcasieu	98NR70	107	0.90	4.5	4.7	3.7	67
Jefferson	98NR107	106	1.42	3.5	5.6	5.8	69
Jefferson	98NR19	102	1.12	4.1	5.4	5.2	78
Calcasieu	98NR71	95	0.57	4.0	4.4	3.7	75
Orleans	98NR25	95	0.78	3.4	4.2	5.1	78
Iberia	98NR60	83	0.84	3.2	4.6	4.4	84
Mean		115	1.13	3.4	5.5	5.9	76
CV		15	37.8	35.95	29.1	29.8	13.5
LSD (0.05)		10.5	0.25	1.3	1.2	1.0	4.2

[a]Height was measured from ground to the uppermost leaf tip of the plant (cm).
[b]Spread was measured as an area covered by the plant (m^2).
[c]Vigor, rust reaction, and tiller density were visually estimated on a scale of 1-10 (1 = excellent; 10 = poor)
[d]Flowering date was recorded as days of first panicle emergence after July 1.

lower at the Grand Terre location. Mean height was 137 and 99 cm, and mean spread was 1.8 and 1.1 m^2 for Ben Hur and Grand Terre, respectively. These differences can at least be partially explained by the later establishment date for the Grand Terre location resulting in reduced plant growth for comparable observation dates. However, disease reaction and plant vigor were not as clearly related. Disease reaction was lower at Grand Terre (2.5 vs. 3.8) but vigor rating was also reduced relative to Ben Hur (6.1 vs. 4.9). Although the plants at Grand Terre were not as impacted by disease, this

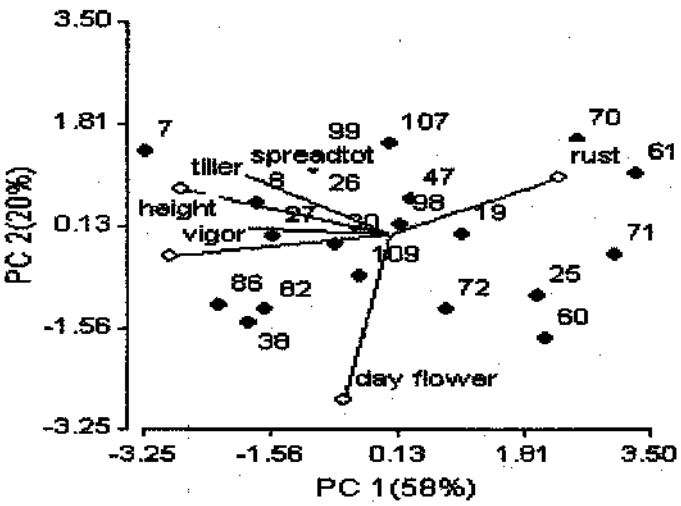

Figure 2. Biplot analysis for 400 cycle 1 *Spartina alterniflora* plants selected from the source population for height, spread, rust reaction, tiller density, vigor, and flowering date.

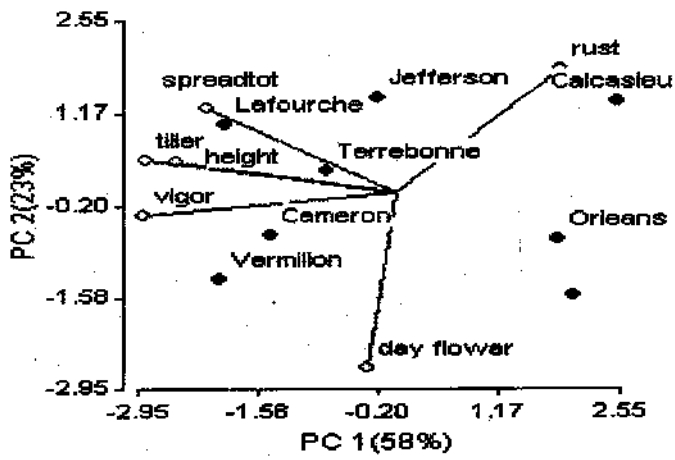

Figure 3. Biplot analysis, by collection site of parental accession, for 400 cycle 1 *Spartina alterniflora* plants selected from the source population, for height, spread, rust reaction, tiller density, vigor, and flowering date.

TABLE 3. PARTIAL CORRELATION COEFFICIENTS, BY COLLECTION SITE, FOR 400 CYCLE 1 *SPARTINA ALTERNIFLORA* PLANTS EVALUATED FOR HEIGHT, SPREAD, VIGOR, TILLER DENSITY AND RUST REACTION. FROM EACH OF 20 PARENTAL ACCESSIONS, 20 PLANTS WERE DERIVED.

Variable	Spread	Vigor	Rust reaction	Tiller density	Days to first flower
Height	0.37**	0.54**	-0.18**	0.48**	0.05 ns
Spread		0.43**	-0.11*	0.48**	0.32*
Vigor			-0.48**	0.60**	-0.1
Rust reaction				-0.21**	0.04
Tiller density					-0.17*

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.

finding was not reflected in overall vigor. Correlations of disease rating and vigor within location were consistent with this finding and did not reveal any significant relationship.

There was a genotype by location interaction for plant height and plant spread. However, plant height at Grand Terre was positively correlated with plant height at Ben Hur ($P < 0.0001$; $r = 0.59$). Similarly, plant spread at Grand Terre was positively correlated with plant spread at Ben Hut ($P = 0.013$; $r = 0.38$) (Table 5). These results would indicate that the interaction was one of magnitude and not direction. Therefore, this observed interaction does not preclude the feasibility of selection in an artificial environment and the expectation of similar relational, although not absolute, genotype performance in a natural environment.

There was no genotype by location interaction observed for disease reaction or plant vigor. Neither disease reaction nor plant vigor was correlated between locations. Plants at Grand Terre, in the restored soil sediment from the Barataria Bay appeared to be less impacted by disease although, as previously mentioned, height and spread measurements and vigor rating at Grand Terre did not indicate any superior growth performance. However, disease reaction was correlated with plant height within the Ben Hur and within the Grande Terre locations. Plant vigor rating was not correlated across or within locations and may not be a useful selection index.

Seed production data for Ben Hur and Grand Terre are presented in Table 6. Means for kernel weight, seed set and germination differed among entries within location but overall means for these variables, across all entries, did not differ between locations. Correlations among the variables kernel weight, seed set and germination, between locations, resulted in a positive relationship between Ben Hur germination rates and Grand Terre germination rates ($P < 0.0001$; $r = 0.62$). No relationship was observed for seed set and kernel weight at Ben Hur relative to seed set and kernel weight at Grand Terre. These results may indicate that seed set and seed weight are influenced by environment to a greater extent than overall germination rate. If this is the case then selection based solely on germination could prove effective.

AFLP Analysis

The selected 26 AFLP primer combinations yielded an average of 93 markers with a range of 58 to 134 (Table 7). A total of 708 polymorphic markers were scored. These molecular markers were sufficient to differentiate all the 41 genotypes. The genetic similarity was calculated using Jaccard's coefficient with all 708 molecular markers, and values ranged from 0.220 to 0.885. The highest level of similarity was between 98NR 7-14 and 98NR 7-15 and the lowest was between 98NR109-11 and the cultivar Vermilion. The goodness-of-fit of the AFLP-generated dataset for the cluster analysis was also supported by a high cophenetic correlation coefficient (0.94) (Rohlf 1997).

The AFLP-based cluster analysis (Figure 4) revealed that at 0.53 level of similarity, two well defined clusters were apparent, but the remaining genotypes did not cluster into large groups and consisted of two or three genotypes, usually defined by the original collection sites. Cluster 1 contained 18 genotypes, which came from contiguous parishes of Lafourche and Terrebonne, while cluster 2 contained 11 genotypes from contiguous parishes Cameron and Vermilion. From the cluster analysis it was possible to determine that 'Vermilion' was genetically different from the rest of the genotypes used in this study. It can be concluded further that none of the collected material was a clone of the Vermilion genotype.

To explore the relationship among genotypes with a similar origin, Jaccard distances were estimated within each accession and among accessions from the same geographic location (Table 8). For this analysis five genotypes were used from each accession 98NR 7, 98NR 8, 98NR 26, 98NR 27, 98NR 47, 98NR 82, 98NR 99; four genotypes from accession 98NR 38, and a single genotype from accession 98NR 109. These results indicate that, for some accessions, the variability among plants within the accession was as large as the maximum variability estimated among all the accessions. Accessions 98NR 27, 98NR 82, and 98NR 99 had larger distances among plants within the accession, compared with accessions 98NR 38 and 98NR7. Accession 98NR7 showed the lowest distances among plants within an accession, including the least differentiated pair of plants from an accession (plants 7-14 and 7-15) (Figure 4). Cluster analysis based on the molecular data reflected a relationship between the geographical origins and genetic differences among the accessions. These results support previous findings of a positive relationship between genetic and geographic distance among populations of S. *altern iflora* (Stiller and Denton 1995, O'Brien and Freshwater 1999, Travis et al. 2002).

DISCUSSION

Coastal erosion, particularly in southern Louisiana, continues at an alarming rate. Efforts to reclaim and re-vegetate these marshland areas using vegetatively propagated plants is

TABLE 4. MEAN VALUES FOR PLANT HEIGHT, SPREAD, DISEASE REACTION AND VIGOR FOR FORTY CYCLE 2 *SPARTINA ALTERNIFLORA* PLANTS AND VERMILION AT TWO LOCATIONS (BEN HUR RESEARCH STATION OF LSU AGRICULTURAL CENTER AND GRAND TERRE ISLAND) IN LOUISIANA DURING 2001.

	Ben Hur				Grand Terre			
Plant	Height (cm)[a]	Spread (m^2)[b]	Disease[c]	Vigor[c]	Height (cm)[a]	Spread (m^2)[b]	Disease[c]	Vigor[c]
98NR7-8	157	3.2	3.5	5.1	124	1.3	2.4	6.3
98NR7-12	159	2.3	3.7	4.9	129	1.4	3.0	6.1
98NR7-14	133	1.5	4.0	4.7	112	1.4	1.5	5.9
98NR7-15	148	2.9	2.8	4.6	118	1.1	2.3	6.1
98NR7-16	146	2.3	3.7	5.1	117	1.3	2.0	5.9
98NR8-4	148	3.8	3.6	5.1	100	0.8	2.6	6.0
98NR8-5	125	2.0	4.0	4.9	107	1.8	2.4	5.7
98NR8-12	160	2.1	4.0	4.7	117	1.1	3.0	6.3
98NR8-13	146	1.9	3.9	4.6	115	0.9	2.8	6.1
98NR8-17	143	2.3	3.7	4.4	122	1.4	2.4	6.4
98NR26-3	94	2.8	3.6	4.9	87	1.2	2.0	5.9
98NR26-4	104	0.7	4.1	5.0	89	1.0	2.6	6.7
98NR26-10	119	1.7	4.1	5.1	87	0.9	2.6	6.1
98NR26-13	167	3.0	3.4	5.0	92	1.1	1.6	6.5
98NR26-18	133	2.1	4.1	5.1	101	1.5	2.0	5.8
98NR27-2	125	2.6	4.5	5.3	88	1.1	2.4	5.7
98NR27-3	111	1.5	3.7	4.9	88	0.7	1.8	6.8
98NR27-13	86	2.9	4.3	4.3	94	1.2	3.2	6.4
98NR27-19	123	2.4	4.2	5.0	83	1.2	2.2	6.0
98NR27-20	131	2.6	4.3	4.6	113	1.5	2.8	6.8
98NR38-4	159	1.1	3.6	4.7	100	1.0	2.6	6.3
98NR38-7	147	1.1	4.0	5.1	97	1.1	3.0	5.6
98NR38-9	134	0.6	4.0	4.8	87	0.8	2.6	5.8
98NR38-16	138	1.3	3.5	4.7	104	1.1	2.2	6.2
98NR47-4	117	1.1	3.5	4.9	88	0.8	2.6	6.2
98NR47-5	135	0.3	4.1	5.0	88	0.5	2.8	6.5
98NR47-14	122	1.1	3.9	4.7	90	1.2	2.3	6.3
98NR47-16	138	1.1	3.5	5.2	109	1.2	1.3	6.1
98NR47-18	136	0.7	3.9	4.9	91	0.8	2.2	5.5
98NR82-3	154	1.4	3.7	4.3	97	0.5	3.0	5.9
98NR82-6	157	1.7	4.1	5.2	99	0.6	2.4	6.0
98NR82-10	243	0.8	3.3	5.2	103	0.8	2.2	5.7
98NR82-19	171	1.1	2.6	4.7	115	1.6	2.6	5.4
98NR82-20	148	2.2	3.4	4.4	87	0.7	3.8	6.3
98NR99-1	108	1.3	4.4	5.4	68	0.6	2.6	5.9
98NR99-2	117	1.3	4.1	5.0	97	0.8	3.4	6.0
98NR99-3	121	2.2	3.4	4.6	87	1.3	1.8	5.8
98NR99-4	132	1.3	4.2	4.9	96	0.9	2.8	6.0
98NR99-18	111	2.0	3.7	4.9	86	1.0	2.6	6.2
98NR109-11	117	2.1	3.9	4.8	89	0.1	3.2	5.8
Vermilion	140	1.0	3.5	4.9	99	1.2	2.4	5.6
Mean	137	1.8	3.8	4.9	99	1.1	2.5	6.1
C.V.	22	53	11.8	18.2	13	49	45	13
LSD (0.05)	55	1.7	0.8	N.S.	21	0.8	N.S.	N.S

[a]Height was measured from ground to the uppermost leaf tip of the plant (cm).
[b]Spread was measured as an area covered by the plant (cm^2).
[c]Vigor and disease reaction were visually estimated on a scale of 1-10 (1 = excellent; 10 = poor).

time consuming and expensive. In addition, the current widespread use of one genotype, 'Vermillion', is a serious threat to maintenance of adequate natural genetic diversity in restored or reclaimed marshland areas. By identifying multiple genotypes, for use in currently accepted restoration methodology, highly significant improvement in genetic diversity could be readily accomplished. Our results have demonstrated that it is possible to identify superior plants from many genetically diverse groups with the objective of maintaining as much natural diversity as possible.

Cluster analysis of the source population and the cycle 1 selections indicated some shift in clustering relative to originating Parishes. For example, Calcasieu and Orleans remained closely clustered as did Jefferson and Lafourche. Iberia, Vermilion and Terrebonne were clustered in the source population (Figure 1) but were clearly separated in

TABLE 5. PARTIAL CORRELATION COEFFICIENTS FOR PLANT HEIGHT, SPREAD, DISEASE REACTION AND VIGOR FOR FORTY CYCLE 2 *SPARTINA ALTERNIFLORA* PLANTS AND VERMILION WITHIN AND BETWEEN TWO LOCATIONS (BEN HUR RESEARCH STATION (BH) OF LSU AGRICULTURAL CENTER AND GRAND TERRE ISLAND (GT), LOUISIANA).

Variable	BH Spread	BH Disease	BH Vigor	GT Height	GT Spread	GT Disease	GT Vigor
BH Height	-0.01	-0.49**	-0.01	**0.59****	0.05	-0.02	-0.18
BH Spread		-0.06	-0.14		**0.38****	-0.15	0.18
BH Disease			0.24			**0.21**	0.14
BH Vigor							**-0.24**
GT Height					0.54**	-0.06	0.03
GT Spread						-0.31*	-0.11
GT Disease							0.06

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.

TABLE 6. MEAN VALUES FOR SEED KERNEL WEIGHT (1000 SEED), SEED SET (5) AND SEED GERMINATION (%) FOR FORTY CYCLE 2 *SPARTINA ALTERNIFLORA* PLANTS AND VERMILION AT TWO LOCATIONS (BEN HUR RESEARCH STATION OF LSU AGRICULTURAL CENTER AND GRAND TERRE ISLAND) IN LOUISIANA DURING 2001.

	Ben Hur			Grand Terre		
Plant	Kernel wt	Seed set	Germination	Kernel wt	Seed set	Germination
	g/1000	%	%	g/1000	%	%
98NR7-8	2.64	62	60	1.53	36	60
98NR7-12	2.38	68	60	1.94	62	58
98NR7-14	1.64	7	56	2.48	43	56
98NR7-15	1.49	27	45	1.87	40	34
98NR7-16	3.09	62	49	2.53	41	49
98NR8-4	1.90	17	58	2.95	60	58
98NR8-5	1.44	59	62	2.45	55	64
98NR8-12	2.02	35	53	2.56	60	53
98NR8-13	3.53	70	53	2.89	63	53
98NR8-17	3.11	56	50	2.61	55	50
98NR26-3	3.11	82	53	2.04	39	76
98NR26-4	1.00	11	47	2.53	72	46
98NR26-10	2.71	29	74	3.88	55	53
98NR26-13	2.23	44	71	2.80	62	47
98NR26-18	3.83	65	66	2.71	58	70
98NR27-2	3.10	81	60	2.59	58	28
98NR27-3	3.69	77	55	2.76	55	78
98NR27-13	3.76	94	84	2.88	80	78
98NR27-19	3.21	57	51	2.76	63	55
98NR27-20	3.14	48	61	2.70	57	51
98NR38-4	2.44	40	38	1.98	44	60
98NR38-7	2.38	29	60	2.32	42	39
98NR38-9	2.98	28	39	1.89	20	43
98NR38-16	1.89	14	43	1.98	7	27
98NR47-4	3.13	70	60	2.38	52	49
98NR47-5	3.94	66	55	2.86	40	49
98NR47-14	5.05	74	75	2.72	54	69
98NR47-16	3.83	68	80	3.31	62	62
98NR47-18	4.00	53	49	2.98	15	40
98NR82-3	3.69	83	49	2.45	54	89
98NR82-6	3.39	81	49	2.61	57	39
98NR82-10	1.67	15	42	3.00	83	49
98NR82-19	3.31	54	96	2.90	60	70
98NR82-20	1.63	50	39	2.19	57	42
98NR99-1	1.67	34	58	2.65	53	58
98NR99-2	1.80	32	34	3.15	39	34
98NR99-3	2.40	56	62	2.84	54	58
98NR99-4	1.76	39	23	2.98	54	3
98NR99-18	1.95	38	35	3.30	60	9
98NR109-11	3.82	67	60	3.11	61	71
Vermilion	2.01	58	73	2.44	54	77
Mean	2.72	51	56	2.62	52	53
C.V.	12.1	20.4	32	11.93	20.5	31
LSD (0.05)	0.4	13.1	17.2	0.3	13.4	17

Table 7. AFLP Polymorphism for 26 selected primer combinations in *Spartina alterniflora*. Forty cycle 2 plants and Vermilion were used for AFLP analysis.

	Selective nucleotides		Number of bands		
Name	*Msel*	*Eco*RI	Total	Polymorphic	Polymorphism (%)
C01	CAA	ACA	72	22	31
C02		ACC	70	18	26
C03		ACG	72	30	42
C04		AGC	84	20	24
C05	CAC	AAC	94	24	26
C06		ACT	58	20	35
C07		ACG	64	28	44
C08	CAG	AAC	90	28	31
C09		AAG	80	20	25
C10	CAT	AAG	104	30	29
C11		ACA	90	24	27
C12		ACT	114	34	30
C13		ACC	90	22	24
C14		ACG	94	38	40
C15		AGG	84	24	29
C16	CTA	ACT	64	18	28
C17		ACA	110	26	24
C18	CTC	AAC	98	34	35
C19		AAG	100	26	26
C20	CTG	AAC	116	32	28
C21		ACC	108	40	37
C22		ACG	104	36	35
C23		AGC	98	20	20
C24	CTT	AAC	122	28	23
C25		ACT	116	34	29
C26		AGC	134	32	24
Total			2430	708	30

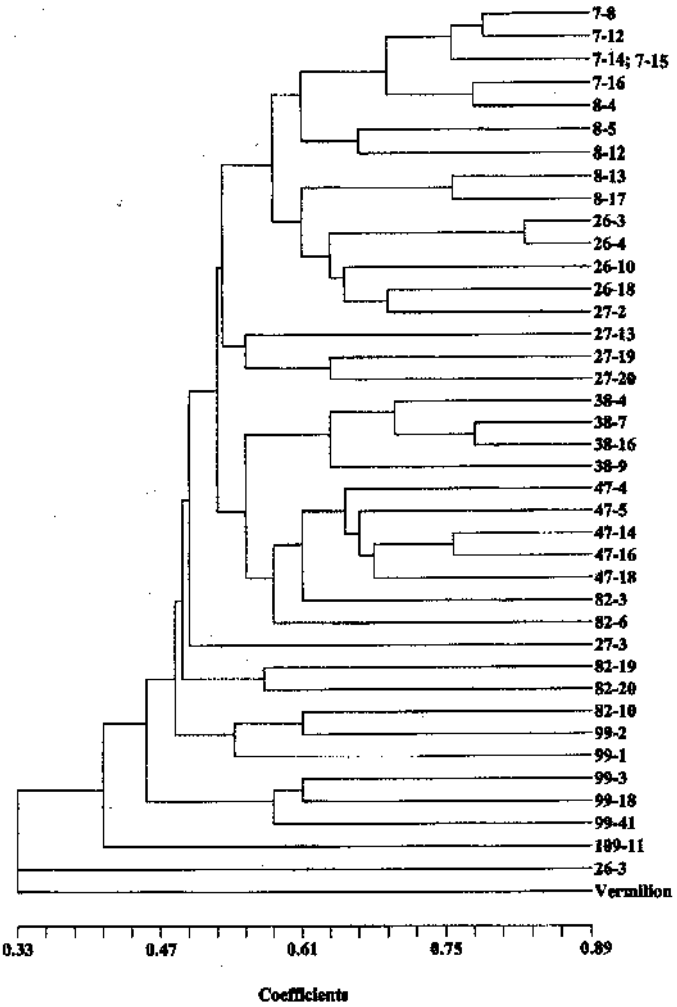

Figure 4. Cluster analysis of 40 *S. alterniflora* genotypes and 'Vermilion' based on 708 polymorphic AFLP markers.

the cycle 1 cluster analysis (data not shown). Given the out crossing ability of this species and its polyploid genome, it should not be surprising that high levels of generational trail diversity would exist resulting in shifts of clustering patterns based on geographic origin. This result would not minimize the need to maintain source plant material from a wide geographic range but it does indicate that morphological trait diversity may not be closely linked to geographic diversity within the geographic confines and sample size of this study.

Given the severity and magnitude of the problem of coastal erosion, non-traditional alternatives to current restoration and reclamation methods need to be explored. Development of S. *alterniflora* populations that could be established from seed and aerially applied would provide a significant savings in time and labor as well as provide a genetically diverse germplasm source for restoration efforts. Previous research by Fang et al. (2004) has indicated that S. *alterniflora* produced less seed when self-pollinated than when cross-pollinated. Somers and Grant (1981) estimated a 20-fold reduction in seed set from self-pollinated inflorescences among a Delaware population. Cross-pollination would insure that any populations developed would be both heterozygous and heterogonous and maximize genotypic progeny variation.

Methods of collecting, storing, and planting S. *alterniflora* seed have been previously reported (Woodhouse et al. 1976). According to Woodhouse et al. (1976), establishment of S. alterniflora from seed has been shown to be economical on dredged material. However, most of the attempts to establish *S. alterniflora* from seed have resulted in moderate success (Mooring et al. 1971, Seneca 1974, Woodhouse et al. 1974, Webb and Dodd 1989). Webb and Dodd (1989) reported that the use of seed to establish S. *alterniflora* on dredged materials in Texas had a high probability of failure and concluded that the use of vegetative material for establishment was much more reliable.

Given the uncertainty of establishment of *S. alterniflora* from seed and the immediacy of the problem, an alternative approach for maintaining genetic diversity, using the current system of vegetative establishment from clones, is being developed. Rather than relying on seed propagation, vegetative clones of superior plants from different sources, both geographically and genetically distinct, will be planted in groups or clusters to facilitate natural . out-crossing. The parent clones selected for this procedure have high seed production capability as well as superior vegetative growth traits. A group

TABLE 8. JACCARD SIMILARITY VALUES AMONG 40 SAMPLED *SPARTINA ALTERNIFLORA* GENOTYPES (WITHIN AND AMONG ACCESSIONS) FROM FIVE LOCATIONS IN LOUISIANA.

Accession	Distance among genotypes	Location	Distance between accessions
98NR 7	0.69	Lafourche	0.56
98NR 8	0.64		
98NR 26	0.60		
98NR 82	0.52	Cameron	0.52
98NR 47	0.65		
98NR 38	0.67	Vermillion	Single accession
98NR 27	0.53	Terrebonne	0.46
98NR 109	Single genotype		
98NR 99	0.54	Jefferson	Single accession

of these plants are currently being increased and are scheduled for release in 2007 by the LSU AgCenter.

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