Coastal Restoration and Enhancement through Science & Technology (CREST)

Final Report

Project: Field evaluation and selection of improved California bulrush and sea oats populations for coastal restoration

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Field evaluation and selection of improved California bulrush (*Schoenoplectus californicus*) **and sea oats (*Uniola paniculata)* populations for coastal restoration**

Coastal erosion is a major threat to the Louisiana economy and environment. This is brought about by both human intervention and natural phenomena such as storms and hurricanes. The latter, being the most destructive, are notorious for causing the erosion of the coastlines and degradation of landmasses. Shoreline erosion rates in the Louisiana coast alone range from 5 to 50 meters per year. Bourne (2000) reported that some of Louisiana's 3,460 km of coastal wetlands have reverted to open water, and he predicted a yearly decline of approximately 65 to 91 km^2. Roughly about 1,800 to 4,500 km^2 of landmasses could vanish in the next 50 years, with an estimated loss of more than $37 billion in public resources and services. Without any potential plant resources and adequate management tools to arrest this disaster, most of these valuable coastlines would gradually diminish in due time. It is thus critical that all available technologies be focused on development of practical solutions to slow the rate of coastal wetland loss and restore the degrading habitats. Many researchers have advocated strategies to utilize indigenous plant species for restoration and reclamation of the coastlines. In this report, we summarize the progress made towards the development of improved plant materials in two native plant species, sea oats (*Uniola paniculata*) and California bulrush (*Schoenoplectus californicus*) to accelerate the coastal restoration efforts.

Section A: Sea oats (*Uniola paniculata)*

Uniola paniculata, commonly known as sea oats, is of considerable economic and ecological importance in dune building and vegetative stabilization projects throughout its native range in the United States. It is a perennial, semitropical, rhizomatous C4 grass with tolerance to a wide variety of harsh environmental conditions including drought, inundation by seawater, salt spray, strong winds, and storm effects. The growth and physiology of sea oats have been investigated in the past, but no study has been directed toward analyzing the genetic variation in natural populations through field evaluation. Genetic variation in adaptive morphological and physiological attributes in relation to its geographical range of distribution is vital for conserving the nations' existing sea oats population as well as their exploitation to revegetate new areas in restoration projects. The knowledge of the genetic diversity is essential for their survival, ecology, management and development of appropriate germplasm for a diverse set of environments. Molecular markers were used to effectively measure variation at individual, population, and geographical levels. Dunes in Louisiana are low, but appear adequate for growth and spread of *U. paniculata*, but natural stands are diminishing. The main barrier to its success here and elsewhere appears to be the limited seed supply. Field trials are needed to identify populations that are adapted to the low dunes of Louisiana and have increased seed yield, to accelerate utilization of sea oats in barrier islands restoration projects. Our objectives are to analyze genetic diversity in sea oats accessions using molecular markers and to identify improved sea oats accessions for Louisiana.

I. Genetic diversity analysis in sea oats

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. All 19 collection sites (Table 1) are well separated physically. Leaf tissues were collected from four-month old, greenhouse grown sea oats seedlings from the above nineteen accessions. From each accession, ten seedlings were sampled randomly with their leaf tissues handled separately for AFLP analysis. AFLP analysis was conducted using 12 *Eco*RI + *Mse*I primer combinations following Subudhi et al. (2005).

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

The silver stained AFLP bands in each gel were scored as present (1) or absent (0) and only distinct polymorphic bands were scored. Binary matrices were prepared for analysis using NTSYSpc (Numerical Taxonomy System) version 2.10t. Jaccards's similarity coefficient was used to estimate similarity among plants within accessions. Similarity matrices were subjected to cluster analysis using the unweighted pair group method with the arithmetic averages (UPGMA) clustering approach and principal coordinate analysis (PCO). Bootstrap analysis was performed to determine the strength of the clusters using software WINBOOT. Analysis of molecular variance (AMOVA) was performed using ARLEQUIN version 2.0 to partition the genetic variation among individual plants, among accessions and among states.

Results

AFLP polymorphism and Analysis of molecular variance (AMOVA)

A total of 703 AFLP bands were generated across all plant samples using 12 primer combinations. Among these, 417 bands were polymorphic with a mean polymorphism rate of 59% ranging from 42-81%. The AFLP markers on an average more polymorphic in the gene pools of populations derived from Florida, Texas, Mississippi, Louisiana, and Alabama gene pools than in those obtained from South Carolina, North Carolina, and Virginia gene pools (data not shown).

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 2). 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.

Table 2 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		

Genetic variation within and between accessions

Jaccard similarity coefficients were calculated between pairs of plants. 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, inter-accession similarity values were relatively lower than intra-accession values. A similar trend was noticed when the accessions from others 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-19, 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-19).

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 3). 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 sea oats were more genetically related compared with those from other states. The sea oats from the state of Virginia was clearly different from other states. Sea oats accessions from Mississippi and Alabama were more similar to one another, as was 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 oat individuals of 19 accessions from 8 states of the United States. In general, the major clusters of sea oats genotypes were supported with high bootstrap values (Fig 1), 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, 1997). The UPGMA-derived dendrogram assigned all the sea oats individuals into four major clusters 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 around at 79.6% genetic similarity levels. Cluster III was separated from Cluster I and II at 79% similarity level where as Cluster IV 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 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 levels.

Table 3 Average Jaccard similarity coefficients between pairs of *Uniola paniculata* plants belonging to the same state (diagonal values in bold) or different state. 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.

	Texas	Louisiana	Virginia	Mississippi	Alabama	Florida	North Carolina	South Carolina
Texas	0.9098±0.0006 (0.8681-0.9574							
Louisiana	0.8883±0.005 (0.8433-0.9318)	0.9014±0.0012 (0.8509-0.9481)						
Virginia	0.8332±0.0007 (0.8065-0.8636)	0.8293±0.0010 (0.800-0.8569)	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± (0.7587-0.9108)	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±0.0010 (0.8629-0.9511)	0.9464±0.0013 (0.9022-0.9848)

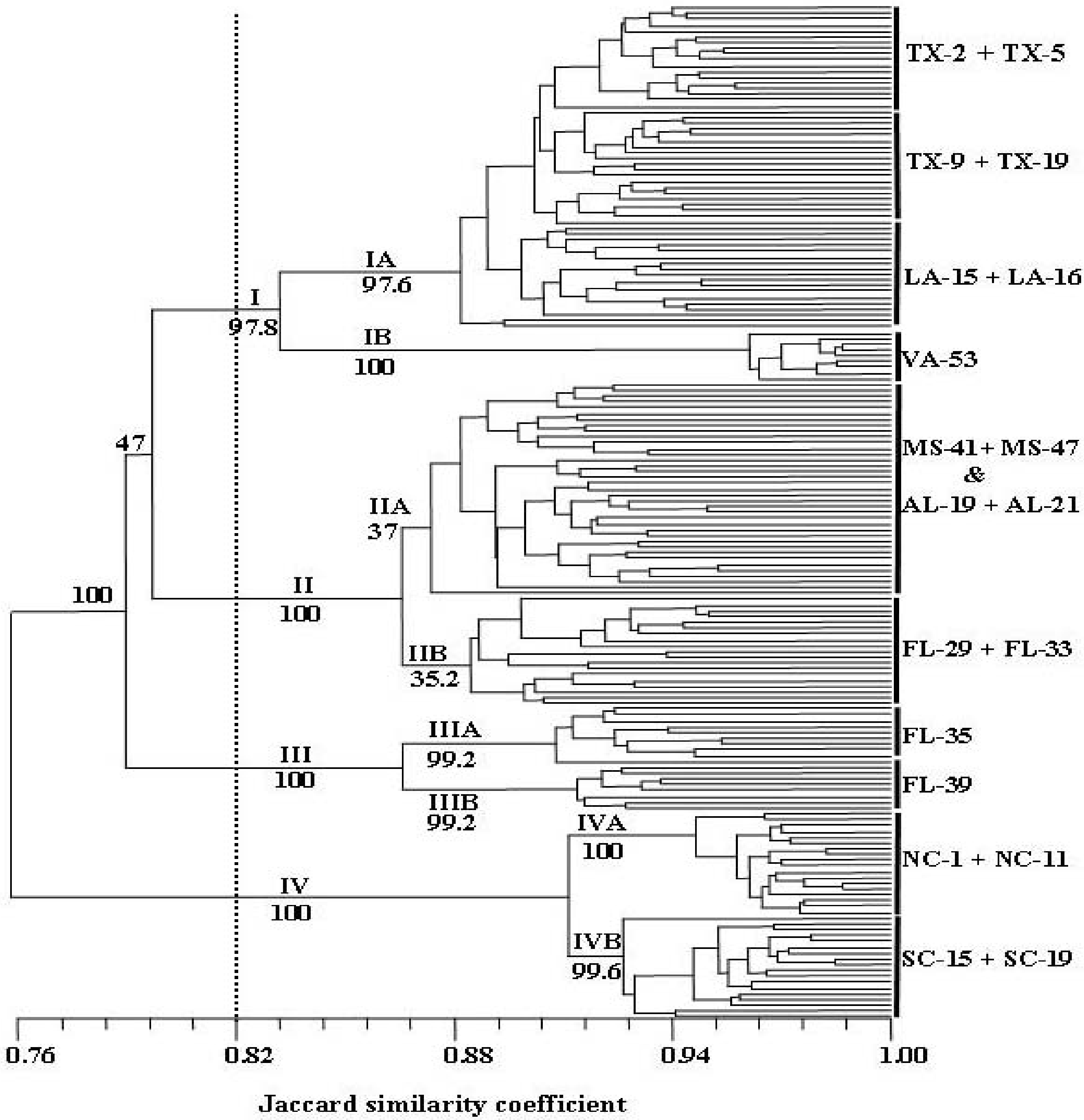

Fig 1 Dendrogram of sea oats (*Uniola paniculata*) individuals belonging to 19 accessions collected from 8 different Gulf coast and South Atlantic States (Texas, Louisiana, Virginia, Mississippi, Alabama, Florida, North Carolina, and South Carolina) produced by UPGMA clustering method based on the Jaccard similarity coefficient matrix derived from 703 AFLP markers. The numbers shown at different nodes represent the bootstrap values based on 500 replications.

__Principal Coordinate Analysis__

Principal coordinate analysis further validated the results of cluster analysis (Fig 2). 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. 2). 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.

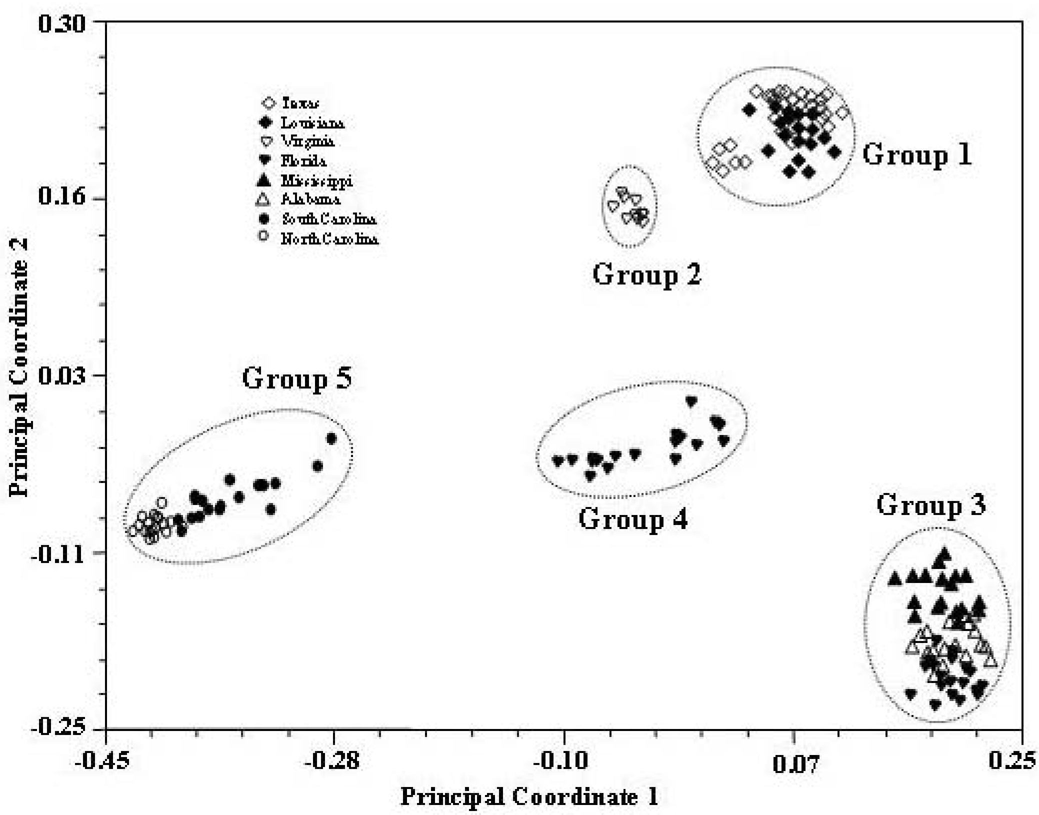

Fig 2 Principal coordinate analysis of sea oats plants from eight different states with the first and second principal coordinates derived from the Jaccard similarity coefficient matrix computed from 703 AFLP markers. Sea oats individuals of all 19 accessions from North Carolina, South Carolina, Texas, 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.

II. Sea Oats Field Trials:

A collection of eighty-six sea oats accessions (Table 4) from eight southeastern Atlantic and Gulf coast states (North Carolina, South Carolina, Virginia, Florida, Alabama, Mississippi, Louisiana, and Texas) were used for field evaluation. Single accession of Caminada, Blustem , and Panicum were used along with the sea oats accessions. Sea oats seedlings were raised in greenhouse of both LSU Agricultural Center and USDA-NRCS Plant Material Center at Galiano. Sea oats plant materials were multiplied by splitting the tillers in the greenhouse. Sea oats plant materials were planted 5 feet apart with three replications at two locations: Holley Beach (Louisiana) and Gulfport (Mississippi). Sea oats seedlings were planted at Holley beach and Gulfport on July 29, 2003 and April 13, 2004, respectively. Observations were recorded on vigor, tillering, and spread using a score of 1-9 (1=best and 9=poorest). A score of 1 indicates high vigor and more tiller density while a score of 9 was allotted for poorest performers. Observations were taken three times over a period of two years.

Table 4 Number of sea oats accessions from eight states for the proposed investigation.

State	Number of accessions
Louisiana	36
Texas	7
Florida	16
Mississippi	2
Alabama	4
North Carolina	17
South Carolina	3
Virginia	1
Total	86

Range for vigor, tiller density and spread ranged from 3 to 8 at Gulfport location. At Holley beach location, mean vigor, mean tillering and mean spread ranged from 3.78 to 7.53, 4.13 to 7.47, and 3.53 to 6.33, respectively. There was high correlation between vigor and tiller density. Analysis of variance indicated that significant genetic variation exists among the accessions. The top ranking 15 accessions at both locations are given in Table 5. Overall, Florida and Louisiana accessions performed well in Louisiana environments. Of the 15 accessions, two Louisiana accessions (LA 16H and LA 16E) and two Florida accessions (FL 30 and FL 39) consistently performed well at both locations. There was significant damage to the field trials by hurricanes Katrina and Rita at both locations. From both locations, just before the hurricane, we collected around 100 best performing accessions and these are being maintained in LSU AgCenter greenhouse. We plant to conduct more extensive trials in future with these selected materials to identify the accessions most suitable for Louisiana environment. In addition, the field experiment will also provide opportunity to select the parents which can be used for initiating a long-term sea oat-breeding program. Hybridization will be done between lines with good adaptation and higher seed set for developing improved sea oats lines/populations.

Table 5 Top 15 performing s sea oats accessions at Holley Beach (LA) and Gulfport (MS). Performance was judged by scoring vigor, tillering, and spread on a 1-9 scale (1=best and 9=poorest). Four accessions in bold italic are common at both locations.

Holley Beach (LA)				**Gulf Port (MS)**			
Accession ID	State of origin	Performance Index*	Rank	Accession ID	State of origin	Performance Index	Rank
LA-26	LA	2.89	1	***LA-16H***	LA	3.17	1
LA-18	LA	3.22	2	LA-16O	LA	3.33	2
FL-39	FL	3.56	3	LA-16	LA	4.02	3
LA-16H	LA	3.64	4	LA-16N	LA	4.08	4
NC-07	NC	3.77	5	***FL-39***	FL	4.15	5
TX-06	TX	3.80	6	LA-16P	LA	4.17	6
LA-16B	LA	3.83	7	LA-16J	LA	4.21	7
MS-47	MS	3.87	8	LA-16F	LA	4.22	8
LA-16E	LA	3.87	9	FL-26	FL	4.24	9
LA-16I	LA	3.88	10	***FL-30***	FL	4.27	10
MS-36	MS	3.92	11	***LA-16E***	LA	4.34	11
FL-34	FL	3.93	12	FL-37	FL	4.34	12
FL-30	FL	3.97	13	LA-16D	LA	4.34	13
TX-10	TX	3.98	14	FL-38	FL	4.36	14
LA-15O	LA	4.00	15	LA-16Q	LA	4.39	15

SUMMARY & CONCLUSION

1. Significant amount of genetic variability observed among the collected accessions will be helpful in selecting superior and adaptable sea oat accessions for the Louisiana environments.
2. Ranking of all accessions based on the field performance revealed four accessions that performed well in both locations.
3. Examination of the different components of the genetic variation by analysis of molecular variance indicated largest proportion of variability at the state level (47.83%) followed by the variation due to individual genotype differences (34.36%), and differences among the accessions within a state (17.81%).
4. The Atlantic accessions were genetically more homogeneous compared to accessions from Gulf Coast states. Florida accessions were most genetically diverse and those from both Carolinas and Virginia harbored less genetic variability. This overall genetic pattern revealed a strong Gulf coast-Atlantic coast or south-north division for *Uniola.*
5. Principal coordinate analysis revealed five major clusters following a clear geographic pattern of genetic structure. This could be due to limited gene flow among populations due to the linear and fragmented distribution of this species.
6. Clear relationship between molecular genetic diversity and geographic source of sea oats populations of the United States revealed in this study should be taken into consideration by the resource managers and commercial nurseries in selecting, moving, and mixing of sea oats plant materials for restoration of new areas without compromising the adaptation and genetic diversity.

Section II: California bulrush (*Schoenoplectus californicus*)

Wave energy is one of the key elements causing coastal erosion. California bulrush (*Schoenoplectus californicus*) is a deep water plant species that can potentially be used as a natural barrier to control erosion along shorelines, canal banks, levees, and other areas of soil-water interface. Capable of growing in 3 foot water depth, bulrush can form extensive colonies parallel to and continuous along shorelines in solid somewhat circular stands. California bulrush can facilitate removal of some toxic metals such as Cu, Pb, Zn, and Me from soil matrix. It improves water quality in wetland construction and reconversion of degraded wetlands. In addition, it provides a favorable habitat for wildlife including some endangered species. Dense stands of California bulrush are efficient users of available nutrients, producing significant amounts of organic matter. The cumulative effects of organic matter production, sediment trapping, and erosion control not only provide shoreline protection but also accelerate sediment accumulation and near-shore building, improve water quality, and promote diverse communities of aquatic life. When planted as continuous vegetative barriers across open water, California bulrush can significantly reduce pond fetch. The resulting calmer environment enhances faster establishment of other vegetation along the shorelines.

Currently, there is no California bulrush cultivar available in Louisiana. Superior California bulrush will provides coastal managers with an additional tool to combat coastal erosion. Native to Louisiana, California bulrush can be used in conjunction with other major marsh plant species to not only reduce erosion but also improve plant diversity, a critical component in establishing productive and sustainable habitats. The potential of California bulrush for erosion control, however, is currently limited by its sensitivity to salinity. California bulrush is a freshwater marsh plant that can only tolerate salt concentrations of up 6.0 parts per thousand (ppt). Greater salt tolerance that allows California bulrush to grow in brackish conditions will increase its role in preserving and restoring salt marshes.

Field Evaluation:

Forty-five California bulrush ecotypes jointly collected by LSU AgCenter and NRCS across its geographical range within Louisiana were evaluated in replicated tests at the Rice Research Station, Rayne, LA, for 2 years. A randomized complete block design with 3 replications was used. Plants from one-gallon pots were transplanted in the field with 15 x 15 ft spacing. California bulrush *cv.* "Restorer" was included in the field trials. Restorer is the only cultivar of *S. californicus*, released in 1993 by the Natural Resources Conservation Service - Georgia Plant Materials Program primarily for use as a wastewater treatment plant species. Four applications of fertilizer (80 lbs/A) were applied during their vegetative growth. Plants were maintained in a permanent flood and occasionally drained during herbicide application to control weeds. The growth parameters measured include (1) plant height (measured from ground level to the uppermost tip (cm), (2) stem density (number of tillers or stems per m^2, (3) stem diameter (measured at 5 cm above water line in mm), (4) spread (measured as an area covered by the plant (m^2), 11 month after planting), and (5) rate of spread (average weekly spread [m^2/week] from 12 to 15 months after planting). Table 1 shows performance of the 45 California ecotypes based on two year observation.

Significant differences in their seed production were also found among the 48 ecotypes evaluated. When germination was measured 1 month after harvest, the average germination rate was 5.1%, ranging from 2.4% to 11.2%. The first peak of germination occurred 4 weeks after planting followed by the second peak at week 8 with twice the rate of the first peak. Seeds from all ecotypes were capable of germinating after 2 years left in the soil.

Table 6 Comparative performance of 45 California bulrush (*Schoenoplectus californicus*) genotypes and *cv* 'Restorer' evaluated for 2 years under freshwater conditions, Rice research Station, Crowley, LA.

Accession	Height (cm)	Stem Density (tillers/m^2)	Stem Diameter (mm)	Spread (m^2)	Rate of Spread (m^2/week)
68268	182.8 a-f	89.6 d-l	13.1b-i	7.5240 a	0.2554 a
68371	177.2 b-g	102.7 b-k	12.2f-k	6.8675 ab	0.2390 ab
68275	190.6 ab	104.5 b-j	12.5 d-k	6.6762 a-c	0.3180 a-c
68284	184.8 a-f	89.7 d-l	13.0 b-i	6.6014 a-c	0.2300 a-c
68279	172.4 d-i	104.3 b-j	13.6 b-i	6.5906 a-c	0.2261 a-c
68309	176.8 b-h	128.8 a-d	12.7 c-j	6.6746 a-c	0.2246 a-d
68293	178.3 b-g	117.2 b-g	14.7 a-f	6.1876 a-e	0.2127 a-e
68329	141.3 j	134.8 a-c	8.7 l	6.2642 a-e	0.2120 a-e
68310	187.8 a-c	80.5 e-m	14.5 a-g	5.9798 a-f	0.2103 a-f
68270	181.1 a-f	111.0 b-i	11.7 h-k	5.9651 a-f	0.2060 a-f
68312	183.9 a-f	104.2 b-j	14.4 b-g	5.8654 a-g	0.2057 a-f
68301	180.7 a-f	140.5 ab	11.6 h-k	5.3817 a-h	0.1848 a-g
68281	177.5 b-g	163.7 a	11.1 i-k	5.4410 a-h	0.1823 a-g
Restorer	164.7 g-i	129.0 a-d	12.4 e-k	5.1756 a-h	0.1767 a-g
68335	166.0 g-i	97.5 b-l	12.1 g-k	4.9488 a-i	0.1748 a-g
68272	165.5 g-i	92.5 c-l	13.1 b-i	5.0768 a-i	0.1733 a-h
68323	186.7 a-d	102.0 b-k	13.1 b-i	4.5813 a-i	0.1659 a-h
68295	181.2 a-f	85.5 d-m	14.1 b-h	4.7305 a-i	0.1655 a-h
68283	180.6 a-f	112.3 b-h	12.1 g-k	4.7410 a-i	0.1612 a-h
68269	165.3 g-i	117.5 b-f	12.4 e-k	4.5774 a-i	0.1610 a-h
68326	177.9 b-g	125.0 a-e	12.5 d-k	4.5044 a-i	0.1604 a-h
68265	177.7 b-g	96.2 b-l	16.9 a	4.7117 a-i	0.1547 a-h
68294	178.7 b-g	80.0 e-m	13.6 b-i	4.4879 a-i	0.1532 a-h
68267	171.7 e-i	77.8 f-m	12.7 c-j	4.6766 a-i	0.1523 a-h
68325	181.5 a-f	110.0 b-i	13.5 b-i	4.2708 b-i	0.1499 a-h
68324	171.2 e-i	78.3 f-m	12.6 d-k	4.1991 b-i	0.1498 a-h
68334	160.4 i	92.5 c-l	10.4 j-l	4.1838 b-i	0.1443 b-h
68274	162.9 hi	61.7 j-m	14.6 a-g	3.8620 b-i	0.1408 b-h
68333	174.2 c-i	71.7 f-m	15.3 a-c	3.9523 b-i	0.1394 b-h
68273	178.9 a-g	82.7 e-m	15.1 a-d	3.9885 b-i	0.1371 b-h
68370	185.4 a-e	53.3 lm	14.2 b-h	3.9388 b-i	0.1350 b-h
68276	175.7 c-h	81.2 e-m	12.4 e-k	3.9900 b-i	0.1348 b-h
68277	176.7 b-h	94.7 c-l	11.6 h-k	3.8713 b-i	0.1348 b-h
68313	181.7 a-f	86.7 d-m	13.8 b-h	3.7041 c-i	0.1277 c-h
68278	192.9 a	78.8 f-m	15.5 ab	3.6962 c-i	0.1269 c-h
68287	175.7 c-h	85.0 d-m	14.6 a-g	3.5601 c-i	0.1249 c-h
68271	170.7 f-i	80.2 e-m	14.8 a-e	3.3923 d-i	0.1177 d-h
68282	180.5 a-f	71.2 g-m	14.7 a-g	3.2444 d-i	0.1138 e-h
68336	172.3 e-i	63.8 j-m	13.5 b-i	3.2383 d-i	0.1119 e-h
68298	171.4 e-i	65.2 i-m	14.6 a-g	3.1219 d-i	0.1082 e-h
68330	171.7 e-i	76.5 f-m	10.1 kl	3.0525 e-i	0.1057 e-h
68299	176.0 c-h	70.3 h-m	12.2 f-k	2.9119 f-i	0.1028 f-h
68337	184.2 a-f	82.2 e-m	12.8 c-j	2.8014 g-i	0.0966 gh
68327	185.0 a-f	57.5 k-m	13.2 b-i	2.6540 hi	0.0931 gh
68280	171.3 e-i	41.7 m	12.9 c-j	2.5388 hi	0.0895 gh

68328	181.2 a-f	66.0 i-m	11.9 h-k	1.8978 i	0.0664 h
Mean	176.3	92.13	13.1	4.5610	0.1577.5
CV (%)	5.52	34.43	13.6	47.30	47.16
LSD (0.05)	11.2	37.51	2.05	29492	0.1029.8

Means with the same letter(s) do not significantly differ at 5% level (Duncan's multiple range test).

Multi location trials

Twelve lines selected based on preliminary field tests are currently undergoing multi-location trials. Nine lines (68268, 68274, 68275, 68279, 68281, 68284, 68293, 68309, and 68310) were selected based on productivity performance. An intermediate performer 68328 and low performer 68371 along with the released cultivar "Restorer" were included as controls. Multi-location trials were conducted at brackish and fresh marsh locations within the Chenier and deltaic plains. Three locations were selected for these trials; Pointe Aux Chenes (Houma) and Cameron terraces for brackish environment and Avoca island terraces near Morgan City for fresh water environment. Automated data recorders were installed to continuously record salinity and water. Additional eight lines, which were selected from the salinity screening studies (0 to 18 ppt) conducted in the laboratory and greenhouse, were also included in the multi-location trials. The two year multi-location data will be used to select the most promising line for release to the public and use in erosion control and marsh restoration.

Salt Tolerance Evaluation

Forty-eight NRCS California bulrush (*Schoenoplectus californicus*) accessions, collected from various growing regions in Louisiana that experienced high salinity, were evaluated for their agronomic performance and their salt tolerance. Salt tolerance evaluation was carried out in the greenhouse using a randomized complete block design with 3 replications. Each entry was grown under flooded conditions and continuously exposed to four salt concentrations of 0, 6, 12, and 18 parts per thousand (ppt) for 9 months. To simulate ocean conditions, salinity was created using "Instant Ocean Synthetic Salt Mix" (Premium Aquatics, Inc.). There was a significant difference in salt tolerant levels among the accessions. Among 46 entries tested, eight accessions appear to possess higher salt tolerance. At a salt concentration of 18 ppt, most entries could not survive while the eight tolerant lines continued to produce small shoots. These lines were increased in the field and currently being evaluated in multi-location trials under brackish environments.

Cellular Selection to improve salt tolerance

To further improve salt tolerant levels, a tissue culture protocol to facilitate cellular selection has been established from five most salt tolerant California bulrush lines previously identified through greenhouse screenings. Calli were induced from immature flowers of approximately 1 inch in length using Murashige & Skoog (MS) medium supplemented with 4 parts per million (ppm) of 2,4-D. Plant regeneration was obtained by plating the calli on N6 basal medium containing plant growth regulators (0.5 ppm NAA and 2 ppm BAP). Cellular selection was conducted by exposing calli to a toxic salt concentration for 8 weeks. The surviving calli were rescued and placed on regeneration medium.

Marker analysis

AFLP (Amplified Fragment Length Polymorphism) markers were used to evaluate genetic diversity among 48 California bulrush accessions. AFLP markers had high reproducibility. Using the Licor sequencer, two primer combinations can be run simultaneously. Nine primer combinations amplified a total of 842 fragments of which 621 were polymorphic. There were significant genetic differences among the accessions. Results from marker analysis indicated that the average pair-wise genetic similarity was 0.749 ± 0.027 and ranged from 0.59 to 0.94. The total gene diversity across all accessions (H_t) was 0.289.

Unique DNA markers for five California bulrush promising elite lines have been developed. These markers can be used to identify each elite line with an extremely high degree of confidence. These molecular markers can be used in conjunction with other standard visual observations to solve any discrepancies or disputes that might be encountered in the field. They can be used during varietal certification in the future. These unique molecular markers are SCAR markers, derived from random amplified polymorphic DNA (RAPD) markers, containing 30 to 34 bp long primers. In addition, a combination of SSR (small repeat sequence) markers along with amplified fragment length polymorphism (AFLP) could also provide sequence differences to uniquely associated with individual elite lines. These PCR based-DNA marker IDs provide an inexpensive, rapid, and reliable method to identify each line. It only needs a tiny bit of leaf tissue that can be collected at any plant growth stage and can be conducted in a very short period of time. A similar identification method has been used successfully for many years in crop industries such as a grape industry.

Marker work initiated through this research can be used as a basis to improve future selection, quantify genetic diversity, trace the origin of cultivars, and identify hybrids and off-types. A simple database containing DNA fingerprints of 48 California bulrush ecotypes has been developed using AFLP, RAPD, and SSR markers. This database can be expanded and used as a reference profile for additional collections and as new varieties becoming available in the future.

Summary of findings for coastal managers

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, helps to establish fore dunes by intercepting and stabilizing drifting sand with its roots, rhizomes, and tillers. Fore dunes 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 elucidated in this report will help development of suitable germplasm for restoration efforts. Phenotypic variability for some notable attributes that are important for propagation and establishment in sea oat 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.

U. paniculata is regarded as a precious coastal resource. Sea oat populations have greatly declined in Louisiana due to the loss of elevated dunes on barrier islands. Based on field trials conducted at two locations, Holley Beach, Louisiana and Gulf Port, Mississippi, it is clearly evident that Florida and Louisiana accessions are best suited for planting in Louisiana and Mississippi. Sea oats in Louisiana are poor seed producers, and most of the material planted in locales such as Grande Isle comes from Florida. The field trials provided evidence that Louisiana accessions are more adapted to low sand dunes and survive better in Louisiana. Although our field trials were seriously damaged by hurricanes, Katrina and Rita, we were able to collect several best performing accessions from both locations just before the hurricane. These are being maintained and multiplied at LSU AgCenter greenhouse for additional trials. These germplasm will also be used in sea oats breeding program to develop improved seed producing sea oats lines which are adapted to Louisiana. Development of seed yielding sea oats types will be particularly helpful to raise the sea oats seedlings economically by addressing the concern of the regulators about the nurseries collecting seeds from the dwindling natural stands. The new insights regarding the extent of diversity in sea oat 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.

Development of salt tolerant California bulrush germplasm will accelerate marsh restoration effort

California bulrush has a significant potential for rehabilitating disturbed coastal sites. It is a perennial grass-like herb native to Louisiana with an extensive network of rhizomes that form dense colonies from the growth of adventitious shoots. It produces seeds that may be used for aerial seeding to economically control erosion over large areas. The potential of California bulrush for erosion control is limited by the fact that it is a freshwater marsh plant that can only tolerate salt concentrations of up 6 ppt (part per thousand). Greater salt tolerance in California bulrush will increase its role in preserving and restoring salt marshes. With that in mind, we conducted a series of investigations in California bulrush toward development of salt tolerant lines with improved vegetative vigor for use in coastal restoration projects in Louisiana. These investigations are collection and evaluation of California ecotypes, multilocation trials, salt tolerance screening, and genetic diversity study. We collected 45 California bulrush ecotypes from its native habitat range within Louisiana. Since field test provide critical information regarding the performance of improved salt tolerant California bulrush accessions under natural conditions, we conducted replicated tests at the Rice Research Station, Rayne, LA, for 2 years. Significant differences in growth and seed production were found among the ecotypes. Greenhouse evaluation of 46 entries resulted in identification of eight accessions with higher salt tolerance. These salt tolerant lines were increased in the field and currently being evaluated in multi-location trials under brackish environments at two locations. Cellular selection is also continuing to improve salt tolerance. Based on the field and greenhouse trials, several accessions are now available for further multiplication and use in coastal restoration.

There are concerns about loss of natural diversity when only a few select genotypes are used in coastal restoration. Therefore, we used molecular markers to analyze genetic diversity among California bulrush lines. There were significant genetic differences among the accessions. Unique DNA markers for five California bulrush promising elite lines have been developed. These markers can be used to identify each elite line with an extremely high degree of confidence. Marker work initiated through this research can be used as a basis to improve future selection, quantify genetic diversity, trace derivation of cultivars, and determine the status of the plants as hybrid, off-types, and cultivars.

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