

## Louisiana smooth cordgrass:

### Genetic evaluation based on DNA fingerprinting

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**A**dvancement in plant biotechnology research has created powerful DNA-based molecular markers, small fragments of DNA that can be visualized following separation according to their molecular size by electrophoresis.

These markers can be utilized to precisely identify organisms, distinguish between apparently similar individuals, determine hybrid purity, estimate genetic variation among genotypes of any organism, or monitor diversity within plant populations (William *et al.*, 1990; Dawson *et al.*, 1993, Hu and Quiros 1991).

#### On the mark

One place where these markers promise to be of value is in work being done with Louisiana smooth cordgrass.

The work was born in the on-going efforts to re-vegetate land to combat coastal erosion and wetlands deterioration in Louisiana. Coastal marshland losses in Louisiana are estimated to be 16,000 to 20,000 acres a year (USGS, 1985). Because the state's coastal marshes cover more than four million acres, Louisiana alone contributes more than 80 percent of the coastal wetlands loss in the U.S. annually, and continuing losses will have devastating impacts.

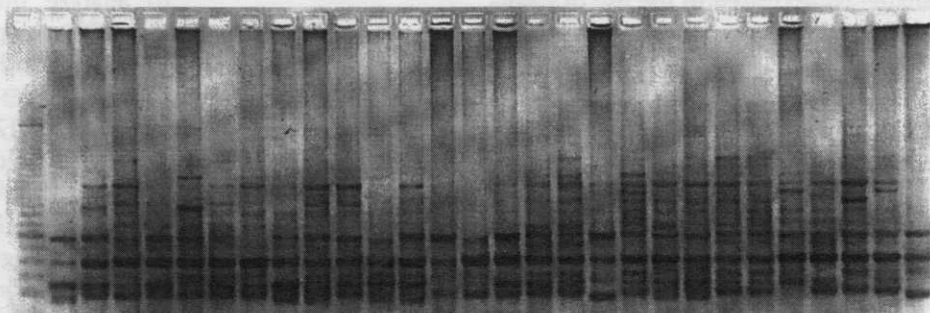
Louisiana's coastal marshlands and water bodies are a prodigiously productive estuarine system that support an abundant population of fish, shellfish, and wildlife. Currently, Louisiana provides 30 percent of the country's annual commercial harvest of fish and shellfish and 40 percent of its furs. Louisiana's estuarine system overwinters 66 percent of the migratory birds that use the Mississippi Flyway.

Constructing physical barriers and building riprap structures using donated Christmas trees have been used to rebuild Louisiana's eroding coastal marshes in limited areas. Despite their effectiveness, they are expensive to build and require continuous maintenance. Their impact is considered too small to compensate for such a high rate of current coast-wide land loss.

Smooth cordgrass (*Spartina alterniflora*) can work well, especially at receding shorelines, on barrier islands, and in deteriorating marshes that give away to open shallow water. It is a highly productive species, palatable to fish, wildlife, and livestock. This species is capable of trapping sediment, and can tolerate a wide

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M 1 2 3 4 5 6 7 8 9 10  
 11 12 13 14 15 16 17 18 19 20 22 23 24 25  
 26 27 28 29



**Figure 1. Electrophoretic analysis of DNA amplification products on 29 smooth cordgrass accessions. M=size marker**

range of environmental conditions, including submersion for 12 hours a day, pH 4.5 to 8.5, and salinity from 10 to 60 ppt (parts per thousand).

It colonizes mudflats and sandflats in saline or brackish water. Smooth cordgrass is, therefore, highly suitable for erosion control and estuarine reclamation.

### **Molecular markers**

Molecular markers may provide specific types of ecological indicators for monitoring the integrity of natural ecosystems and the sustainability of ecosystems that are affected by external factors.

Molecular markers can be generated from various molecular procedures, including random amplified polymorphic DNAs (RAPD). RAPD utilizes short, random DNA primers to hybridize with complementary DNA strands anywhere in the genome. The resulting intervening DNA is amplified by polymerase chain reaction (PCR).

*Markers are not affected by a plant's age or the environment.*

The amount of DNA needed for PCR amplification is very small, and the procedure is simple, rapid, inexpensive, and reproducible. The main advantage of RAPD analysis is that it does not require prior knowledge of the organisms under investigation. This is ideal for smooth cordgrass in which genetic information is very limited.

The objective of this study was to utilize RAPD analysis to evaluate the genetics of Louisiana smooth cordgrass. The genetic evaluation includes characterization of genetic diversity, spatial distribution based on closely related grouping, and potential use of marker-based identification.

### **Cordgrass genetic diversity**

Recent incidence known as "brown marsh" or "marsh dieback", first noticed in the spring of 2000, affected extensive coastal marsh regions in Louisiana where smooth cordgrass is the predominant species. The brown marsh has occurred several times in the past, but the size of the last incidence was unprecedented. The most affected areas were the salt marshes between the Mississippi and Atchafalaya Rivers.

Possible causes of this phenomenon have not been determined, however, the incident raised the question about genetic diversity of smooth cordgrass in Louisiana.

Genetic diversity has been a critical and debated issue related to the use of plants for coastal stabilization and wetlands reclamation.

The use of a single cultivar over large areas for erosion control could have serious consequences in the future. Loss of genetic diversity has been widely recognized as a major threat for adaptive potential and the maintenance of species. Populations with low genetic diversity could be more vulnerable to a sudden change in macro/micro climate or other irregularly occurring environmental stresses such as disease, insects, drought, salinity, and fire.

To effectively manage genetic diversity requires the ability to identify genetic variation. Genetic diversity is measured traditionally based on the differences in morphological, physiological, and agronomic characters or can be determined based on pedigree information. A similar objective can be obtained using DNA fingerprinting techniques.

The random amplification of DNA polymorphisms (RAPD) provides an unbiased method of quantifying genetic diversity. This molecular technique therefore can be applied as a complementary strate-

gy to traditional approaches in the management of plant genetic resources. Estimates of genetic distance can be used to evaluate genetic diversity among smooth cordgrass accessions

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To analyze the genetic relationship among 95 smooth cordgrass accessions, a total of 12 primers were used to generate DNA fragments. Figure 1 shows DNA fragments of accession 1 to 29. A total of 225 fragments were obtained with the 12 primers. Among these, 136 fragments (60 percent) were polymorphic. The size of fragments ranged from approximately 180 to 2,300 base pairs.

The proportion of smooth cordgrass accessions with polymorphic RAPD fragments ranged from one percent to 88 percent (representing fragments that were rare to those that were common) with a mean frequency of 40 percent. A total of

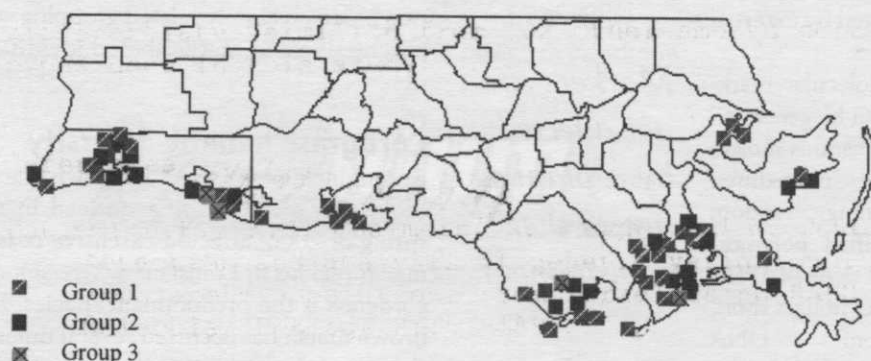


Figure 2. Distributions of smooth cordgrass accessions based on their genetic groups



4,465 pairwise comparisons based on 136 polymorphic RAPD markers were made.

Overall data suggested that Louisiana smooth cordgrass exhibited a large amount of genetic diversity. See Figure 1.

### Genetic distribution of Louisiana smooth cordgrass

Cluster analysis revealed that 95 smooth cordgrass accessions used in this study fell into three genetic groups, each of which is separated by genetic distance of more than 0.90.

Plotting the members of each group according to their origins indicated that there was no apparent specific genetic pattern associated with geographical location (Figure 2). Each group occupied a large area and was well-dispersed across coastal region. This finding suggests that seed may play a significant role in smooth cordgrass colonization of salt marshes along Louisiana's coast.

Even though Louisiana smooth cordgrass has been known to be generally sterile with little or no seed production, careful searching eventually yielded a few exceptionally fertile plants that produce viable seeds. Smooth cordgrass seed has some degrees of dormancy. Since the seed is light and remains dormant during winter, it could potentially spread by floating significant distances.

Utilization of a single smooth cordgrass cultivar for salt marsh reclamation and erosion control has raised concern. The main concern is related to the genetic of the cultivar used and possible negative

impacts associated with the introduction of the cultivar on the genetic diversity in and around the target areas.

Currently, Vermilion is the only available smooth cordgrass cultivar in Louisiana. It has excellent plant vigor and other growth characteristics.

Results from cluster analysis indicate that Vermilion belongs to group 2. In order to maintain genetic diversity, it appears that the use of Vermilion for erosion control over large areas should be accompanied by other cultivars that represent groups 1 and 3. See Figure 2.

Distribution of each genetic group across marsh coastal regions provides information on how future smooth cordgrass cultivars might be developed with regards to genetic diversity. Since there are no distinctive genetic patterns associated with any region, there is no apparent need to develop cultivars for specific locations.

A finer genotype map, however, might be needed to facilitate a better design for developing new cultivars that will maintain existing genetic diversity in the target areas. The map can be also used for monitoring the integrity of smooth cordgrass

*Currently, Vermilion is the only available smooth cordgrass cultivar in Louisiana.*

### REFERENCES

Dawson, J.K., K.J. Chalmers, R. Waugh, and W. Powell. 1993. Detection and analysis of genetic variation in *Hordeum spontaneum* populations from Israel using RAPD markers. *Molecular Ecology* 2:151-159

Hu, J. and J. Quiros. 1991. Identification of broccoli and cauliflower cultivars with RAPD markers. *Plant Cell Reports* 10:505-511.

United State Geological Survey (USGS), 1985.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*. 18(22):6531-6535.

under its natural ecosystem and suitability of the ecosystems as affected by the introduction of new cultivars.

### Addressing questions of identity

Molecular markers can be used to resolve a dispute over plant identity. They involve identification of DNA segments unique to a particular individual or cultivar. Once the markers have been identified, genetic identification is relatively fast and reliable. The procedure requires only a small amount of plant tissues that can be taken at any plant growth stage. In addition, markers can also be used to confirm the purity of cultivars being used.

In many cases, the use of the cultivar Vermilion is required in contracts to revegetate with smooth cordgrass. To differentiate cultivar Vermilion from non-Vermilion genotypes based on physical characteristics at early growth stages can be challenging. Molecular markers can be used to solve this problem. Evaluation of 66 RAPD primers yielded three primers that produce unique DNA bands that differentiate Vermilion from 102 other smooth cordgrass types used in this study.

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