*J. Aquat. Plant Manage.* 46: 60-67

# Genetic Diversity in Smooth Cordgrass from Brown Marsh Areas of Louisiana

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## ABSTRACT

Smooth cordgrass (*Spartina alterniflora* Loisel.) is the single most important grass species in salt marshes along the Gulf of Mexico and the eastern United States coastline. In spring 2000, coastal Louisiana experienced rapid and large scale damage to its salt marshes due to a brown marsh event. We analyzed the genetic relationship among the *S. alterniflora* samples collected from the severely affected dead zones and lightly affected peripheral transition zones of brown marshes and compared it with those from healthy marsh areas. Forty *S. alterniflora* individuals collected from 18 brown marsh and 6 healthy non-brown marsh sites were fingerprinted using amplified fragment length polymorphism (AFLP) markers. Twelve *Eco*RI/*Mse*I primer combinations produced 684 scoreable fragments, of which 59.9% were polymorphic. Each *S. alterniflora* individual was clearly distinguished by its unique AFLP fingerprint. Individuals from healthy marshes were clearly separated from individuals of brown marsh-affected areas in UPGMA cluster analysis. Individuals in closer proximity to each other tend to be more genetically related than those sampled in distantly located sites. Partitioning of the genetic variability by analysis of molecular variance (AMOVA) showed that the within-group variance component was high (91%) compared to the between-group component (9%). This preliminary study suggests that the surviving brown marsh individuals are genetically different from those of healthy marshes, and their survival could be due to favorable combination of genes responsible for tolerance to multiple abiotic stresses. However, further confirmation by DNA assay and field evaluation using large sample size is necessary to identify die-back tolerant genotypes for marsh restoration projects.

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*Key words:* genetic diversity, marsh die-back, salt marsh, smooth cordgrass, *Spartina alterniflora,* wetland restoration.

## INTRODUCTION

Smooth cordgrass (*Spartina alterniflora* Loisel.) is the single most important grass species in salt marshes along the Gulf of Mexico and the eastern United States coastline (Valiela et al. 1978). Salt marshes are of major ecological importance because they provide habitats for numerous fish, birds, mammals, and invertebrates, and act as the primary producer of organic matter for coastal food chains. Healthy marshes also provide important defenses against coastal storms and hurricanes. Because of its vigorous spreading ability, strong underground rhizome growth, and stress tolerance (Anderson and Treshow 1980), *S. alterniflora* is widely used to prevent soil erosion and restoring wetlands along coastal areas (Woodhouse et al. 1976).

The term "brown marsh" refers to a rapid and large-scale browning and die-back of the Louisiana salt marshes that appears periodically and was particularly severe during spring 2000. This phenomenon normally occurs on a small scale during fall, but the extent of damage in 2000 was so severe that it was imperative to investigate (http://www.brownmarsh.net/). Although this brown marsh phenomenon was observed along the entire Louisiana coastline, salt marshes between the Atchafalaya and the Mississippi river were the most severely affected. As the dieback spread, approximately 390,000 ac were affected, with varying levels of vegetative destruction. About 110,000 ac were severely impacted, of which at least 17,000 ac were converted to open mud flats with little or no vegetation. Any further incidence and spread of marsh dieback without a timely recovery of the impacted areas could significantly contribute to the large scale erosion of coast line already observed in this region. Continued loss of this valuable resource threatens the economy and environment of coastal Louisiana.

The cause of the 2000 marsh dieback is still unknown but is widely believed to be the effect of a series of biotic and abi-

otic stresses, including prolonged drought, high salinities, heat, evaporation, and extremely low fresh water discharges from the Mississippi and Atchafalaya rivers. Extensive sampling from the brown marsh areas in summer 2000 indicated that aboveground and belowground plant tissues were damaged, providing little likelihood of plant recovery in the severely impacted areas. A lack of *S. alterniflora* seed bank and limited natural seedling recruitment in dieback areas from adjacent healthy plants, low seed viability, and specific conditions required for germination may limit this method of recovery (Booth and Hendry 1993, Travis and Hester 2005).

As the major native constituent of the salt marshes, *S. alterniflora* is planted in many wetland restoration and creation projects on both the Atlantic and Gulf coasts of the United States. There is a high risk of deterioration of marshes from the pressure of biotic and abiotic stresses; this is particularly true in Louisiana, where the majority of the marsh restoration projects use the clonally propagated single genotype 'Vermilion' (released by the USDA-Natural Resources Conservation Service in 1989). A lack of genetic diversity could increase risk of further deterioration of restored and new wetland areas from biotic and abiotic stresses. The harmful effect of these stresses on marsh productivity and longevity can be buffered and reduced by planting genetically diverse and superior *S. alterniflora* individuals. *S. alterniflora* clones available in adjoining states may not be suitable for the local environments because environmental stresses can elicit differential response from diverse genotypes (Smith and Proffitt 1999). The lack of diverse and suitable plant material capable of tolerating disturbances such as brown marsh poses a serious challenge to resource managers of coastal restoration projects. To meet this challenge, a breeding program for *S. alterniflora* has been initiated at the Louisiana State University Agricultural Center. The occurrence of marsh dieback offered an excellent opportunity for us to identify unique individuals from the surviving *S. alterniflora* genotypes.

Molecular markers have been widely used to analyze population structure and genetic diversity in ecologically important plant species (Parker et al. 1998). Random amplified polymorphic DNA (RAPD) markers were employed to examine genetic diversity in *S. alterniflora* populations introduced to Willapa Bay, Washington (Stiller and Denton 1995), and RAPD analysis of *S. alterniflora* collected along the Atlantic and Gulf Coasts revealed three separate groups correlating to geographic regions (O'Brien and Freshwater 1999). This marker system was also utilized to study the extent and degree of hybridization between the invading *S. alterniflora* and native *S. foliosa* in San Franscisco Bay, California (Ayres et al. 1999, Daehler et al. 1999). Amplified fragment length polymorphism (AFLP; Vos et al. 1995) is a high throughput molecular marker system that effectively generates distinct DNA fingerprints in closely related genotypes. It is both PCR- and restriction-based and generates a large number of stable genetic markers compared with RAPD analysis. Most AFLP markers follow Mendelian inheritance and can be used for population genetic studies (Mueller and Wolfenbarger 1999, Ronikier 2002). AFLP has been used to evaluate the genetic relatedness of a wide range of plant species, including *S. alterniflora* (Perkins et al. 2002, Travis et al. 2002, Ryan et al. 2007). Perkins et al. (2002) assessed the genetic diversity among *S. alterniflora* samples collected from neighboring marshes in Mobile Bay that were native or created with introduced clones. Similarly, Travis et al. (2002) used AFLP markers to evaluate the impact of wetland restoration on genetic diversity by analyzing *S. alterniflora* samples from restored and undisturbed reference sites in southwestern Louisiana.

Aerial survey of brown marsh-impacted regions indicated a few isolated circular patches of green or yellow-green *S. alterniflora* vegetation. These plants either escaped the unfavorable effects that precipitated the die back or were genetically unique to tolerate these unfavorable effects. Information regarding the genetic uniqueness of these surviving *S. alterniflora* plants will be essential for identifying tolerant genotypes and developing improved populations of *S. alterniflora* for vegetative restoration and protection of the Louisiana coastline. Therefore, the objective of this study was to analyze the genetic relationship among the few surviving S. *alterniflora* plants taken from the highly affected dead zones, the lightly impacted peripheral brown marsh transition zones, and from healthy non-brown marsh areas.

## MATERIALS AND METHODS

### Sample Collection

In late summer 2000, 18 dieback sites were identified across the Terrebonne and Barataria basins (Figure 1). An additional site was added later during a second survey in spring 2001. Surviving ramets of *S. alterniflora* were collected from each site from the distinct interior dead zone where the dieback impact was severe and from the transition zone on the periphery of these dead patches that experienced less severe damage. The distance between samples taken from dead and transition zone varied from site to site and ranged from 10 to 50 m (Table 1). Individual samples were labeled with the prefix BMD or BMT to designate the brown marsh dead zone or brown marsh transition zone, respectively. Samples were then taken to a greenhouse at the Golden Meadow Plant Materials Center, Galliano, Louisiana, and reestablished in containers. Two samples, BMT-13 and BMD-21 from the transition zone and dead zone respectively, did not survive. Therefore, paired samples from both dead zone and transition zone were available for 16 sites. In addition to these 34 brown marsh genotypes, six more plants, including the cultivar 'Vermilion,' were drawn from six geographically separated healthy marsh areas across southern Louisiana (Figure 1). Samples from healthy marshes were designated with a prefix HM. Four of these plants (HM-44, HM-49, HM-75, HM-90) were from sites in southwestern Louisiana located farther from the brown marsh areas and two (HM-50 and Vermilion) were collected from sites close to brown marsh affected areas. Young green leaf tissues (approximately 5-10 gm) were collected from each sample and stored at -80 C until DNA extraction.

### DNA Isolation and AFLP Protocol

Leaf tissues from each individual were ground into a fine powder in liquid nitrogen using a mortar and pestle, and ap-

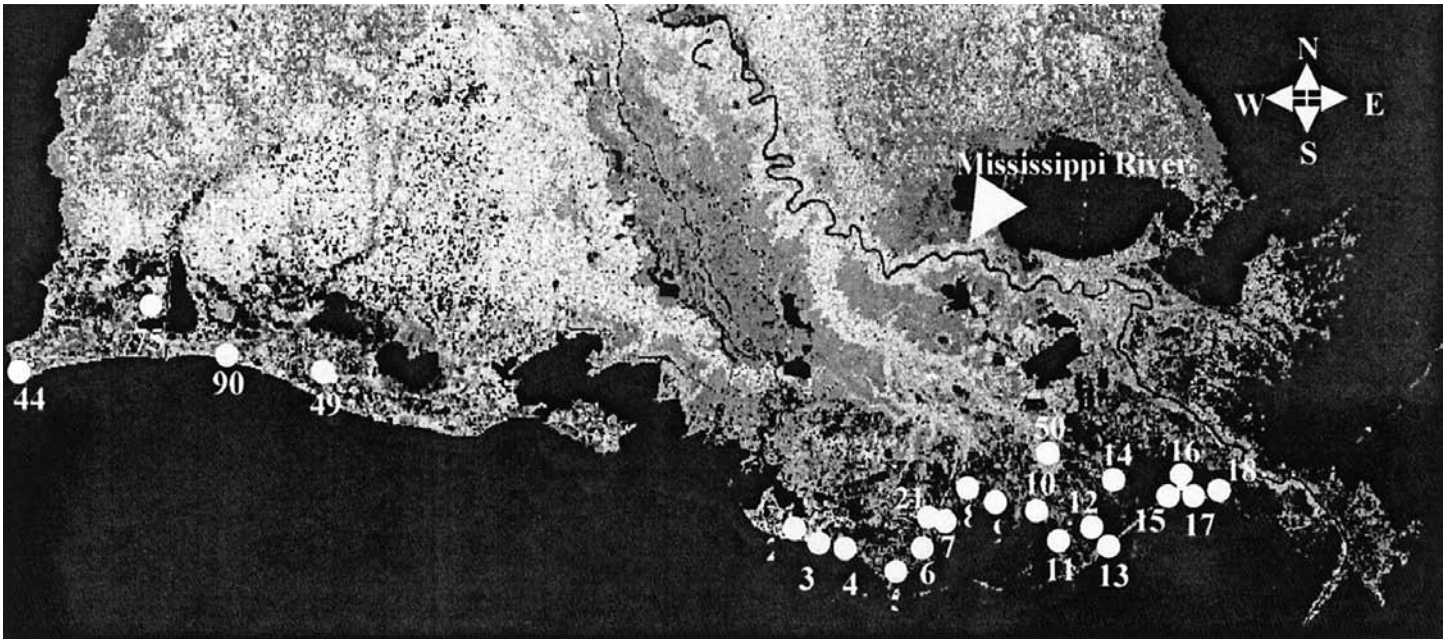


Figure 1. Sampling sites in brown marsh and healthy marsh areas plotted with LOSCO Environmental Baseline Inventory Dataset "Thematic Mapper Image of Louisiana, UTM15 NAD83, LOSCO (1999) [la_south]. Coordinates of each site are given in Table 1. Samples were collected from interior dead zones and transition zones at each site. Due to closeness between dead zone and transition zone samples in each site, sites were labeled without BMD or BMT prefix.

proximately 100 mg of the powder was used to isolate genomic DNA using the GenElute™ Plant Genomic DNA kit (Sigma-Aldrich®, St. Louis, MO) following instruction provided by the manufacturer. The quality and concentration of DNA was determined by running a 1% agarose gel; the final concentration of the DNA was adjusted to 50 ng/µl.

The AFLP protocol described by Subudhi et al. (2005) was followed. Twelve *Eco*RI+*Mse*I primer combinations (Table 2) were used for selective amplification using a PTC-100 thermocycler. The thermocycler was programmed as follows: cycle 1: 30 sec at 94 C, 1 min at 65 C, 1 min at 72 C; cycles 2 through 13: annealing temperature reduced by 0.7 C in every successive cycle; cycles 14-36: annealing at 56 C; final extension for 5 min at 72 C and hold at 4 C. AFLP reaction products (12 µl) were added to an equal volume of loading dye (0.025% each of xylene cyanol and bromophenol blue, deionized formamide, 10 mM EDTA). Samples were denatured for 4 min at 95 C immediately before loading and were placed on ice while loading into the gel. We analyzed 3 µl of AFLP reaction products on 6% denaturing polyacrylamide gels and electrophoresed in 0.5× TBE at 50 C, 110 W for 2 h. A BioRad Sequi-Gen® (38 by 50 cm) unit was used to run the gels. Silver staining of the gels was done manually following the procedure of Fritz et al. (1999). After gels were developed, a sheet of 3 mm Whatman white paper was placed under the developed gel to visualize the AFLP bands; gels were then scanned directly using a scanner.

### AFLP Scoring and Data Analyses

Saved images of the silver stained gels of 12 primer combinations were scored as 1 or 0 for presence or absence of bands, respectively. All clear and unambiguous monomorphic and polymorphic fragments were scored. Binary matrices were made for statistical analyses using NTSYSpc version 2.1 (Exeter Software, Setauket, NY) (Rohlf 1997). Dice (1945) coefficient of similarity was used to estimate similarity among the genotypes, and cluster analysis was performed following the unweighted pair group method with arithmetic averages (UPGMA; Sneath and Sokal 1973). Analysis of molecular variance (AMOVA) was applied using ARLEQUIN version 2.0 (Schneider et al. 2000) to determine the amount of genetic variation among individual genotypes and groups (healthy marsh, brown marsh-dead zone, and brown marsh-transition zone). Genetic diversity was evaluated among the three sample groups on the basis of average heterozygosity values (Lynch and Milligan 1994) and proportion of polymorphic loci (Hartl and Clark 1997). The proportion of polymorphic loci (P) was estimated as the number of loci at which the most common allele had a frequency of less than 95% divided by the total number of individuals in the samples.

## RESULTS

### AFLP Polymorphism in *S. alterniflora*

The protocol for AFLP analysis was optimized for *S. alterniflora*, and 12 *Eco*RI-*Mse*I primer combinations used in this survey yielded a total of 684 markers, of which 410 were polymorphic (Table 2). The total number of amplified bands per primer combination ranged from 46 for *Eco*RI-ACG/*Mse*I-GAC to 80 for *Eco*RI-AAC/*Mse*I-CTC. The polymorphism rate was highest in the primer combination *Eco*RI-ACG/*Mse*I-CAC (83.3%) and lowest in *Eco*RI-ACT/*Mse*I-CAT (35.9%), with an average of 59.9%.

TABLE 1 LIST OF *S. ALTERNIFLORA* SAMPLES COLLECTED FROM BROWN MARSH AND HEALTHY MARSH AREAS OF LOUISIANA, UNITED STATES AND LOCATION COORDINATES OF COLLECTION SITES.

| Sample name | Time of collection | Site of collection | Latitude (D°M'S") | Longitude (D°M'S") |
|---|---|---|---|---|
| BMD-2 | Fall 2000 | Brown Marsh | 29 14 48 N | 91 08 37 W |
| BMT-2 | Fall 2000 | Brown Marsh | 29 14 48 N | 91 08 37 W |
| BMD-3 | Fall 2000 | Brown Marsh | 29 11 50 N | 91 05 21 W |
| BMT-3 | Fall 2000 | Brown Marsh | 29 11 50 N | 91 05 21 W |
| BMD-4 | Fall 2000 | Brown Marsh | 29 10 40 N | 90 59 26 W |
| BMT-4 | Fall 2000 | Brown Marsh | 29 10 40 N | 90 59 26 W |
| BMD-5 | Fall 2000 | Brown Marsh | 29 06 03 N | 90 48 49 W |
| BMT-5 | Fall 2000 | Brown Marsh | 29 06 03 N | 90 48 49 W |
| BMD-6 | Fall 2000 | Brown Marsh | 29 10 56 N | 90 43 20 W |
| BMT-6 | Fall 2000 | Brown Marsh | 29 10 56 N | 90 43 20 W |
| BMD-7 | Fall 2000 | Brown Marsh | 29 15 02 N | 90 37 58 W |
| BMT-7 | Fall 2000 | Brown Marsh | 29 15 02 N | 90 37 58 W |
| BMD-8 | Fall 2000 | Brown Marsh | 29 21 20 N | 90 33 18 W |
| BMT-8 | Fall 2000 | Brown Marsh | 29 21 20 N | 90 33 18 W |
| BMD-9 | Fall 2000 | Brown Marsh | 29 18 22 N | 90 28 00 W |
| BMT-9 | Fall 2000 | Brown Marsh | 29 18 22 N | 90 28 00 W |
| BMD-10 | Fall 2000 | Brown Marsh | 29 16 22 N | 90 18 57 W |
| BMT-10 | Fall 2000 | Brown Marsh | 29 16 22 N | 90 18 57 W |
| BMD-11 | Fall 2000 | Brown Marsh | 29 11 11 N | 90 14 20 W |
| BMT-11 | Fall 2000 | Brown Marsh | 29 11 11 N | 90 14 20 W |
| BMD-12 | Fall 2000 | Brown Marsh | 29 13 23 N | 90 07 36 W |
| BMT-12 | Fall 2000 | Brown Marsh | 29 13 23 N | 90 07 36 W |
| BMD-13 | Fall 2000 | Brown Marsh | 29 10 11 N | 90 04 30 W |
| BMD-14 | Fall 2000 | Brown Marsh | 29 21 47 N | 90 02 59 W |
| BMT-14 | Fall 2000 | Brown Marsh | 29 21 47 N | 90 02 59 W |
| BMD-15 | Fall 2000 | Brown Marsh | 29 19 13 N | 89 50 27 W |
| BMT-15 | Fall 2000 | Brown Marsh | 29 19 13 N | 89 50 27 W |
| BMD-16 | Fall 2000 | Brown Marsh | 29 20 50 N | 89 49 06 W |
| BMT-16 | Fall 2000 | Brown Marsh | 29 20 50 N | 89 49 06 W |
| BMD-17 | Fall 2000 | Brown Marsh | 29 19 09 N | 89 41 43 W |
| BMT-17 | Fall 2000 | Brown Marsh | 29 19 09 N | 89 41 43 W |
| BMD-18 | Fall 2000 | Brown Marsh | 29 18 44 N | 89 46 35 W |
| BMT-18 | Fall 2000 | Brown Marsh | 29 18 44 N | 89 46 35 W |
| BMT-21 | Spring 2001 | Brown Marsh | 29 15 32 N | 90 39 47 W |
| 'Vermilion' | Fall 2000 | Healthy Marsh | 29 26 56 N | 90 16 05 W |
| HM-50 | Fall 2000 | Healthy Marsh | 29 26 56 N | 90 16 05 W |
| HM-44 | Fall 2000 | Healthy Marsh | 29 43 08 N | 93 51 14 W |
| HM-49 | Fall 2000 | Healthy Marsh | 29 42 14 N | 92 45 54 W |
| HM-75 | Fall 2000 | Healthy Marsh | 29 54 36 N | 93 22 59 W |
| HM-90 | Fall 2000 | Healthy Marsh | 29 45 43 N | 93 07 26 W |

## Genetic Diversity in *S. alterniflora* from Brown Marsh and Non-brown Marsh Areas

All 40 *S. alterniflora* individuals were placed into three groups, brown marsh-dead zone (BMD), brown marsh-transition zone (BMT), and healthy marsh (HM), for comparison. Genetic diversity within each group was assessed initially on the basis of average heterozygosities and the proportion of polymorphic loci (Hartl and Clark 1997; Table 3). The average heterozygosity (H) value of BMD was highest followed by BMT and HM. This trend was not consistent when the proportion of polymorphic loci (P) was considered. Following 0.95 criterion, 356 of 684 fragments were polymorphic in BMT, whereas only 290 and 214 fragments were polymorphic in BMD and HM, respectively. The proportion of polymorphic loci (P) was highest in the BMT (0.5204), followed by BMD (0.4239) and HM (0.3129). Relative frequencies of monomorphic and polymorphic fragments among these three groups were tested by a chi-square contingency analysis, and a significant difference was observed ($\chi^2 = 60.644$, d.f. = 2, $P < 0.001$).

Genetic similarity estimates, measured as Dice coefficients using the whole data set ranged from 0.8036 to 0.9853 (Table 3). When within-group variability was examined, the samples from BMT revealed more variation (0.8766) but with a greater range (0.8456 to 0.9853) compared to BMD and HM samples. The mean Dice estimates within BMD or HM were similar, but the range was greatest in BMD. Interestingly, the range of differences in similarity values among the HM individuals was the least, but the mean value was similar to BMD and greater than BMT. Analyses of the among-group genetic variation revealed that average genetic similarity was not significant among the three groups (data not shown).

TABLE 2 LIST OF SELECTIVE *ECO*RI AND *MSE*I PRIMER COMBINATIONS (THREE SELECTIVE NUCLEOTIDES) USED TO GENERATE AFLPS IN *S. ALTERNIFLORA* AND THE POLYMORPHISM RATE FOR EACH COMBINATION.

| | Primer combinations | | No. of variable (polymorphic) bands | No. of fixed (monomorphic) bands | Total number of bands | Polymorphism rate |
|---|---|---|---|---|---|---|
| | *Eco*RI | *Mse*I | | | | |
| 1 | ACG | CAC | 45 | 9 | 54 | 83.3 |
| 2 | ACG | CAA | 38 | 24 | 62 | 61.3 |
| 3 | ACT | CAT | 19 | 34 | 53 | 35.9 |
| 4 | ACG | CAT | 37 | 13 | 50 | 74.0 |
| 5 | ACA | CAA | 25 | 40 | 65 | 38.5 |
| 6 | ACG | AGC | 32 | 22 | 54 | 59.3 |
| 7 | AAC | CTC | 44 | 36 | 80 | 55.0 |
| 8 | ACG | GAC | 27 | 19 | 46 | 58.7 |
| 9 | ACG | CTT | 26 | 21 | 47 | 55.3 |
| 10 | ACG | CTG | 44 | 12 | 56 | 78.6 |
| 11 | ACC | CTG | 39 | 19 | 58 | 67.2 |
| 12 | ACT | CAC | 34 | 25 | 59 | 57.6 |
| | | Total | 410 | 274 | 684 | 59.9 |
| | | Average | 34.2 | 22.8 | 57 | 59.9 |

## Cluster Analysis using UPGMA

Genetic differences among the 40 individual genotypes were clearly visible in UPGMA analysis (Figure 2). This analysis revealed that each of the 40 *S. alterniflora* individuals is genetically distinct, indicating a high level of diversity. All 40 individuals could be grouped into five clusters. All six HM samples belonged to cluster I with two subclusters of three individuals each. Vermilion was grouped with HM-75 and HM-90 in cluster IA, and genotypes HM-50, HM-44, and HM-49 were grouped in the IB cluster. There was no obvious separation of BMD and BMT individuals into separate groups, and all 34 individuals could be grouped into 4 different clusters. Cluster II was the largest, with four subclusters composed of 14 BMD and 11 BMT individuals. Cluster III included three BMD and three BMT genotypes clearly separated into subclusters IIIA and IIIB. The individuals BMD-14, BMD-15, and BMD-18 were in subcluster IIIA, and BMT-12, BMT-14, and BMT-15 were in subcluster IIIB. Cluster IV comprised only the genotype BMT-3, and Cluster V comprised two individuals, BMT-2 and BMT-4. The high cophenetic correlation coefficient of 0.882 indicated a good fit of the AFLP data for cluster analysis and thus strongly supported the reliability of clusters based on the generated data.

We examined the genetic similarity estimated between the paired BMD and BMT samples for 16 locations. The BMD-9/BMT-9 pair was the closest pair (0.9706) and BMD-4/BMT-4 pair was the most distant pair genetically (0.8403). Most of these BMD-BMT pair similarity estimates were higher than the average between groups estimates. Even though the physical distance separating these BMD-BMT pairs was less, they were not clustered together in most cases.

## Partitioning of Genetic Variation by AMOVA

Analyses of molecular variance (AMOVA) were employed to partition the total genetic variation (Table 4). A significantly large component of total genetic variation was contributed by variation within groups (90.8%). Variation among the three groups accounted for only 9.2% of overall variance but was nevertheless significant ($P < 0.001$). Values of $F_{ST}$ revealed a low-to-moderate level of differentiation among BMD, BMT, and HM samples. The rate of differentiation between the BMT and BMD zone samples was very low ($F_{ST}$ = 0.0334), and it was 5- to 6-fold less differentiated than between HM and BMD-BMT samples ($F_{ST}$ = 0.1860 between HM and BMD and 0.1488 between HM and BMT). The overall level of differentiation among all three groups of *S. alterniflora* was 0.09193, indicating an average migration rate among groups on the order of 2.46 migrants per generation [where Nm is the migration rate defined as $(1\text{-}F_{ST})/4F_{ST}$ (Wright 1969)].

TABLE 3 DIVERSITY WITHIN BROWN MARSH AND HEALTHY MARSH GROUPS OF *SPARTINA ALTERNIFLORA* AS REFLECTED BY THE AVERAGE HETEROZYGOSITY (H), PROPORTION OF POLYMORPHIC LOCI (P), AND SIMILARITY VALUES (DICE COEFFICIENT).

| Group | Size of group | H | P | Similarity within the group (mean and range)* |
|---|---|---|---|---|
| Brown marsh-dead zone | 17 | 0.3851 | 0.4239 | 0.9036 (0.8036-0.9679) |
| Brown marsh-transition zone | 17 | 0.3697 | 0.5204 | 0.8766 (0.8456-0.9853) |
| Healthy marsh | 6 | 0.3609 | 0.3129 | 0.9032 (0.8822-0.9268) |

*Range of similarity values are in parentheses.

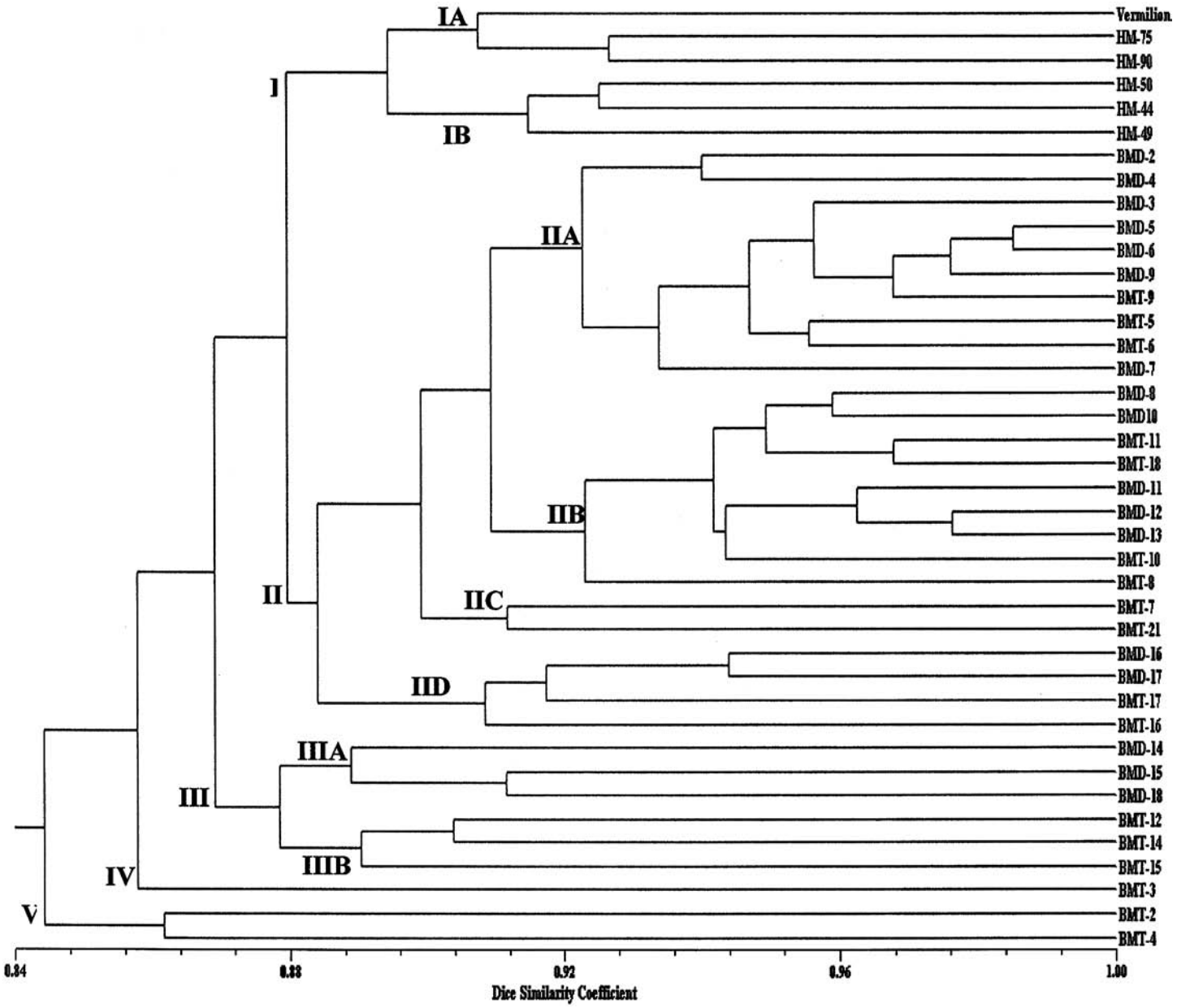


Figure 2. Dendrogram of 34 brown marsh and six healthy marsh *Spartina alterniflora* genotypes produced by UPGMA clustering method based on the Dice similarity coefficient matrix derived from 684 AFLP markers. Individuals with prefix BMD or BMT are from the dead zone or transition zone of the brown marsh, respectively. Vermilion is a released clone for vegetative propagation and the genotypes HM-50, HM-44, HM-49, HM-75, and HM-90 were collected from healthy marsh areas in southern Louisiana.

## DISCUSSION

### AFLP Polymorphism, Population Structure, and Genetic Diversity in *S. alterniflora*

Few studies involving RAPD (Stiller and Denton 1995, O'Brien and Freshwater 1999) and AFLP markers (Perkins et al. 2002, Travis et al. 2002) in *S. alterniflora* are available. The polymorphism rate observed in the present study is comparable to that of Perkins et al. (2002), who observed 63% polymorphism among seven populations sampled from native and created marshes. While both Travis et al. (2002) and Stiller and Denton (1995) reported only 41% polymorphism with AFLP and RAPD markers, respectively, O'Brien and Freshwater (1999) could identify 75% polymorphism in a study involving RAPDs.

Our results showed that genetic diversity in the brown marsh was comparable to the level of diversity observed in *S. alterniflora* sampled from healthy marsh areas. This conclusion was corroborated from the mean Dice coefficient values of each of the groups (Table 3). The range of similarity coefficient values is of the same order as those of Stiller and Den-

TABLE 4 ANALYSIS OF MOLECULAR VARIANCE (AMOVA) FOR 40 BROWN MARSH AND HEALTHY MARSH *S. ALTERNIFLORA* GENOTYPES USING 684 AFLP MARKERS. ALL 40 SAMPLES WERE GROUPED INTO THREE CATEGORIES: BROWN MARSH-DEAD ZONE, BROWN MARSH-TRANSITION ZONE, AND HEALTHY MARSH. STATISTICS INCLUDE SUMS OF SQUARED DEVIATIONS (SSD), VARIANCE COMPONENTS ESTIMATES, THE PERCENTAGE OF THE TOTAL VARIATION CONTRIBUTED BY EACH COMPONENT AND THE PROBABILITY (P) BASED ON 1000 PERMUTATIONS.

| Source of variation | Degrees of freedom | SSD | Variance components | % of Total | P |
|---|---|---|---|---|---|
| Among groups | 2 | 241.02 | 5.43 | 9.19 | <0.001 |
| Within groups | 37 | 1983.71 | 53.61 | 90.81 | <0.001 |
| Total | 39 | 2224.73 | 59.04 | | |

ton (1995) but is much higher than the values obtained by O'Brien and Freshwater (1999). *S. alterniflora* samples from transition zones were relatively more diverse compared to the other two groups, as revealed from the proportion of polymorphic genes. The relatively low proportion of polymorphic genes among the HM group is probably due to the small sample size; however, the average heterozygosity values were higher than those reported by Travis et al. (2002) in *S. alterniflora*. The comparison among the groups and the similarity of trends clearly indicate significant genetic diversity is available for exploitation by aquatic plant managers.

The UPGMA clustering provided important information about the genetic relationship among the genotypes sampled from the brown marsh. Because samples were collected from the dead zone and transition zone in contiguous patches, the dead zone plants can be considered as a subset of genotypes of the population of plants of that particular site. Because plants in the transitional zone did not experience stress as severe as plants in the dead patches, the transitional zone should have maintained the entire range of genetic variability, including the plants lost during the brown marsh event. This could explain the higher diversity exhibited by the BMT group as compared to the BMD group. Survival of the plants in the dead zone could be attributed to the favorable combination of genes that are responsible for tolerating abiotic stresses occurring during brown marsh events.

The fact that healthy non-brown marsh genotypes are cleanly separated into a single group (Figure 2) confirmed differences in their overall genetic makeup compared with the brown marsh samples. Two of these healthy non-brown marsh samples (50 and Vermilion) were collected from places closer to brown marsh affected areas, and these two samples still did not group with the BM samples in UPGMA clusters. There was no clear genetic differentiation among brown marsh genotypes based on dead zone or transition zone. The open-pollinated nature of *S. alterniflora* in this clonally propagated species (Somers and Grant 1981) may be the reason for the increased level of diversity between BMD and BMT samples from several sampling sites observed in this study. Although the bulk of the genetic variation (91%) occurred within groups, a significant amount of variance (9%) could be attributed to between group components (Table 4). Higher level of within-population variance was also reported by Travis et al. (2002). Cluster analysis provided some evidence of underlying subpopulation structure based on geography. Group IIA individuals are from the sites in close proximity to each other, and a similar pattern was also noted for Group IIB. Groups IID and III are physically close to each other as well. Our treatments of dead zone and transition zone are intermixed within the underlying subpopulation structure, which probably arose through limited gene flow (isolation by distance) among the groups of plants we analyzed.

## Implications for Wetland Restoration

There is now tremendous interest in wetland creation and restoration activity in the United States to prevent further wetland loss. With increased environmental perturbation due to natural factors, increased plant and genotypic diversity is needed for enhanced fitness and longevity of salt marshes (Seliskar 1995, Seliskar et al. 2002). Genetic variability helps maintain high fitness potential among individuals with distinct genotypes for out-crossing. Particularly during periods of rapid environmental change, genetic diversity is crucial for natural selection and maintenance of population viability (Hamrick et al. 1991, Richards et al. 2004).

Increased diversity in the planting materials in wetland restoration projects has also been recommended to avoid inbreeding and to increase the ability of marsh population to adapt to environmental disturbances (Travis et al. 2004). Selection of *S. alterniflora* lines with desirable characteristics will have significant implications for ongoing marsh creation and restoration efforts (Gallagher 1995). Because the goal of wetland restoration and creation is to achieve functional equivalence with native local marshes, it is essential to genetically improve adaptive traits in native *S. alterniflora* plant populations. This preliminary study suggests that the surviving brown marsh individuals we sampled, were genetically distinct from those of healthy marshes and may have favorable combination of genes for adaptation in unfavorable marsh environments. The results obtained from this investigation, however, should be viewed with caution due to the limited number of samples analyzed. Further confirmation and field evaluation using large sample size is needed to identify suitable native genotypes for marsh restoration projects.

## ACKNOWLEDGMENTS

The authors wish to thank Alan Shadow and Gary Fine for technical assistance to complete this investigation. Financial support for this study from the Louisiana Department of Natural Resources is gratefully acknowledged. This manuscript is approved for publication by the Director of Louisiana Agricultural Experiment Station as manuscript number 07-14-0415.

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