# Genetic Evaluation of California Bulrush and Seed-based Propagation of Smooth Cordgrass

#### **Herry Utomo**

Rice Research Station LSU AgCenter







## **Project Goals**

- Develop California bulrush cultivars for use in coastal marsh restoration.
- Employ cellular selection to improve salt tolerance in California bulrush.
- Evaluate natural genetic diversity of smooth cordgrass using DNA markers.
- Develop seed-based propagation, seed harvest, handling, and storage to support an aerial seeding application.

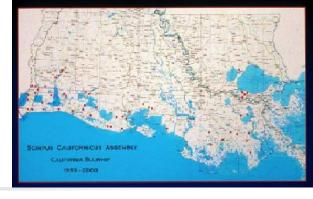


#### **Field Trials**

- ☑ 10x10 ft spacing
- ☑ RCBD, 2 blocks, 3 reps w/in block
- ☑ Loc: RRS plots; Planted: May 26, 04

#### **Parameters:**

- ✓ Spreadability (area of spread, rate of spread, stem density)
- ☑ Stem diameter
- ☑ Plant height
- ☑ Biomass
- ☑ Seed production and germination rate
- ☑ Disease resistance





#### **Field Trials**

Source	df	Height	Stem Diameter	Area of Spread	Rate of Spread	Biomass	Stem Density
Block	1	111.80	47.87 **	86.59 **	63.01 **	350.66 *	15792.62**
Rep	2	148.52	1.16	0.44	0.74	19.66	719.89
Entry	45	484.88 **	13.99 **	10.41**	7.53 **	103.38 **	3621.67**
Error	223	97.43	3.02	4.35	3.23	57.15	943.97
Total	271						

<sup>\*,\*\*</sup> Significant at the 0.05 and 0.001 probability level, respectively.

### **Field Trials**

Accession				Rice Res	Rice Research Station site				
	Sprd. (m²/yr)	Hgt. (cm)	Stem dent. (/m²)	Stem diam. (cm)	Tillers (per m²)	Seed prod. (g)	Vigor	Dis. rating	
68268	6.3848	186.5	33.8	13.13	26.3	337.6	6.4	2.4	
68309	5.6159	176.2	48.3	12.73	39.2	306.4	6.7	2	
Restorer	4.4184	164.8	33.2	12.4	29.3	262.8	6.5	2.2	
LSD (0.05)	1.9721	14.3	14.7	2.03	10.9	43.1	n.s.	n.s.	
CV	43.82	7.14	29.67	13.56	27.45	45.46	14.52	23.21	



### **Cultivar Development**

#### **MULTI-LOCATION TRIALS**

#### 3 locations:

Pointe Aux Chenes, Avoca Island, and Wildlife refuge Cameron.







## **Field Trials - Cameron 2007**

Accession	Stem Count			
	Anova	GLM, LSMEAN		
68268	81.67	86.63 ± 10.47		
68309	76.83	93.36 ± 10.98		
Restorer	43.33	62.0 ± 12.35		
LSD (0.05)	33.57			
CV	94.97	55.53		



#### **Cellular Selection**

- Protoplast screening.
- 823 plants were recovered.
- Greenhouse tests.

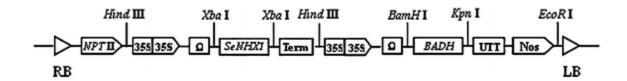




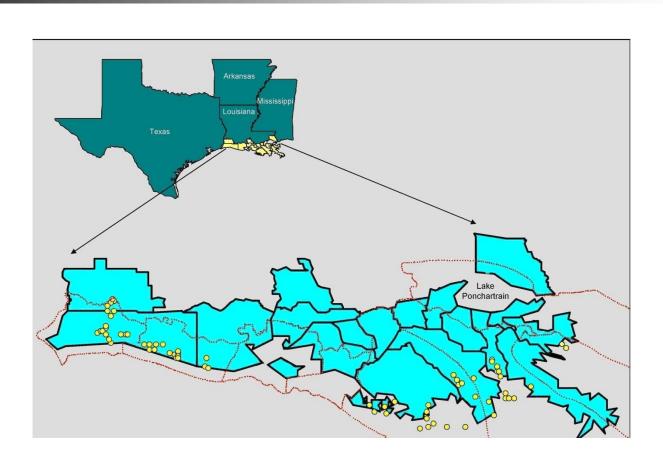


#### On Going experiments

- Trait Stability tests Progeny of the in vitro salt tolerant lines is being used to determine the stability of the trait.
- Established tissue and protoplast culture to be used to facilitate gene transformation.







# Oligonucleotide sequence of adaptors and primer combinations used in AFLP analysis

ner	Sequence
Msel	5'-GACGATGAGTCCTGAG-3'
<i>Eco</i> RI	3'-TGCTACTCAGGACTCAT-5' 5'-GACTGCGTACC-3'
	3'-CTGACGCATGGTTAA-5'
M50/E36 <sup>†</sup> M62/E36	M-CAT/E-ACC M-CTT/E-ACC
M59/E38	M-CTA/E-ACT
M62/E40	M-CTT/E-AGC
	Msel  EcoRI  M50/E36 <sup>†</sup> M62/E36  M59/E38

# Polymorphic loci and genetic diversity of 4 groups of *Spartina alterniflora* accessions according to their geographical origin

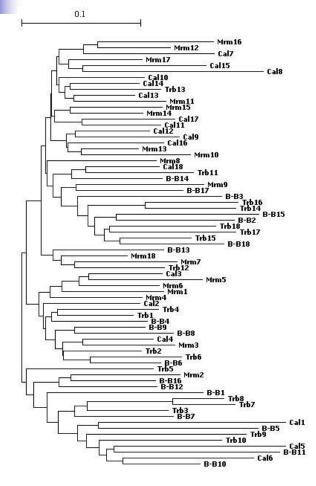
Population/ Basin	Poly- morphic	%	H <sub>s</sub>	na	ne	h	1
Barataria-Breton	157	62.8	0.2168	1.6280	1.3699	0.2168	0.3246
Terrebonne	144	57.6	0.1984	1.5760	1.3396	0.1984	0.2971
Mermentau	138	55.2	0.1878	1.5520	1.3250	0.1878	0.2806
	152	60.8	0.2026	1.6080	1.3465	0.2026	0.3042

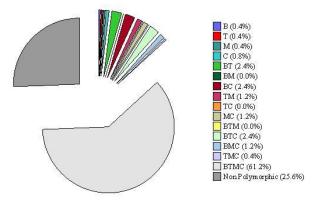
Average  $H_s = 0.2014 \pm 0.0327$   $H_t = 0.2159 \pm 0.0365$  $G_{st} = 0.0671$  Unbiased measures of identity and genetic distance among basin groups of *Spartina alterniflora* accessions.

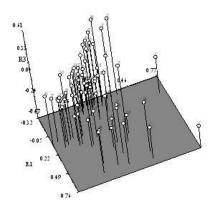
	Barataria- Breton	Terrebonne	Mermentau	Calcasieu
B-Breton	-	0.9819	0.9751	0.9761
Terrebonne	0.0812	-	0.9677	0.9708
Mermentau	0.0252	0.0329	-	0.9839
Calcasieu	0.0242	0.0297	0.0163	-

## AMOVA and partitioning AFLP variation in *Spartina alterniflora* accessions from 4 coastal Louisiana basins

Source of Variation	df	Sum of Square	Variant Component	Percentage Variation	Р
Among basins	3	116.29	0.836	3.41	< 0.001
Within basins	68	1612.39	23.711	96.59	< 0.001
Total	71	1728.68	24.548		
Fixation index (F	$(s_t) = 0.034$	1			









## **Summary of findings**

- Phenetic analysis did not indicate any association between AFLP pattern and basin-based geographic origin.
- Most diversity (96.6%) resided within each basin.
- Use of samples collected from relatively large areas within a basin for habitat restoration and erosion control is sufficient for maintaining high levels of genetic diversity.
- Inclusion of plant samples from other basins will not likely increase genetic diversity.
- Choosing parental lines from different coastal Louisiana basins for breeding purposes may not significantly increase genetic variability produced among the progeny lines.



#### Seed-based Propagation of Smooth cordgrass

- Seed production aspects
- Seed handing and storage





- Seed dormancy. A cold stratification of 2-3°C for a month to break the dormancy.
- Unorthodox traits, long-term storage of S. alterniflora is very challenging.
- Seed cannot be maintained in a desiccated condition in a prolonged period of time.
- The seed remains viable for 6 months under cool and humid conditions, but its viability becomes rapidly deteriorated thereafter.
- Identified a genotype that can tolerate up to 2 yrs of storage conditions.
- To find Plant GRs that can slow down the seedling growth while maintaining its high seedling establishment rates





#### **Seed-based Propagation of Smooth cordgrass**



Foundation plots of poly-cross populations: 2 locs. - Rice Research Station, Crowley (0.25 acre) and the Ben Hur Farm, (0.25 acre)

Seed harvested in the first week of November 2007.

Currently undergoing stratification at 2°C and 100% humidity.

Direct seeding of blend seeds will be evaluated in three locations; Rice Research Station and two intertidal sites in the Fourchon/Bayou Lafourche, LA.

Three seeding rates (75, 50, and 25 seeds per square foot): January, March, and May 2008)

Data collected: seedling survival, seedling vigor, spread, % coverage, plant height, and seed production.

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### Smooth cordgrass vs. rice

	Yie	eld		Area planted
Crop	Lbs/A	No. seed (million)	Seeding rate	(using seed harvested)
Smooth cordgrass	146.9	20.2	16 lbs/A (2.2 mil. seeds/A)	9.2 A
Rice	5800.0	106.3	120 lbs/A (2.2 mil. seeds/A)	48.3 A



#### An alternative approach

Up to the present date,

- Sprigs, plugs, or potted plants of *S. alterniflora* are used for erosion control and habitat restoration.
- Labor intensive 25 to 125 hours per acre.
- Mechanized digging and planting reduce about half the time.
- Expensive Labor requirements and plant cost from can easily approach \$2,700 to \$3,500 per acre.
- Assessiblitity issues.

Seed-based aerial seeding in an alternative to overcome these problems.



