ANNUAL PROGRESS REPORT

DEVELOPMENT OF STRESS TOLERANT SEASHORE PASPALUM FOR GOLF COURSE USAGE

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Objective 1. Establish an extensive collection of genetic material

A total of 66 ecotypes (cultivars) has been collected thus far; an additional six vegetative ecotypes (2 from Israel and 4 from Brazil) are in quarantine at Beltsville, MD (Table 1). In the working collection, 4 accessions came from Africa, 7 from Argentina, 4 from the Caribbean region, 4 from Georgia, 26 from Florida, 4 from Texas, 10 from California, 1 from Australia, 2 from Arizona, and 4 from Hawaii. Textures range from very fine (suitable for greens) to intermediate, to coarse (similar to St. Augustinegrass), to very coarse ornamental types.

A major collection trip was made to the Ft. Myers, FL area during July 1993. Alden Pines Golf Course on Pine Island in north Ft. Myers is 100% seashore paspalum (course was established in 1981). I selected 15 ecotypes from greens, tees, fairways, roughs, and cart paths. I also collected 2 ecotypes from Sanibel Island, and one from Marco Island in the Ft. Myers area. At the International Turfgrass meetings at Palm Beach in July, I collected 5 ecotypes from the Breaker's ocean course, as well as one type from the Gulf Stream Golf Course south of Palm Beach. Two ecotypes were collected in late June from Galveston Island, TX. Dave Kopec in Arizona has gratuitously furnished his collection of 15 accessions (designated K1-13 and K16-17) that he has accumulated over several years.

Two collection trips are planned for 1994. A 2-week trip to Hawaii involving all islands is planned from January 16-29, 1994 and another trip to coastal Georgia, including Sea Island, will be made sometime during the summer, 1994.

On October 15th, I received the accessions from quarantine handling at Glenn Dale, MD. The Israeli genotypes (-1,-2) and the Brazilian genotypes have been transplanted in the greenhouse. Two Brazilian cultivars are definitely P. vaginatum, one is P. distichum, and the other is suspected to be P. dilatatum.

Objective 2. Improve adaptability of the species

A. Acid soil stress tolerance

Various cultivars have been established at pHs ranging from 3.9 to 6.5. All plantings were made on high bulk density soils with low organic matter. Thus far, I have found that the genotypes have no problem rooting in Georgia soils, and that they can withstand very severe prolonged (6 weeks) drought stress with high heat (>90°F) and humidity (>50%). They are, at least, equal to the hybrid bermudas in tolerance to drought. All plots were maintained at 1" mowing height. Persistence and quality factors will be monitored over a 3-year period.

B. Winterhardiness

The cold thermal threshold for this species (based on Adalayd) has been documented in the literature as 17-18°F. Twenty-five accessions were subjected to normal field winter exposure in one field on the Georgia station. Two separate 1992-1993 winter cold-shock and recovery cycles of 16°F hit the field plots.
PI364368 (Mozambique) was killed; Parrish HS was severely damaged, but recovered. PI364985 (South Africa) has less winter-kill than Parrish HS and recovered faster. Sixteen genotypes were planted at Bledsoe farm at pH 4.0 on a 5° slope and all plots survived one episode of 12°F cold-shock.

On June 9th, 33 accessions were planted at the Blairsville Mountain Station at an elevation of about 1100 feet. This location is subjected to 0° and below temperatures during the winter. Winter survival ratings will be recorded during June 1994.

I have ordered a cold chamber to begin initial determination of cold thermal thresholds for each accession in the collection. A Ph.D. graduate student (Cesar Cardona) will begin these evaluations during January-March, 1994. The chamber will also be used to initiate cold tolerance improvement of the species, based on techniques modified from C. Taliaferro's bermudagrass improvement program at Oklahoma State.

C. Wear resistance

No studies have been initiated yet, but plots have been established with Bob Carrow and Carrow has an operational "wear" machine that will be used next summer (1994).

Satellite Research

1. Three mother nurseries have been established on station. Fifty of the 66 types have been planted. Plastic will be used to cover these plots this winter, to minimize winter injury or kill. Plans in 1994 include a consolidation of all cultivars into one general area (near the original mother nursery). Also, a grouping of genotypes by texture (fine → coarse) will help standardize mowing and other maintenance requirements.

2. Management studies

   A. In cooperation with B. Carrow, six grasses ('Penncross') bentgrass; 'Tifgreen' bermuda; 'Adalayd', 'SIPV-1', 'Mauna Key', and 'HI-1' seashore paspalums) were planted on a green during June 1993 on 9' x 9' plots with 4 replications and 2 N-fertility treatments (48 total plots). Mowing height will be 5/32". This is a 3-year study to determine management and turf quality factors.

   B. In cooperation with B. Carrow, six grasses ('SIB-2-1' Sea Island bermuda selection, Adalayd, SIPV-1, Mauna Key, HI-1, and 'Tifway' bermuda) were planted on a fairway in June 1993 on 15' x 15' plots, 4 reps, and 2 N-fertility treatments (48 total plots). This is also a 3-year study. Water-use parameters and quality factors will be monitored.

   C. In cooperation with B. J. Johnson, Mauna Key, Adalayd, PI509022, and PI299042 were planted during August 1993 on 3' x 24' plots with 3 reps. Thirteen herbicide treatments will be imposed on these plots during 1994. A similar test involving 4 additional cultivars will be established during the summer 1994. These will be 3-year studies on herbicide management. Tim Murphy evaluated Manage 50W (Monsanto 12051) herbicide on 4 paspalums (PI509021, PI509022, PI299042, SIPV-1) during June 1993. All cultivars tolerated the new herbicide quite well.

   D. In cooperation with Kris Braman, 21 cultivars have been evaluated from free-choice armyworm moth oviposition. Mean number ($n=12$) of larvae per pot revealed a significant difference among cultivars, with Fidalayel and 561-79 having no larvae and PI364985 and SIPV-2 having the most. The preliminary results suggested ovipositional preference or differential larval survival. The cultivars were also evaluated for tawny and southern mole cricket infestation (single cultivar, 10-day exposure, no-choice test, $n=4$). Tawny mole crickets caused a significantly greater reduction in quality ($\bar{x}=4.06$ vs. 6.70, respectively; uninfested control = 8.00, i.e., 10.00 was maximum quality) than southern mole crickets; both produced a significant loss of quality, compared to uninfested controls. Cultivars ranged from 8.00 quality rating (tolerant) for Tropic Shore to a minimum rating of 4.50 (susceptible) for Glenn Oaks Adalayd from southern mole cricket feeding. For tawny mole cricket infestation, cultivars ranged from 6.25 (tolerant) for Excalibre and SIPV-1 to 1.25 (susceptible) for Glenn Oaks Adalayd.

   Future research will be directed to determination of mechanism(s) of resistance to mole crickets, white grubs, surface feeding caterpillars, and spittlebugs.

E. In a preliminary shade test (~30% light) under mature oak trees, 21 paspalums were compared to zoysiagrass and St. Augustinegrass. After 2 years, only Temple 1 was still alive among the paspalums and it did not maintain adequate turf quality, compared to zoysia and St. Augustine.

F. In cooperation with Larry Shuman and preliminary investigations of using seashore paspalum to clean up heavy-metal-contaminated soils, nutrient solution experiments were conducted using paspalum, bermudagrass, St. Augustinegrass, and centipedegrass. Incomplete nutrient solutions were used to prevent the metals from precipitating or being complexed by chelates used for Fe. The solutions contained 400 μM KNO_3, and 100 μM $MgCl_2$ in order to prevent loss of root integrity. In separate experiments, Zn was added at rates up to 200 μg/mL, Pb up to 20 μg/mL and Cd up to 50 μg/mL and plants were subjected to the solutions for 4 days. Net root length and root weights were measured, along with plant height and dry weight.

Paspalum was the only one of the three to show tolerance of Zn levels up to 500 μg/mL as determined by root weights and root lengths. The plant weight and height measurements for these experiments were too variable to be used to test tolerance. For Pb, St. Augustinegrass and centipedegrass were tolerant up to 10 μg/mL, but the others were not tolerant even at 5 μg/mL. For Cd, the centipedegrass data is not yet available; however, bermudagrass showed some tolerance up to 5 μg/mL. Paspalum and St. Augustinegrass were tolerant of only 2.5 μg/mL.

These experiments are continuing and will include Ni and Cu. The next set of experiments will "fine-tune" these results by using lower metal rates, as determined by the highest tolerance levels in these preliminary tests. A greenhouse pot experiment with these metals will follow.

G. An initial overseeding study was established during October 1993 involving 10 paspalums varying in texture from fine to coarse. Two grasses (Med 32 and Fults puccinellia distans) were planted in 3' wide bands across cultivars. Additional cool-season species will be added as needed. Data will be collected beginning in January 1994.

3. Biotechnology studies

A. A Ph.D. graduate student (Cesar Cardona) is developing a tissue culture regeneration system as a preliminary step prior to gene transformation research later. He has used various explant sources (immature embryos, meristematic stem tissues, spikelets) and has had some success in coercing embryogenic calli and initial regenerated seedlings. The protocol is still being refined, since each cultivar responds differently and bacterial contamination has been a major problem with two cultivars. A stable, reliable regeneration system should be in place by late summer 1994.

B. A post-doctoral student shared with the S-9 USDA Plant Genetic Resources Conservation Unit-Griffin (J. Liu) has run RFLPs and RAPDs on 80 Paspalum species; in addition, he is currently running RAPDs on 47 seashore paspalum cultivars. Eventually, a genome map will be constructed on Paspalum, and a cDNA library will be developed using Excalibre seashore paspalum. During 1994, and in conjunction with Steve Kresovich, microsatellites will be used to definitively "fingerprint" each accession of seashore paspalum. This information will be used to 1) determine relatedness (diversity) among the cultivars (especially since many accessions from the U.S. are thought to be derivatives from Adalayd-Excalibre), and 2) provide a reliable scientific determination for adding unique accessions to the USDA paspalum collection. Three diploid x diploid (one involving Paspalum vaginatum) crosses

were accomplished during the winter 1992-1993. F1s have been grown out and F2 seed have been collected. Gene mapping will follow using these populations.

C. DNA content of seashore paspalum cultivars is being determined via flow cytometry in conjunction with Bob Jarret (USDA-Griffin) and Marianne O'Prisko (Texas A&M University Ph.D. Student). These data will provide some relationships among ploidy levels of different Paspalum species and hopefully aid in future interspecific crossing studies to transfer various traits.

4. General breeding studies

A. In conjunction with Marianne O'Prisko (Texas A&M University), preliminary studies in the greenhouse have revealed that seashore paspalum has either a temperature-induced trigger (cold shock of $\leq 60^{\circ}F$), or a photoperiod-sensitivity trigger ($\leq$11 hours light, or $\geq$16 hrs light) to induce flowering. Some accessions require one or both triggers for inducement, while others are totally photoperiod and temperature insensitive. Eventually, all accessions will be evaluated for these traits.

B. Paspalum districhum is the fresh-water, aquatic biotope species that is similar to P. vaginatum except the former has no salt tolerance and, theoretically, has greater cold tolerance, since it is normally found further inland. I have 21 accessions from the Plant Introduction collection that have been germinated in the greenhouse and will be used for interspecific mating with P. vaginatum. Both species are diploids. The crossing will be done during the winter 1993-1994.

C. Circular polycross blocks were planted at Bledsoe research farm during July 1993. Twelve blocks using 2-parent groups in various combinations (SIPV-1, HI-1, Glenn Oaks, Temple 1, Mauna Key, K2-K5, K-7, K-9, PI377709, PI299042, PI509020, PI509021) were established to assess this technique for intraspecific mating.

D. An initial harvest of seeds from about 30 cultivars was made in September 1993. Seed viability will be checked to assess seed production capability.

Seashore paspalum Research Funding - 1993

1.	U.S. Golf Association, Green Section Research	$20,486
2.	Georgia Seed Development Commission (1 year)	8,000
3.	Georgia Turfgrass Foundation Trust, Inc. (1 year)	7,500
	1993 Total	$35,986

Table 1. Ecotypes currently in the seashore paspalum collection.

Identification	Origin
PI299042	Zimbabwe
PI364368	Mozambique
PI364985	S. Africa
PI377709	S. Africa
PI509018†	Argentina
PI509020	Argentina
PI509021	Argentina
PI509022	Argentina
PI509023	Argentina
310-79	Argentina
561-79	Argentina
SIPV-1‡	Georgia
SIPV-2‡	Georgia
Glenn Oaks‡	Georgia
AM-3554	Georgia
Taliaferro	Florida
Parrish HS	Florida
AP1-11‡	Florida
AP 13-16‡	Florida
SAN-1	Florida
SAN-3	Florida
Marco-4	Florida
Breaker 1-5	Florida
Gulf Stream‡	Florida
Temple-1	Texas
Temple-2	Texas
GAL-1	Texas
GAL-2	Texas
Fidalayel‡	California
Excalibre‡	Australia
K1-9‡	California
K10-K13	Caribbean
K16-K17	Arizona
HI-1	Hawaii
HI-2	Hawaii
Mauna Key	Hawaii
Tropic Shore	Hawaii
Israel 1-2§	Israel
Brazil 1-4§	Brazil

†At least 3 ecotypes have been selected out of this accession.

‡Suspected close derivatives or selection from original Adalayd introduced from Australia

§Currently still in quarantine at Beltsville, MD