ANNUAL PROGRESS REPORT

CONCERNING

BREEDING AND EVALUATION OF COLD TOLERANT BERMUDAGRASS VARIETIES

SUBMITTED BY:

Dr. C. M. Taliaferro
Plant Breeder and Geneticist

Dr. J. A. Anderson
Plant Stress Physiologist

Dr. M. P. Kenna
Turfgrass Scientist

Oklahoma Agricultural Experiment Station
Oklahoma State University

Jointly Sponsored By:

United States Golf Association

and

Oklahoma Agricultural Experiment Station

October 25, 1987

EXECUTIVE SUMMARY

ANNUAL PROGRESS REPORT

BREEDING AND EVALUATION OF COLD TOLERANT BERMUDAGRASS CULTIVARS

PRINCIPLE INVESTIGATORS: Dr. C. M. Taliaferro, Dr. J. A. Anderson, and Dr. M. P. Kennaa

REPORT PERIOD: November 1, 1986 to October 30, 1987

RESEARCH PROGRESS:

1. Currently we have accomplished a 3-fold increase in basic fertility for a bermudagrass breeding population while making improvements in texture.

2. Progress was made developing reliable and rapid screening methods for cold tolerance. These methods allow for laboratory and greenhouse testing of genotypes, without field test winters.

3. Successful tissue culture regeneration of plants from immature inflorescences was achieved. This is an important first step in developing haploid plants, and subsequent homozygous lines for production of uniform F_1 hybrid progeny.

4. Several genotypes are currently being screened in replicated turfgrass variety trials. Texture and turf quality are approaching that of 'Midiron' and U-3. Rate of coverage from vegetative material for these genotypes was superior to 'Tifgreen', 'Tifway', and 'Midiron'.

5. Several plants from the breeding populations were identified as potential parents for use in synthesizing new seeded varieties. Seed from these crosses will be evaluated this year in Stillwater, Oklahoma, and increased for evaluation next year by outside cooperators.

6. A total of 2,500 new F_1 progeny were established in field nurseries for evaluation of basic fertility and growth habit.

7. Approximately 100 plants were selected for preliminary cold hardiness testing this winter, and for inclusion in replicated turf trials spring, 1988.

I. INTRODUCTION

Research with bermudagrass (Cynodon dactylon) and other Cynodon species has been underway for more than 20 years at Oklahoma State University (OSU). During the mid 60's, a world-wide collection of Cynodon germplasm was assembled at OSU and used in a comprehensive biosytematic study of the genus by J. R. Harlan, J. M. J. de Wet, Wayne Huffine, and others. A breeding program was initiated in the late 60's with emphasis on improving forage quality of pasture-type bermudagrass varieties. The varieties 'Hardie' and 'Brazos' were subsequently released in 1974 and 1984, respectively.

A by-product of the forage quality breeding work on bermudagrass pasture varieties was the identification of winter hardy germplasm with relatively good fertility (i.e., percent of florets setting seed). Two germplasm lines from this program were used as parents in the synthesis of 'Guymon', which is a coarse-textured, but very winter hardy seed-propagated bermudagrass.

Presently, there is no cold-tolerant, seed-propagated, fine-textured turf bermudagrass variety available for use in the northern half of the bermudagrass belt. Consequently, the primary objective of this research is the development of such varieties.

II. RESEARCH PROGRESS

A. Breeding and Development

Two cycles of phenotypic recurrent selection for basic fertility (as indicated by average seed weight/head) and growth habit were completed on a broadbased bermudagrass population previous to this report period (Figure 1). This population was synthesized from cold-tolerant, relatively fertile accessions from our Cynodon germplasm. The most significant result of the two cycles of selection was a 3-fold increase in the basic fertility. An obvious response to selection for growth habit also occurred. Plants selected for finer texture tended to produce progeny with finer texture in the C_1.

Selected plants from the C_1 population were polycrossed in 1986 to produce the C_2 population comprised of 672 plants. A field nursery containing the C_2 population was established during the spring 1987. This population is currently being evaluated for fertility and growth habit (Figure 2). Additionally, plants other than those from the phenotypic recurrent selection nurseries were polycrossed to provide progeny populations from which to select. A total of 2,500 F_1 progeny were established into field nurseries during spring, 1987. Approximately 100 plants were selected from these progeny populations for inclusion in replicated turf trials in spring, 1988.

Several plants from the breeding populations were identified as potential parents for use in synthesizing either single or polycross progeny populations for evaluation as possible new varieties. Polycross seed from some of these parents was obtained this year. In

1988, isolated field plantings will be established to obtain additional single and polycross seed.

B. VARIETY TRIALS

The reproductive characteristics of bermudagrass make the utilization of desirable genotypes possible at any time. The ability to vegetatively increase single plants on a field scale allows for the development of new varieties which could be established by sod, plugs, or sprigs. Sexual self-incompatibility allows for the synthesis of new seeded varieties from parental plants that combine well to produce adequate seed yields and desirable progeny populations.

Some plants in our present populations are approaching the desired combination of characteristics (i.e., winter hardiness, good fertility, and fine texture). Replicated turfgrass evaluation trials were established in May and June of 1986, and July 1987. Entries in one of the 1986 trials included the national bermudagrass variety trial (28 accessions), other bermudagrass varieties (6 accessions), and experimental bermudagrasses developed at OSU (10 accessions). Turfgrass quality was evaluated during April through September of 1987, and leaf width was determined for each variety to help sort out textural differences among the varieties (Figure 3).

Among the vegetatively propagated bermudagrasses, 'Hilltop', 'Texturf', 'Tifgreen', 'Vamont', 'Tufcote', and 'Tifway' were the best performers (Table 1). The recently released variety, NMS-1, ranked the highest among seed propagated entries. The 'Guymon' variety has a wide leaf blade which results in coarse texture. It consistently ranks low in turf quality ratings for this reason. The winter of 1986-87 was mild and none of the bermudagrass varieties winter killed.

Superior parents from the breeding nurseries were established in a replicated turfgrass evaluation trial in July 1987 (Figure 4). Rate of establishment, turfgrass quality, color, texture, and fall dormancy were evaluated this summer and fall (Table 2). Some experimental bermudagrass varieties (i.e., OK 1-7, OK 41-3, and OK 45-3) are approaching the texture and turfgrass quality of 'Midiron' and 'U-3'. However, these experimental varieties are still much coarser than 'Tifgreen' or 'Tifway'. The rate of establishment for all experimental varieties was superior to the four commercially available varieties.

C. COLD HARDINESS

Many workers have developed laboratory procedures to evaluate cold hardiness levels of turfgrasses and cereals. Relative hardiness estimates, rather than absolute hardiness level, were usually determined. Exposure of sprigs or plugs to one treatment temperature in a freezer gives relative hardiness. However, absolute hardiness levels requires removing samples sequentially over a series of temperatures.

Two independent procedures were evaluated for determining cold hardiness levels of 'Midiron' and 'Tifgreen'. The electrolyte leakage test used bermudagrass crowns removed from the soil, thoroughly washed,

and placed in test tubes (Figure 5). When equilibrium was reached at -3° C in an ethylene glycol bath, chips of ice were dropped in the tubes to prevent supercooling. This ensured evaluation of freeze tolerance rather than avoidance of freezing (Figure 6).

Crowns were held overnight at -3° C. Samples were removed the next day at 2° C intervals while the bath was cooled at a rate of 2° C/hour. Following slow thawing at 0° C, 20 ml of distilled water was added to the tubes containing the crowns. Electrical conductivity of the water in the tubes was measured 24 hours later. Samples were then heat-killed in an autoclave and electrical conductivity readings were taken 24 hours later.

Response data were expressed as the ratio of electrical conductivity following exposure to freezing to the value after being heat-killed. The killing temperature was determined as the warmest treatment level resulting in 40% or greater loss of total electrolytes. This value was found to correspond most closely to the value with the greatest slope in a plot of electrolyte leakage vs. treatment temperature.

The second procedure evaluated regrowth of bermudagrass plugs in the greenhouse after they were exposed to freezing temperatures in an air cooled, low temperature chamber. Samples were removed at selected temperatures measured by thermocouples inserted into each soil cylinder. Samples were then thawed at room temperature, placed in 15 cm pots containing commercial soil mix, and transferred to a greenhouse. Survival was visually determined 3 to 4 weeks later as regrowth, and the critical temperature was the coldest treatment temperature permitting at least one viable shoot.

Electrolyte leakage and regrowth tests were in close agreement, differing in hardiness estimates by 0.1 to 1.4° C. 'Midiron' was found to be hardier than 'Tifgreen' on all dates tested. Differences in killing temperatures of 2 to 4° C were indicated by electrolyte leakage, and 0 to 2.3° C for regrowth. The period of greatest freeze tolerance for both 'Midiron' and 'Tifgreen' was December and January. Tifgreen retained hardiness to about -5° C through mid-April, and lost all freeze tolerance by June 4. Midiron exhibited a killing temperature of -7° C in mid-April, and retained freeze tolerance to -5° C on June 4.

The advantage of the electrolyte leakage test is the rapid turn around time for determining cold hardiness (i.e., 2 days). Regrowth tests using plugs requires more time (3 to 4 weeks) and also utilizes a large amount of greenhouse space. If time and space are not limiting, the regrowth test may provide a slightly more accurate determination of field cold hardiness. A disadvantage of the electrolyte leakage test is the time required for tissue preparation. For relatively small samples where a quick answer is needed, the electrolyte leakage test will work very well.

D. TISSUE CULTURE

Tissue culture techniques for several agronomic and ornamental crops were developed over the past several years by other researchers.

However, very little research work has been attempted with bermudagrasses. An important potential use of tissue culture in our breeding program would be as a tool to develop homozygous lines. The highly heterozygous nature of bermudagrass creates problems when developing uniform F_1 hybrid populations. Haploids from tissue culture methods could produce homozygous lines by chromosome doubling with cholchicine. Single-cross combinations of homozygous parents would then produce uniform F_1 hybrid populations for commercial use.

Success was achieved using tissue culture to regenerate bermudagrass explants from very young inflorescence tissues (Figure 8). All regenerated (R_1) plants appear to be phenotypically identical to their R_0 parent. The mode of regeneration was documented as somatic embryogenesis. This was a major accomplishment toward the development of tissue culture as an important tool in the breeding program.

Experiments are currently underway to develop techniques for regenerating haploid plants from anther culture. Callus cultures were successfully produced from bermudagrass anther explants, but plants have not regenerated from these cultures at this time (Figure 9). During the summer, a technique was also developed for <u>in vitro</u> germination of mature bermudagrass pollen grains. The growing pollen tube may possibly be used as explant tissue for developing haploid callus. Preliminary attempts did not produce callus cultures, however, several different modifications of this technique will be investigated. The ability to initiate callus production, and subsequent regeneration of plants from gametophytic tissue, would be a major accomplishment toward the development of haploid plants.

III. RESEARCH PLANNED

A. Breeding and Development

1. Selected plants from the C_1 and C_2 cycles will continue to be polycrossed and also combined in specific single-crosses to provide seed for evaluation of turf performance, and to test combining ability.

2. Isolated field plantings of parents will be established to increase the amount of polycrossed and single-crossed seed available for variety testing.

3. The third cycle of selection for basic fertility and growth habit will be completed in 1988.

4. Around 2,500 F_1 progeny from the mating of plants not originating from recurrent selection populations will be monitored for basic fertility and growth habit.

B. VARIETY TRIALS:

1. Continue evaluating bermudagrass plants selected from C_1 and C_2 populations for turfgrass characteristics and persistence.

2. Potential parental lines will be increased for evaluation by outside cooperators. Materials and protocol will be available during late winter, 1988.

3. Start evaluating experimental seeded varieties in Stillwater in 1988. We also plan to enlist the aid of cooperators in the southwest, Arizona and California, for evaluation of these materials for commercial seed production potential.

4. Establish a replicated turf trial of 100 superior bermudagrass genotypes in summer 1988.

C. COLD HARDINESS

1. Continue to develop the laboratory procedure for rapid screening of cold hardiness.

2. Cold hardiness evaluations will be made on an expanded number of varieties using regrowth tests. Ten superior genotypes from the breeding program will be tested along with 'Midiron', 'Tifgreen', and U-3.

3. Conduct a preliminary screen for cold hardiness on 100 genotypes this winter.

4. Conduct a preliminary study on the relationship between water stress and cold hardiness.

D. TISSUE CULTURE

1. Work will continue on development of techniques to regenerate plants from anther culture for potential production of haploid plants.

2. Continue investigations on in vitro pollen germination and production of haploid plants from pollen tube tissue.

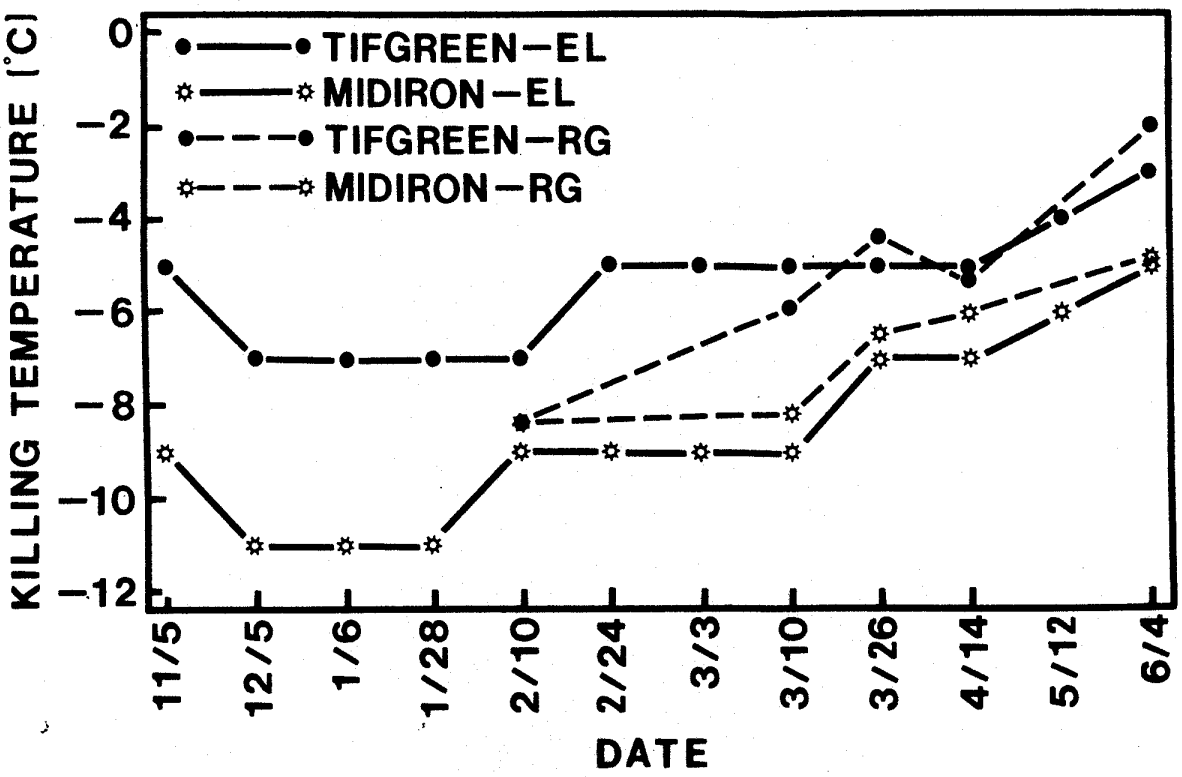

Figure 7. Killing temperatures of 'Midiron' and 'Tifgreen' bermudagrass vs. date. Electrolyte leakage and regrowth tests were in close agreement, differing in hardiness estimates by 0.1 to 1.4° C.

Table 1. Bermudagrass Variety Trial, Spring and Summer 1987. Oklahoma Turfgrass Research Center, Stillwater, OK.

No.	Entry	Leaf width*	Turfgrass Quality**				
			April	May	June	August	Mean
		-- mm --					
28	HILLTOP	3.1	5.3	6.0	5.3	7.0	5.9
36	TEXTURF	3.8	5.3	6.3	5.3	6.3	5.8
2	MSB 20	2.1	5.3	5.7	4.7	7.3	5.8
23	A 29	2.7	5.0	6.0	5.3	6.7	5.8
37	TIFGREEN	2.0	4.7	6.3	3.3	8.0	5.6
15	VAMONT	3.9	5.7	4.7	4.7	6.7	5.4
6	NM 43	2.2	4.7	6.0	4.0	7.0	5.4
16	RS 1	3.3	5.7	5.0	4.7	6.3	5.4
42	TUFCOTE	2.7	5.0	6.0	5.7	5.0	5.4
44	E 29	2.9	5.3	6.0	4.3	6.0	5.4
11	NMS 1	3.7	5.0	4.3	5.3	6.3	5.2
1	TIFWAY	2.3	4.7	5.7	3.7	6.7	5.2
25	NM 507	3.2	4.0	4.7	4.7	7.3	5.2
40	OK 86 12	2.7	4.7	5.0	4.7	6.3	5.2
24	NM 72	2.7	4.3	3.7	5.3	7.3	5.2
27	NMS 2	3.7	4.7	4.0	4.7	7.0	5.1
8	FB 119	2.7	5.0	4.7	4.3	6.0	5.0
20	NMS 14	3.2	5.0	4.3	5.0	5.7	5.0
45	A 22	2.4	5.0	5.0	4.3	5.7	5.0
47	OK 86 11	4.3	4.0	4.7	5.0	5.7	4.8
39	MSB 30	3.9	4.0	4.7	5.0	5.3	4.8
26	NMS 4	3.7	4.0	4.3	4.7	5.7	4.7
3	OK 86 3	3.0	4.3	4.0	4.3	6.0	4.7
34	TIFWAYII	2.2	4.0	5.3	3.3	6.0	4.7
21	OK 86 4	3.6	5.0	4.3	4.0	5.0	4.6
12	MSB 10	3.0	4.0	4.7	3.3	6.0	4.5
43	CT 23	2.4	4.3	5.0	3.7	5.0	4.5
5	MIDIRON	3.2	4.0	4.7	3.7	5.3	4.4
7	OK 86 9	3.2	3.7	4.0	4.7	5.3	4.4
30	OK 86 2	4.4	3.7	4.0	4.0	6.0	4.4
35	U-3	2.6	3.7	4.7	4.0	5.3	4.4
32	OK 86 5	3.6	3.7	3.7	4.3	5.7	4.3
38	NM 471	2.8	3.7	4.0	3.7	6.0	4.3
9	NM 375	3.7	3.3	3.3	4.0	6.7	4.3
46	MIDWAY	3.6	4.0	4.3	4.0	5.0	4.3

* Leaf width was measured on the fourth leaf from newest leaf. Means are for three samples from each plot averaged over three replications.

**Turfgrass quality where 1 = poor to 9 = excellent.

Table 1. (Continued) Bermudagrass Variety Trial.

No.	Entry	Leaf width*	Turfgrass Quality**				
			April	May	June	August	Mean
		-- mm --					
13	SUNTURF	2.6	4.3	3.7	3.3	5.7	4.3
31	OK 86 8	3.9	3.3	3.7	4.0	5.7	4.2
29	OK 86 1	4.3	3.7	3.7	3.7	5.3	4.1
41	NMS 3	3.3	3.7	3.3	3.7	5.0	3.9
14	GUYMON	4.6	3.0	3.3	2.7	5.3	3.6
17	OK 86 6	4.6	3.0	3.3	3.3	4.7	3.6
19	OK 86 10	3.9	3.3	3.0	3.3	4.7	3.6
33	ARIZ COM	3.7	3.3	3.0	3.0	5.0	3.6
48	OK 86 7	4.0	2.7	3.3	3.0	5.3	3.6
	LSD (0.05)	1.4	1.3	1.4	1.5	1.4	1.2
	CV (%)	20.1	19.2	19.5	23.3	15.7	-

* Leaf width was measured on the fourth leaf from the newest leaf. Means are for three samples from each plot averaged over three replications.

**Turfgrass quality where 1 = poor to 9 = excellent.

Table 2. Experimental Bermudagrass Cultivar Trial. Oklahoma Turfgrass Research Center, Stillwater, OK.

Entry	Coverage			Dormancy	Color*		Texture**	Quality+	Cold Hardiness++
	Aug.	Sep.	Oct.		Sep.	Oct.			
	%				rating				
OK 1-7	89	92	99	25	5.2	5.2	4.7	6.7	3
OK 1-8	29	51	84	14	3.5	3.2	5.0	5.5	2
OK 2-2	69	84	94	30	4.2	4.0	5.2	5.7	3
OK 40-3	78	85	93	28	5.5	5.5	5.2	6.0	3
OK 41-3	70	75	91	28	5.2	6.2	4.2	6.0	2
OK 42-3	75	81	91	27	4.5	7.2	4.5	4.5	1
OK 45-3	80	89	98	38	4.7	6.0	4.7	7.0	2
OK 47-3	84	88	94	24	5.0	4.0	5.2	5.5	3
OK 47-4	74	84	93	15	2.2	3.5	6.0	6.2	3
OK 8-7	65	76	90	18	4.0	3.5	6.7	5.0	3
Tifgreen	34	48	64	17	4.5	2.7	1.0	6.0	2
Tifway	15	30	40	9	4.7	2.0	1.5	5.7	-
U-3	45	58	78	9	4.5	4.2	3.0	6.5	2
Midiron	31	40	55	30	3.7	5.2	3.0	5.0	4
Mean	56	65	78	21	4.2	4.4	4.4	5.5	-
LSD(0.05)	16	14	12	17	2.5	2.2	1.3	2.1	-
CV(%)	20	16	11	15	41.5	35.1	20.0	26.5	-

* Color rated on a scale of 1 to 9 where 1 = dark green and 9 = yellow green.

** Texture rated on a scale of 1 to 9 where 1 = fine and 9 = coarse.

\+ Quality rated on a scale of 1 to 9 where 1 = poor and 9 = excellent.

++ Cold hardiness where 1 = killed in the range from -3.8 to -4.5° C, 2 = -4.3 to -5.0, 3 = -4.9 to -6.1, and 4 = -6.3 to -7.1 on September 30, 1987.

Figure 1. Two cycles of phenotypic recurrent selection for basic fertility and growth habit were completed on a broadbased bermudagrass population. The most significant result of the two cycles of selection was a 3-fold increase in the basic fertility.

Figure 2. Selected plants from the C_1 were polycrossed to produce the C_2 population comprised of 672 plants. A field nursery containing the C_2 population was established during the spring 1987.

Figure 3. Replicated turfgrass evaluation trials were established in May 1987. Turfgrass quality was evaluated during April through September of 1987.

Figure 4. Superior parents from the breeding nurseries were established in a replicated turfgrass evaluation trial in July 1987. Rate of establishment, turfgrass quality, color, texture, and fall dormancy were evaluated.

Figure 5. The electrolyte leakage test used bermudagrass crowns removed from the soil, thoroughly washed, and placed in test tubes.

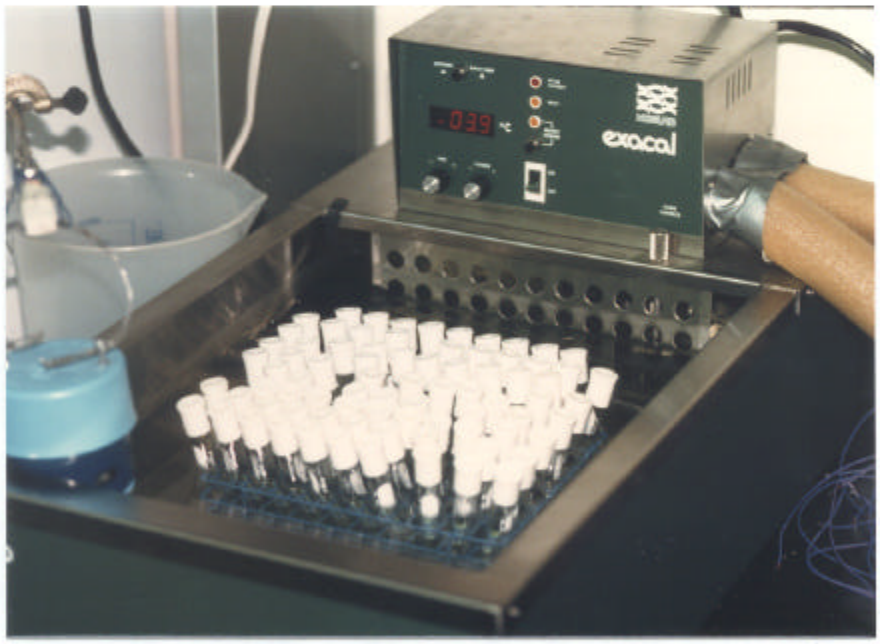

Figure 6. An ethylene glycol bath was used to reach an equilibrium temperature of -3° C. Ice chips were dropped in the tubes to prevent supercooling. This ensures evaluation of freeze tolerance rather than avoidance of freezing.

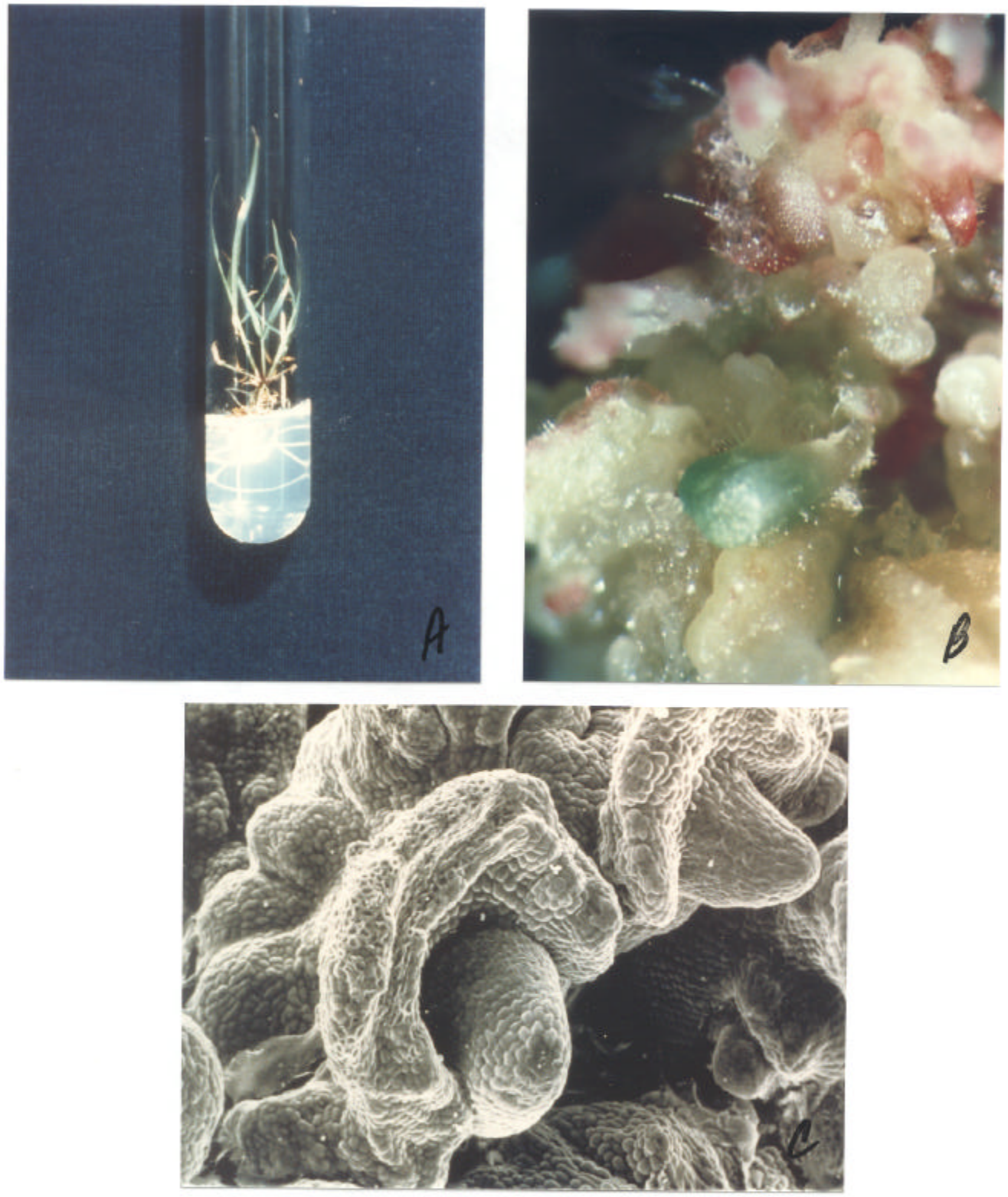

Figure 8. Success was achieved using tissue culture to regenerate bermudagrass explants from very young inflorescence tissues (A). The mode of regeneration was documented as somatic embryogenesis (B - close-up of callus, and C - scanning electron micrograph of somatic embryo).

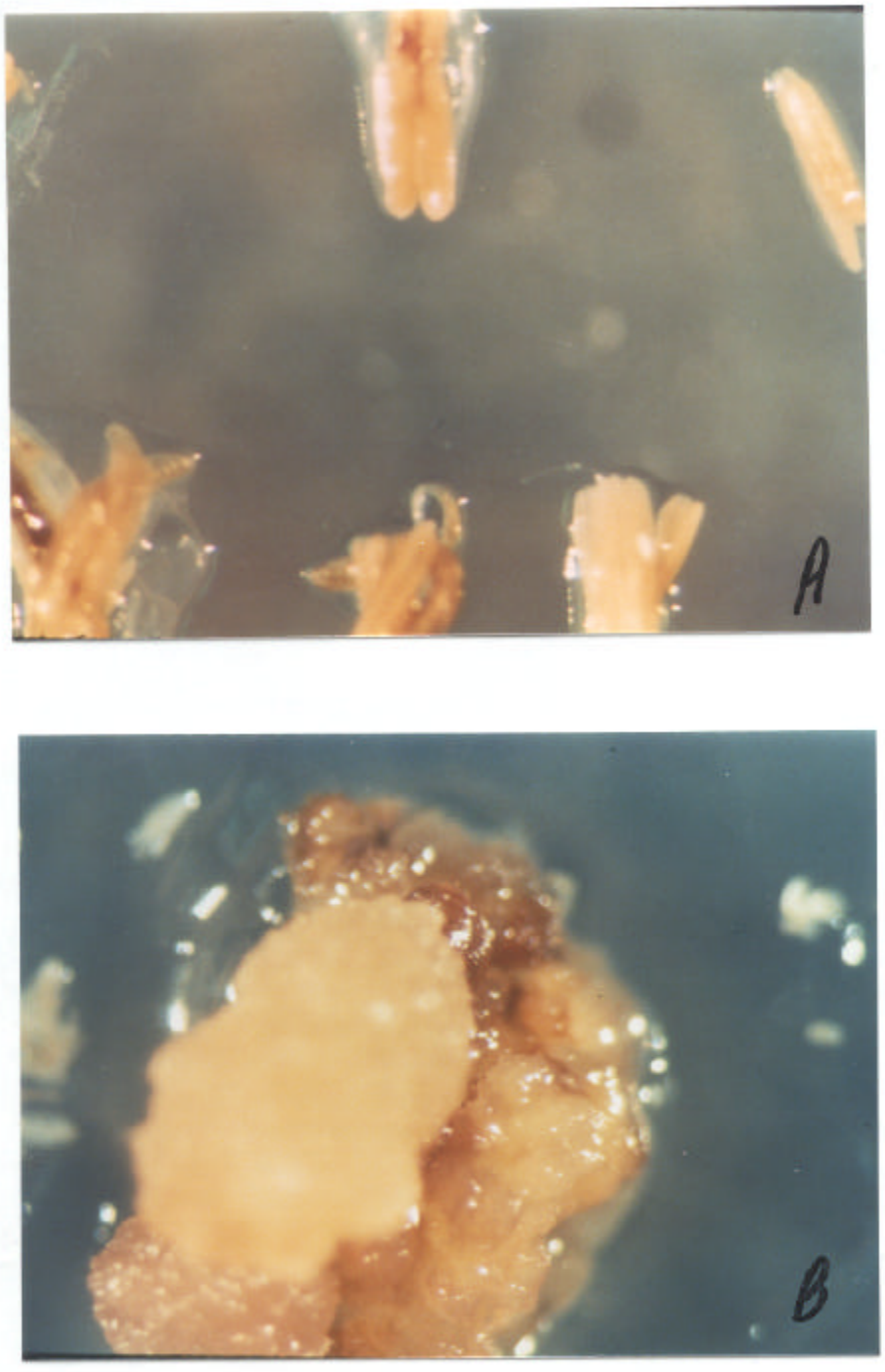

Figure 9. Experiments are currently underway examining if plants can be regenerated from anther culture to produce haploid plants (A - anthers in culture, and B - callus forming from anther tissue).