

SEMI-ANNUAL PROGRESS REPORT
CONCERNING
BREEDING AND EVALUATION OF COLD-TOLERANT BERMUDAGRASS VARIETIES

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EXECUTIVE SUMMARY
SECOND SEMI-ANNUAL PROGRESS REPORT
BREEDING AND EVALUATION OF COLD TOLERANT BERMUDAGRASS CULTIVARS

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REPORT PERIOD: February 1, 1986 to October 30, 1986

Presently there is no cold-tolerant, seed-propagated, fine-textured turf bermudagrass variety available for use in the northern half of the bermudagrass belt. The basic objective of research jointly sponsored by the United States Golf Association and the Oklahoma Agricultural Experiment Station is the development of such varieties.

To date we have identified cold-tolerant bermudagrass plants with good fertility, incorporated them into breeding populations (germplasm pools), and completed two cycles of selection for increased basic fertility and growth characters. Significant response to selection has been documented. An additional cycle of selection will begin next spring.

Significant progress was made in recent months in tissue culture research with bermudagrass. Plants have been regenerated from very young inflorescence explants, and work underway with other explant tissues, including anthers, appears promising. Regeneration of plants from anthers would provide opportunity for development of haploid plants (plants containing half the normal chromosome number) which have several potentially significant benefits to breeding.

Preliminary research was initiated recently to characterize the self-incompatibility mechanism in bermudagrass. Bermudagrass plants typically are strongly self-incompatible, thus will not produce seed when self-pollinated. Although we know the mechanism exists, very little is known about how it works or about its genetic control.

Development of a reliable laboratory technique for measuring cold tolerance in bermudagrass would be of immense value in screening plants for the bermudagrass breeding program. The necessary equipment has been obtained and Dr. Jeff Anderson, stress physiologist in the Department of Horticulture and Landscape Architecture, has initiated experiments to develop the procedures. Development of a laboratory procedure would enable cold hardiness determinations without relying on the occurrence of test winters.

I. INTRODUCTION

This semi-annual report of progress is for the period February 1, 1986, to October 30, 1986. The contractual agreement between the United States Golf Association and the Oklahoma Agricultural Experiment Station for breeding and evaluation of cold-tolerant, seed-propagated turf bermudagrass varieties was effected February 1, 1986. This was the first year of support for the project. Co-investigators in the project at the time of approval were Dr. A. D. Brede, Turfgrass Scientist and Associate Professor in the Department of Horticulture and Landscape Architecture, and Dr. Charles M. Taliaferro, Plant Breeder (grasses) and Professor in the Agronomy Department. As defined in the original proposal, the major division of responsibility between the co-investigators was the breeding work (Taliaferro) vs. comprehensive evaluation of breeding lines for turf characteristics (Brede). Dr. Brede resigned his position with the Oklahoma State University in early September to accept a position with a private firm. Dr. Brede is a very capable, energetic scientist whose talents will be missed; but his departure does not detract from our ability to fulfill the objectives of the research as outlined in the contractual agreement, nor should it result in any delay. The Department of Horticulture and Landscape Architecture is now in the process of refilling the position vacated by Dr. Brede. Dr. Mike Kenna, Turfgrass Extension Specialist in the Horticulture & Landscape Architecture Department, is supervising ongoing research activities in the interim. Either Dr. Kenna or the individual replacing Dr. Brede will assume the responsibilities for evaluating advanced lines from Dr. Taliaferro's breeding program.

II. IMPLEMENTATION

A. BACKGROUND

As indicated in the initial progress report, research with bermudagrass (Cynodon dactylon) and other Cynodon species has been underway at the Oklahoma State University (OSU) for more than 20 years. Beginning in the mid-60's a worldwide collection of Cynodon germplasm was amassed at OSU and used in a comprehensive biosystematic study of the genus by J. R. Harlan, J. M. J. de Wet, Wayne Huffine, and others. This work elucidated biosystematic relationships between taxa within the genus and culminated in its revision. Using the germplasm collection amassed by Harlan et al., a bermudagrass breeding program was initiated at OSU in the late 60's with emphasis on improving forage quality in pasture-type varieties. This effort has produced the 'Hardie' variety, released in 1974, and 'Brazos' bermudagrass, which was cooperatively tested and released (1984) by the Oklahoma, Texas, and Louisiana Experiment Stations. Recurrent selection for high forage quality and adaptation in germplasm pools derived from crossing high-quality, non-winter hardy plants with lower-quality, winter hardy ones has resulted in progressive improvement for those characters and associated performance.

A by-product of the breeding work to incorporate higher nutritive value into adapted pasture varieties was the identification of winter hardy germplasm with relatively good basic fertility (% of florets setting a seed). Two such germplasm lines were used as parents in the synthesis of the recently released 'Guymon' variety. Guymon is a relatively coarse-textured, but very winter hardy seed-propagated variety. It is for use in soil stabilization, and as general-purpose turf in the northern portion of the bermudagrass belt where present commercially available seeded varieties are not adapted. Winter hardy accessions with reasonably good fertility were incorporated into a basic germplasm pool which is now being subjected to phenotypic recurrent selection for increased fertility. Two other important characteristics being modified via selection within this breeding population are cold tolerance and growth habit. The fundamental objective is to produce a fine-textured, winter hardy, seed-propagated variety fully acceptable for use as a turf in the northern half of the bermudagrass belt.

B. PROGRESS TO DATE

As indicated in the 1st semi-annual report, two cycles of selection for basic fertility (as indicated by average seed weight/head) and growth habit have been completed. The C₀ (initial) population, comprised of 1047 plants, averaged 101 mg seed/5 inflorescences (range 0-460), while the C₁ population (517 plants, Fig. 1) averaged 365 mg (range 0-647). Thus, there was a 3-fold difference in seed weight/5 heads between the two populations. Selected plants from the C₁ population were polycrossed this summer (1986) and will be used to establish a C₂ population next spring. Because the size of individual inflorescences varies considerably within and among bermudagrass plants, the percentage of florets setting a caryopsis is the preferred indicator of basic fertility. With the financial support now provided by the USGA, we will measure % seed-set of individual plants in our breeding populations. To do this, multiple (usually 5) inflorescences are collected from individual plants, the number of florets/head determined, then the florets are separated from the rachilla by use of a rub-board, separated into empty and filled by air (South Dakota seed blower), and finally the number of seed is determined with the aid of an electronic seed counter (Fig. 2).

There was also an obvious response to selection for growth habit. Plants with less vigorous growth and/or finer texture tended to reproduce similar progeny phenotypes as indicated by the appearance of plants in the C₁ population and progeny testing of selected plants from that nursery (Fig. 3). Although population improvement of bermudagrass for basic fertility and growth characteristics via recurrent selection is a desirable long-term goal, the reproductive characteristics of the species make possible the utilization of desirable genotypes (plants) at any point in time during the breeding program. The reproductive characteristics are: (1) ability to vegetatively propagate single plants on a field scale, and (2) the strong self-incompatibility mechanism of most plants. Because of these characteristics, any two plants that combine well as

parents to produce adequate seed yields and desirable progeny populations can be used as parents to synthesize a new variety. Pedigreed propagating stock of each of the parent plants may be increased asexually and planted as a mixture to establish seed production fields. We find some plants in our present populations that approach having the desired combination of characteristics that we seek, i.e., good fertility, winterhardiness, and fine texture.

The addition of new germplasm to existing breeding populations is a continuing effort and, to that end, evaluation of 115 spaced plants derived from seed sold commercially as seed from U-3 bermudagrass (a vegetatively propagated variety) was begun this summer. The plants were established in May 1985. All plants survived the unusually mild 1985-86 winter at Stillwater, but there were obvious differences in spring recovery, due presumably to differences in cold tolerance. Spring "greenup" of most of the U-3 progeny was delayed compared to that of known winter hardy plants, but a few plants did begin growth in the spring about the same time as known winter hardy types and exhibited uniform "greenup." Differences in characters such as foliage color and texture, sod density, and incidence of leaf spotting disease (Helminthosporium spp.) were also obvious through the growing season. Fertility of the 115 plants was assessed by measuring seed set percentage on five inflorescences collected in July. Percent of florets setting seed ranged from 1 to 96 with a mean of 49. Fifty-three of the 115 plants had seed set above 50%. Five plants had seed set above 90%. These results suggest that selection within this germplasm population for increased cold tolerance, fertility, sod density, and foliage texture is warranted. Ten plants were selected from this nursery and are being evaluated for turf quality.

Success was achieved in tissue culture of bermudagrass explants this summer. Plants were regenerated from explants of very young inflorescences (Fig. 4). The mode of regeneration has been documented as somatic embryogenesis. Experiments are currently underway with explant sources other than young inflorescences. We are particularly interested in regenerating plants from anther culture because of the potential for producing haploid plants. Such plants would serve many uses in genetic studies and breeding, including the potential for developing genetically homozygous plants which might be used in a single-cross combination to produce a uniform F_1 population for commercial use. The tissue culture research is not supported by USGA funds, but is applicable to the basic objective of developing cold tolerant, seed-propagated turf bermudagrass varieties.

Preliminary research of the self-incompatibility mechanism of bermudagrass was initiated recently. The initial objective is to characterize the mechanism by studying pollen germination on stigmatic surfaces and follow growth of pollen tubes through the stigma, style, ovary, and into the embryo-sac in order to determine where blockage occurs in incompatible matings. Our efforts to date have largely been devoted to perfecting techniques for observing pollen germination and growth of tubes. We have ascertained that

pollen grains on stigmatic surfaces via self-pollination do germinate, and growth of tubes into the stigma and style does occur. We have not yet determined how far such tubes penetrate toward the embryo sac, or where fertilization is blocked. Again, this work is not supported by USGA funds, but is also applicable to the breeding effort.

Replicated turf evaluation trials were established in May and June of this year. One of these trials contained the entries in the National Turf Bermudagrass Variety Test and selected parental plants from our breeding program. Ratings of ground cover (rate of spread), color, texture and turf quality of the entries during the establishment phase are given in Table 1. Additional selected plants from the breeding program were established in another replicated test to observe their response under turf maintenance.

III. RESEARCH PLANNED

BREEDING

Recurrent selection for basic fertility, cold tolerance, and turf texture and density will continue. The C3 population of plants will be established in spring 1987 and selections made during the growing season. Further study of the U-3 progeny population will be made to ascertain the amount of genetic variation for the above mentioned characteristics. Superior plants in the existing field population will be selected and polycrossed to generate a new population.

COLD TOLERANCE EVALUATION

Equipment for laboratory measurement of cold tolerance of plants has been purchased with non-USGA funds. Acclimated crowns will be subjected to a series of low temperatures in order to determine the killing temperature. Plant damage at each stress level will be quantified using the electrolyte leakage viability test. Since the cell membrane is the primary site of injury, damage results in a loss of selective permeability. Solutes diffuse from damaged cells into an aqueous bathing solution. The resulting increase in electrical conductivity of the bathing solution is an indication of low temperature damage to the cells of the bermudagrass crowns.

The procedure will be carried out at two-week intervals throughout the winter. In addition to any differences in cold tolerance potential (maximum hardness level in mid-winter), differences in rates of acclimation and deacclimation can be detected. The effect of midwinter thaws on cold tolerance level can also be evaluated.

TISSUE CULTURE

Tissue culture research with bermudagrass will continue. Emphasis will be placed on anther culture and the derivation of haploid plants. Plants regenerated through tissue culture,

especially those from another culture, will be characterized for chromosome number. If haploids are found, attempts will be made to double the chromosome number via colchicine.

SELF-INCOMPATIBILITY

Efforts to characterize the self-incompatibility mechanism in bermudagrass will also be continued next spring. Controlled self- and cross-pollination of selected bermudagrass clones will be made to investigate the extent of the self-incompatibility mechanism.

Table 1. Ratings of entries in the national bermudagrass variety test for establishment and turf quality characteristics. Stillwater, OK¹.

Entry #	Strain	Ground Cover				Color ²	Texture ³	Turf Quality ⁴
		6-11-86	6-25-86	7-15-86	8-11-86			
		- - - - - % - - - - -						
1	CT-23	7	32	60	93	6.3	7.0	7.0
2	NM-43	18	37	63	94	5.3	9.0	7.7
3	NM-72	18	33	57	89	5.3	6.7	6.3
4	NM-375	15	27	40	63	7.3	6.0	4.3
5	NM-471	15	37	48	88	7.7	8.0	6.3
6	NM-507	17	37	52	95	8.3	7.7	8.0
7	Vamont	32	62	78	97	4.0	4.7	5.7
8	E-29	20	48	57	96	5.7	6.0	6.3
9	A-29	23	43	68	97	5.3	7.0	7.0
10	RS-1	26	52	82	98	4.7	5.7	6.3
11	MSB-10	13	33	57	89	6.7	7.3	6.7
12	MSB-20	17	32	75	89	6.3	8.3	7.7
13	MSB-30	22	40	52	89	7.7	7.3	6.0
14	A-22	20	45	68	94	5.7	7.3	7.0
15	Texturf-10	22	48	83	97	8.0	6.7	7.7
16	Midiron	10	25	48	87	6.0	6.0	5.3
17	Tufcote	25	45	63	93	6.0	6.3	6.0
18	Tifgreen 328	25	40	65	97	5.3	8.7	7.7
19	Tifway 419	13	18	43	86	7.0	7.0	6.3
20	Tifway 11	16	33	45	96	6.0	6.7	7.0
21	NMS 1	13	38	71	96	6.0	4.3	5.7
22	NMS 2	25	50	72	98	5.3	4.7	6.0
23	NMS 3	18	40	60	87	5.7	6.0	5.7
24	NMS 4	15	38	67	84	6.0	5.7	5.7
25	NMS 14	20	40	57	97	6.3	3.7	5.0
26	Arizona Common	25	57	73	98	4.3	3.7	4.0
27	Guymon	11	28	47	82	6.0	2.0	3.6
28	FB-119	17	40	87	99	5.3	6.0	6.0
29	OK-86-1	32	47	58	88	6.0	3.3	4.7
30	OK-86-2	21	57	70	95	6.7	3.3	5.3
31	OK-86-3	35	50	63	93	6.7	5.3	5.7
32	OK-86-4	35	60	70	89	7.0	4.7	5.7
33	OK-86-5	38	55	75	93	6.7	5.3	5.3
34	OK-86-6	32	40	48	84	8.0	3.0	4.0
35	OK-86-7	32	45	58	87	7.3	3.3	5.0

Table 1. (continued)

Entry #	Strain	Ground Cover				Color ²	Texture ³	Turf Quality ⁴
		6-11-86	6-25-86	7-15-86	8-11-86			
		----- % -----						
36	OK-86-8	38	48	60	83	8.7	4.7	5.0
37	OK-86-9	33	50	58	94	6.3	4.7	5.0
38	OK-86-10	32	40	45	75	6.7	4.0	4.3
39	OK-86-11	38	67	78	95	7.3	3.3	5.3
40	OK-86-12	30	52	72	95	6.3	7.0	7.0
41	Sunturf	22	45	63	94	7.0	8.3	7.7
42	U-3	18	45	60	92	5.7	5.7	6.3
43	Midway	13	32	45	90	5.3	6.3	6.7
46	Hilltop	25	62	87	99	6.0	5.3	6.3

¹Established 21 May 1986 by plugging on 1 ft. centers.

²Rated on a scale of 3-9, where 3 = yellow green and 9 = dark green.

³Rated on a scale of 1-9, with 9 representing finest texture.

⁴Rated on a scale of 3-9, with 9 representing best quality.



Fig. 1. A typical bermudagrass plant population in one of our breeding nurseries.



Fig. 2. Basic fertility of individual bermudagrass plants is determined by ascertaining the percentage of florets that set seed. Here a technician counts seeds with the aid of an electronic counter.



Fig. 3. Plants selected for finer texture and dwarfness produce similar progeny phenotypes as indicated by the smaller plants in this photograph. These dwarfy plants are progeny from selected smaller plants.

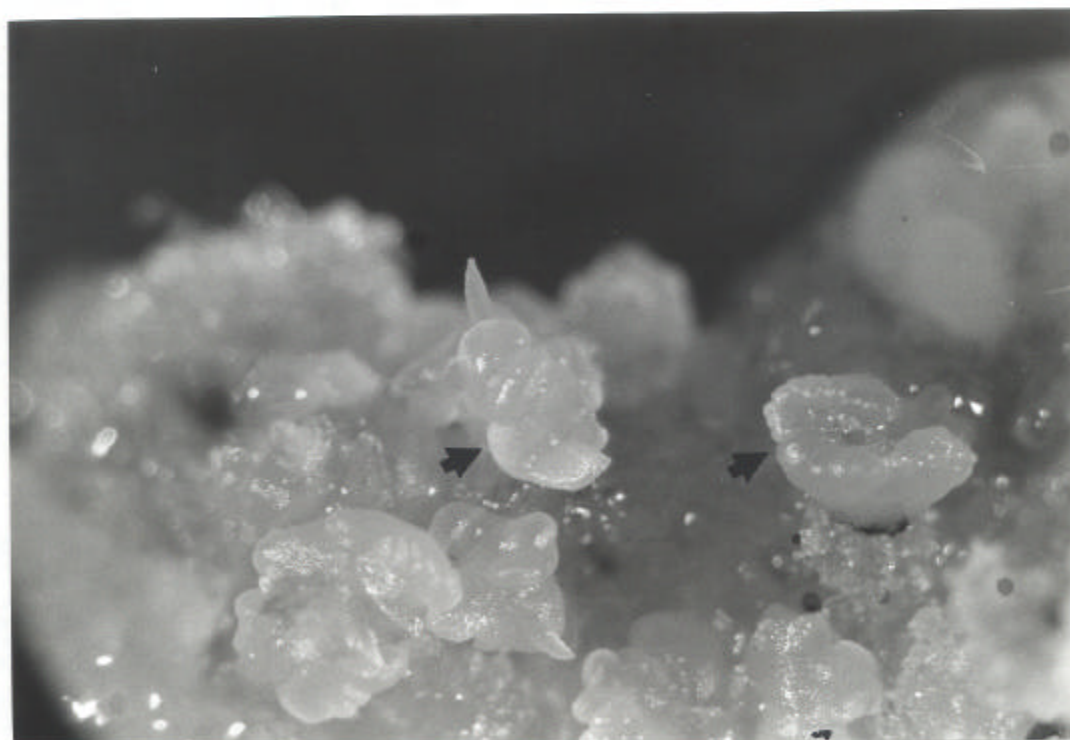


Fig. 4. Callus tissue from a very young bermudagrass inflorescence. Arrows indicate embryoids.