CALLUS INITIATION AND PLANTLET REGENERATION OF APOMICTIC BLUESTEM GRASSES (*BOTHRIOCHLOA* SPP.)

By

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(BOTHRIOCHLOA SPP.)

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PREFACE

The author wishes to express her sincere thanks and appreciation to her major adviser, Dr. Charles M. Taliaferro for his advice, assistance, and patience during the course of this research. Appreciation is also extended to Dr. Becky Johnson for her suggestions, and the use of her lab and equipment throughout the study, and Dr. Earl Mitchell for his time and use of his laboratory facilities.

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CHAPTER I

INTRODUCTION

This thesis is composed of three chapters. Chapter I contains a description of the material utilized in this research. Chapter II consists of general information and a review of literature pertinent to the research; and Chapter III is presented as a manuscript for publication in a professional journal.

Thirty years ago it was estimated that over twelve million acres in the southern Great Plains needed to be taken out of cultivation and seeded to permanent grass cover. It was also suggested that additional millions of acres should be established to perennial grasses for an indefinite period of time in order to adjust crop acreages in line with production needs and to help preserve or improve the soil (9).

Recently, the group of grasses dubbed the "Old World" bluestems have been the subject of extensive agronomic study and have aroused the interest of farmers, ranchers, and soil conservationists (14). These grasses, mainly Asiatic and European in origin, have an environmental habitat ranging

from quite restricted to comparatively cosmopolitan (8). The great variability among the many Old World bluestem strains offers versatility in their use and range of adaptation, which is of particular importance in the southern Plains area, a region of high risk agricultural production due to frequent, often severe droughts, high summer temperature, and periodically harsh winters (16). In their natural habitats and in the southern Great Plains, most of the grasses react ecologically as plants suited to some stage of secondary succession; they do not behave as climax plants but tend rather to increase and thrive under grazing and other disturbances (10). However, in a 36-year species adaptation test in Dallam County, Texas, the bluestem grasses were found to dominate both grazed and ungrazed plots and had actually spread into plots planted to other native or introduced grass species (6).

Old World bluestem grasses complement native rangeland grasses in forage-livestock production systems (17). They may be of greatest value on marginal farmland, interspersed among rangelands, or to overseed brush infested or depleted pastures (17). They are not meant to replace native range but are recommended for use in those areas where farming of annual crops is not only unprofitable, but may lead to excessive wind and water erosion (16).

Old World bluestems are perennial warm-season bunchgrasses with a C-4 photosynthetic pathway (19). These

grasses begin spring growth in late March or April in Oklahoma and are ready for grazing in late May or early June. Stocking rates on a seasonal basis can range from two acres/steer to two steers/acre when irrigation is available; and with the addition of nitrogen fertilizer, beef production in excess of 200 lbs./acre can be attained (15). In the Dallam County, Texas, study, two of the Old World bluestem varieties had the highest forage yields of the established grasses under both grazed and ungrazed conditions (6).

All warm-season grasses, including Old World bluestems, are highest in forage quality (crude protein and digestability) in spring and early summer (May-June) when green leaf material is most available (15). During this time the grasses are in the early boot stage, and the crude protein content of the bluestems may be as high as 12-14% (15). Following seedhead formation, however, the crude protein content will drop, often to levels as low as 5-6% (15).

Use of Old World bluestems has been restricted due to their indeterminate flowering habit and the consequent difficulty of harvesting and processing the chaffy seed, but recent advances in harvesting and cleaning equipment have reduced this problem (2, 3, 4, 20). The attributes of Old World bluestems, including good forage yield potential, acceptable forage quality, the ability to increase in stand density, and superior ability to tolerate various stresses

without loss of stand (18), make them especially advantageous as pasture grasses despite their limitations in seed production.

Botany

Old World bluestems constitute an array of grasses of the Bothriochloininae tribe, forming the Bothriochloa-Dichanthium-Capillipedium complex (12). Developing independently from a common ancestor (13), the three genera are linked together by the compilospecies, Bothriochloa intermedia (12). This particular species acts as a bridge between the morphologically distinct genera, Capillipedium and Dichanthium (5). While most grass species in a genus share some genetic information with each other, intergeneric crossing of the kind encountered in the Bothriochloininae makes taxonomic classification difficult (1).

Apomixis is the common, if not predominant, mode of reproduction, in the Bothriochloas (10). Although there exist some classical types of Old World bluestems, which are sexually reproducing diploids (2n=2x=20), the majority are polyploids ranging from tetraploid (2n=4x=40) to octaploid (2n=8x=80) forms (5). Several of the tetraploids are considered facultative apomicts, reproducing by sexual and asexual means, but higher ploidy levels tend to be obligate apomicts. Most apomictic grasses are pseudogamous, and

pollen is required for seed formation (11). Sexual and asexual embryo sacs are formed in all the Bothriochloas (10). In obligate apomictic plants the asexual sacs are more numerous and/or more precocious than in the facultative apomicts (11), and will crowd out the sexual sac not allowing it to develop (10).

Varietal Information

Four Old World bluestem accessions were used in this research. Two accessions belong to the taxon Bothriochloa ischaemum (L.) Keng and two belong to Bothriochloa intermedia (R. Br.) A. Camus. The following descriptions were taken form Dr. Jack Harlan's article on "Natural Introgession Between Bothriochloa ischaemum and Bothriochloa intermedia in West Pakistan" (7) and describe the two species worked with in this study.

Bothriochloa ischaemum (L.) Keng is a Eurasian grass naturally distributed from the Atlantic coasts of Europe to the Pacific shores of Asia. Plants are erect tufted perennials. The leaves are narrow, mostly basal and sparse. Inflorescences consist of several unbranched false racemes on an axis distinctly shorter than the longer racemes. Most plants of this species are tetraploids (2n=4x=40) and reproduce by obligate apomixis. Typically, the plants are more winter hardy than other Bothriochloa species. Accessions of B. ischaemum var. ischaemum evaluated in this

research were 8793 (PI 301535) from Afghanistan, and 8911c (PI 301573) from Pakistan.

Bothriochloa intermedia (R. Br.) A. Camus is a more tropical species ranging from S. Africa across India, Malaya, Indonesia, and New Guinea to Australia. This species is tremendously variable, rather anomalous and difficult to characterize. Generally the inflorescence consists of an axis as long as or longer than the longest racemes. The lowermost racemes are often branched and leaves are rather broad, abundant, cauline, and aromatic or pungent to the taste. Harlan's collection of B. intermedia (R. Br.) A. Camus consisted mainly of tetraploids (2n=4x=40), but pentaploids (2n=5x=50), hexaploids (2n=6x=60) and octaploids (2n=8x=80) had also been obtained. The species is predominantly apomictic; but some of the tetraploids are facultative, reproducing sexually at a low frequency. Plants are only semihardy to non-hardy. Accessions of B. intermedia var. indica used in this research were 8873b (PI 301539) and 8894d (PI 301551). Both plants originated from Pakistan.

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CHAPTER II

GENERAL INFORMATION AND LITERATURE REVIEW

Tissue culture offers potential for use in plant breeding as a means of enhancing selection efficiency and for rapid clonal propagation of desirable genotypes. Genetic variability has been greatly reduced in many crop species due to widespread cultivation of a limited number of cultivars (18). Smith (38) [as cited in Ahloowalia (2)] stated that the production of new and novel variants through tissue culture via anther culture, protoplast fusion, formation of callus from embryos, endosperms and seedlings, and transformation of cells with exogenous DNA has created new experimental possibilities in plant breeding and genetics. Using tissue culture techniques, therefore, has the potential of (i) augmenting the available gene pool, (ii) quickening clonal propagation of a desirable genotype and (iii) hastening the release of a new variety (5).

Tissue culture is promising as a tool enabling selection at the cellular levels for traits such a tolerance to minerals, heat, herbicides, and winterhardiness. It also has potential for screening of seed samples for fungus contamination (13), and in meristem tip culture to eradicate

viruses from vegetatively propagated species (14).

The Gramineae tribe contains a large number of agriculturally important species, and although the monocots are more recalcitrant, in vitro propagation among the grasses may prove to be particularly useful (18). Polyploids and unique variation in chromosome numbers, contained within several of the grasses may be helpful for initiation of genetic variation in those species (18).

A number of difficult problems persist in tissue culture practices which prevents the realization of the full potential of this important technology. Plant regeneration in vitro is sometimes sporadic and transient with the tissues often losing their morphogenetic competence after a few subcultures and only a few selected genotypes of each species responding optimally (30). However, the initiation and growth of callus and subsequent regeneration of viable plants are prerequisits to genetic improvement of plants via tissue or cell culture (39).

Media

The composition of the medium is very important in successfully culturing plant tissues. Each tissue type requires a different formulation depending on whether the objective is to obtain optimum growth rate of the callus or to induce shoot formation (33). Perhaps the most frequently

used and cited medium for a wide range of plant genera and species, including many agronomic crops, is that published by Murashige and Skoog (MS) (31). Modified versions of this medium have promoted plant regeneration in Italian ryegrass [Lolium multiflorum Lam.] (15), Guinea grass [Panicum maximum Jacq.], proso millet [P. millaceum L.] (5), sorghum [Sorghum bicolor (L.) Moench] (6), and napier grass, [Pennisetum purpureum Schum.] (23). Chu et al. (9) developed the N6 medium for anther culture of rice [Oryza sativa subsp. Keng], and both MS and N6 media were used for somatic embryogenesis and plant regeneration in maize [Zea Mays L.] (30), napier grass (42), and bermudagrass [Cyndon dactylon (L.) Pers.] (3).

A one-half strength MS medium, sometimes referred to as Ahloowalia, has proven useful in the regeneration of wheat [Triticum aestivum L.] (1), and two varieties of red fescue [Festuca rubra L. var commutata Gaud.], and [F. rubra L. var trichophylla Gaud.] (39). After a callus culture has been established, the Ahloowalia medium is used to initiate shoot differentiation. Other modifications of the MS medium, such as Linsmaier and Skoog's (LS) medium (27), have the same inorganic constituents as MS medium but differ in organic nutrients. The LS medium has been used successfully in the regeneration of plantlets in big bluestem [Andropogon gerardii Vitman.] (8), and indiangrass [Sorghastrum nutans (L.) Nash] (7). It has also been used for long duration,

high-frequency plant regeneration studies in wheat, rice, oats [Avena sativa L.], proso millet, and pearl millet [Pennisetum typhoides Burm. Stapf et. Hubb.] (32).

Other types of media have been developed by tissue culturists. Schenk and Hildebrand (36) devised a medium (SH) for both monocots and dicots, and this was used in the regeneration of orchardgrass [Dactylis glomerata L.] (11), establishing callus cultures of tall fescue [Festuca arundinacea Schreb.] (13), and in the culturing of several dicots including soybeans [Glycine max cv. 'Hood'] and cotton [Gossypium hirsutum cv. 'Auburn 56'] (36). Gamborg et al. (B5) medium (20) and Nitsch's medium (34) are two more media that have also been successfully used in tissue culturing.

Explants

In many plant species, particularly those of the Dicotyledonae, all tissues taken at various stages of development have successfully produced somatic embryos in culture (4). This is apparently not true for many monocotyledonous species, especially members of the Gramineae (4). With these species the actively growing regions of the plant have been most responsive to tissue culture techniques (4).

In the grasses, mature and immature embryos are

commonly used as explants to initiate in vitro cultures (10). Callus formation and plant regeneration from immature embryos has been achieved in wheat (1), barley [Hordeum vulgare L.] (22) and pearl millet [Pennisetum americanum (L.) K. Schum] (40, 41). Mature caryopses served as the explants for orchardgrass (11), red fescue (39), tall fescue [Festuca arundinacea Schreb. cv. 'Ky 31'] (29), and Italian ryegrass (12); and virus free plants of Italian ryegrass were obtained from meristem tip cultures (14).

In the Gramineae, young leaves have been induced to form somatic embryos in sorghum (43), Guinea grass (21), Italian ryegrass (12), and sugarcane [Saccharum officinarum L.] (24). Calli were established from internode and peduncle explants of Italian ryegrass x tall fescue F1 hybrids (26). Plants were regenerated from young inflorescence explants of big bluestem (8), indiangrass (7), pearl millet (40), creeping foxtail [Alopecurus arundinaceus Poir.], crested wheatgrass [Agropyron cristatum (L.) Gaertn.], green needlegrass [Stipa viridula Trin.], smooth bromegrass [Bromus inermis Leyss.], and western wheatgrass [Agropyron smithii Rydb.] (28). Use of anther explants for the production of haploids is still relatively new in forage grasses. Protoplast isolation and culture has thus far been limited to a small, though ever increasing, number of plant species (16).

Fungi and bacterial disinfection of plant parts serving

as explants is critical. Fresh plant tissue is usually less contaminated than dormant material, but the latter can withstand more rigorous disinfection treatments without damaging the cellular tissue of the host plant (10). In general the younger the tissue the more likely it will be to produce embryogenic callus (4), and the larger the original explant the slower the proliferated growth.

Growth Regulators

According to Skoog and Miller (37), [as cited in Flick et al. (18)] growth regulator concentrations in the culture medium are critical to the control of growth and morphogenesis of the tissue. Fujimura and Komaine (19) [as cited in Ammirato (4)] found that in cultures derived from differentiated tissues, growth regulators in the medium, especially auxins or auxin in combination with cytokinin, appeared to be essential for the onset of growth and induction of embryogenesis.

In comparing the effects of various auxins on somatic embryogenesis and root formation in carrot tissue cultures, Kamada and Harada (25) stated that phenoxyacetic acid derivatives such as 2,4-D (2,4-dichlorophenoxyacetic acid) were more effective in inducing embryo formation than the naturally occurring IAA (indole-3-acetic acid), or the weaker synthetic auxin, NAA (napthalene acetic acid). After

callus formation, however, it was necessary to transfer the explants to auxin free medium for rapid development of the somatic embryos. Studies suggest that 2,4-D may be necessary for the promotion or development of single cells to cell clusters, which once transferred to an auxin-free medium, are capable of embryo formation (19). According to Reinert (35), [as cited in Kamada et al. (25)] treatments with 2,4-D seem to favor formation of so-called embryonal calli; but the regulator's prolonged presence in the culture medium inhibits actual development of embryos. It seems the appropriate sequential changes in the level of endogenous hormones, especially auxins, at least during crucial periods, are essential factors for the induction, initiation, and development of somatic embryos (25).

Two media are usually required for regeneration of forage grasses. The first is a primary medium with a high auxin concentration for initiating and maintaining callus (4). The second medium has a lower concentration of auxin, or none at all, for inducing somatic embryo maturation and the consequential formation of plants (4). The presence of a cytokinin in the secondary medium may promote formation of embryos by enhancing cell division. A typical example is the role of 2,4-D, IAA, and kinetin in the proliferation, maintenance, and differentiation of ryegrass cells in cultures. In these cultures relatively high auxin concentrations and very low kinetin produced callus, while

low auxin and high kinetin concentrations produced shoots (2).

The auxin for the primary and secondary media may be the same or different; but of all the auxin or auxin-like plant growth regulators, 2,4-D has proven to be the most useful, being used in 57.1% of successful embryogenic cultures according to Evans et al. (17) [as cited in Ammirato (4)].

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CHAPTER III

CALLUS INITIATION AND PLANTLET REGENERATION OF APOMICTIC BLUESTEM GRASSES (*BOTHRIOCHLOA* SPP.)

Abstract. Explants from immature inflorescences of Old World bluestem grasses, *Bothriochloa* spp., produced callus tissue on modified MS media with a high level of growth regulators. The compact "embryogenic" callus underwent further organization, and when transferred to medium with a reduced level of 2,4-D and/or supplemented with zeatin formed shoots. The shoots were placed on medium devoid of 2,4-D and supplemented with 1 mg/l NAA in order to enhance root development. Plantlets obtained were placed in soil, and after a brief adjustment period, transported to a greenhouse. In May 85, plants were transferred to a field nursery at the Agronomy Research Station, Stillwater, Oklahoma, and phenotypic variants were noted.

Introduction

Introduced bluestem grasses *Bothriochloa* spp. are widely

used in the southern Great Plains of the USA for pasture and soil stabilization. Desirable attributes of these perennial, warm-season bunchgrasses include good forage yield potential, acceptable forage quality, ability to increase in stand density and tolerance to stresses such as heat, drought and close grazing [12]. The principal taxa currently being used commercially are B. caucasica (Trin.) C. E. Hubbard and B. ischaemum (L.) Keng, both of which reproduce by obligate apomixis [4, 5]. However, germplasm within B. intermedia (R. Br.) A. Camus also has potential for use in the southern Great Plains. Mode of reproduction in this taxon is typically facultative or obligate apomixis [4].

Improvement of plants through conventional means depends on availability of natural genetic variation and its manipulation through breeding and selection. Although there is much genetic variation in Bothriochloa the apomictic mode of reproduction in the genus precludes, or at least makes unfeasible, cultivar improvement via conventional breeding. Application of tissue culture techniques to the genetic improvement of these grasses, thus seems to be especially appropriate. Such techniques could potentially be used to induce variation into apomictic biotypes and to provide the basis for developing screening procedures at the cellular level. This paper reports the results of studies designed

to identify an in vitro culture system for callus induction and plant regeneration in two species of Bothriochloa.

Materials and Methods

Young unemerged inflorescences collected from single plants (genotypes) of two accessions of Bothriochloa ischaemum var ischaemum (L.) Keng [8793 (PI 301535) and 8911c (PI 301573)] and two accessions of Bothriochloa intermedia var indica (R. Br.) A. Camus [8873b (PI 301539) and 8894d (PI 301551)] were used in these studies. Accession 8793 came from Afghanistan. Accessions 8911c, 8873b and 8894d originated in Pakistan.

Shoots containing immature inflorescences were gathered between 7:00-8:00 a.m. from each of the four plants growing in pots in a greenhouse. After trimming away excess leaves, surface sterilization of shoots and enclosed young inflorescences was achieved by placing the material in a sterile jar containing a surfactant mixed with distilled water. The jar was agitated five minutes. The surfactant-distilled water solution was replaced with a 10% Clorox, plus surfactant solution and stirred for 2 minutes. After decanting off this mixture, 70% ethanol was added, and the jar was shaken 1.5 minutes. The material was then rinsed 5x with sterile 3x distilled water.

Inflorescences were excised aseptically from the leaf

sheaths and cultured on a solid nutrient medium. Inflorescences longer that 5 mm were placed in autoclaved petri dishes and sectioned into 2-5 mm segments with a scalpel. The initial induction medium consisted of Linsmaier and Skoog (LS) medium [7] supplemented with 5 mg/l 2,4-D (2,4-dichlorophenoxyacetic acid) and containing 30 g/l sucrose with 7 g/l T.C. agar, or one-half strength Murashige and Skoog (1/2 MS) medium [10] supplemented with .5 mg/l 2,4-D, 3.2 mg/l IAA (indole-3-acetic acid) and 1 mg/l kinetin, and containing 15 g/l sucrose with 5 g/l T.C. agar. Both media were adjusted to pH 5.8 before autoclaving 15 minutes at 121 degrees C.

Following a 4-week dark incubation period, test tubes containing newly formed callus were placed in a growth chamber at 25 degrees C with a 16h photoperiod. Callus was transferred onto LS or 1/2 MS with levels of growth regulators the same as or reduced to 1 mg/l or .5 mg/l 2,4-D, respectively. The 1/2 MS also contained 1 mg/l zeatin [6-(4-hydroxy-3-methylbut-2-enylamino)amino purine] filtered into the autoclaved medium. Various changes in the supplements to the basal medium were tested in order to achieve shoot differentiation and plant regeneration. Calli were maintained by subculturing every 20-35 days onto fresh media.

When shoots were detectable, they and the subtending calli masses were transferred to individual test tubes

containing 1/2 MS, supplemented with 1 mg/l NAA (napthalene acetic acid) to promote rooting. After formation of an extensive root system, plantlets were removed from the test tubes. Roots were gently washed to remove all traces of medium. Then plants were placed in a complete soil mixture.

Regenerants were individually labeled to identify from which test tube and parent plant they had originated, and were acclimatized in a growth chamber before being moved to the greenhouse.

On 24 May 85, 522 regenerated plants were transplanted to a Kirkland silt loam soil (Abruptic Paleustoll) field nursery (1 m spacing) at the Agronomy Research Station, Stillwater, Oklahoma. Randomly distributed among the respective regenerated plant populations were 4 ramets of each parental plant. Morphological comparisons were made between parental plants and regenerants to select potential variants.

A second experiment was conducted in order to test various 1/2 strength ammonium:nitrate levels in the medium on callus initiation and growth. Ten x ten grids were prepared using ten ratios of ammonium:nitrate (1:6-1:5). Each ratio was replicated 45 times. The two media from the previous experiment, LS and 1/2 MS, were also evaluated making a total of 900 vials (8 ml medium/vial) in use. Statistically arranged in a randomized block design, the media were randomized in rows; while the ratios were

randomized within the rows. Explants were obtained from accession 8911c or accession 8793; and the vials were individually labeled with the ratio, inflorescence number and its maturity level. Inflorescences were scored visually from least to most mature on a scale of (0-3).

Following initiation of explants to the media, vials were covered with brown paper to remove the light source and placed in a walk-in growth chamber, 30 degrees C. The callus tissue was transferred to fresh media with the nitrogen ratio 1:1, five weeks later, and placed in a growth chamber, 25 degrees C, 16-hr photoperiod. Approximately ten weeks from initiation of the inflorescences fresh weights of the tissue were recorded.

Results and Discussion

Calli developed from the floral primordia and directly from the rachilla or rachis on LS and 1/2 MS media. Large areas of green were visible in the callus cultures after subculturing twice on the 1/2 MS media, although an additional 30 days were required for the greening of calli on LS media. The first shoot was obtained on 1/2 MS 94 days following calli greening, while on the LS medium the first shoot was removed in only 61 days. Zeatin or reduced levels of 2,4-D seem to promote the early occurrence of green spots in calli on 1/2 MS medium, but both media took approximately

150 days before producing the first shoot indicating a certain period of callus "aging" had taken place.

Callus cultures were heterogenous consisting of two distinct proliferation types. Using designations identified by Nabors et al. [11], one type was an "embryogenic" (E) white, compact knobby portion, and the other was a soft translucent gelatinous non-embryogenic (NE) type. NE calli occasionally gave rise to localized areas of E calli, and some of the E calli appeared to turn NE. Aerial roots were initiated in many of the callus cultures, but no shoots or plants differentiated from the areas of extensive root growth.

Date of collecting and inoculating explants proved critical. Explants initiated 8 Aug 84 suffered a high level of contamination. In accession 8793 over 1/2 the material was lost. Comparing culturing dates on rate of contamination in accession 8873b, on 8 Aug 84, 46% of the cultures survived till the first transfer, while in those explants initiated 29-30 Aug 84, 80% of the cultures were transferred.

Exposure of the tissue to light resulted in anthocyanin production. The pigmentation was immediately noticable, and all four accessions exhibited areas of reddish color in their calli and in small projections extending from the cultures. Surfaces later turned dark and no shoots or plants developed from those areas. The four genotypes were

distinguishable during this phase, and showed various degrees of pigmentation at different times. Accession 8894d was noted for producing the least amount. This anthocyanin production seemed to be deleterious to calli growth, but pigmentation is a common feature in cereal and grass cultures [6, 13].

Over 50% of the calli transferred after initiation produced plants in three genotypes (Table I). Accession 8793 had a low 26.7% success rate but suffered the most contamination. Accession 8873b, also suffered contamination from culturing on the first date; but the data were averaged with those on the second dates and a higher 53.6% success rate resulted. Plants 8894d and 8911c had rates of 54.5% and 61.1%, respectively, indicating that plant regeneration should occur in at least 50% of the calli in this particular grass.

Results of the follow-up experiment showed a very obvious genotypic effect (Table II). Accession 8793 responded significantly better in culture than 8911c. The LS medium was approximately twice as effective in initiating callus than 1/2 MS in accession 8793. Maturity of the explants also significantly affected callus production. Less mature inflorescences of both accessions responded best on LS medium. On 1/2 MS medium the second maturity level (1) proved optimal in accession 8793. In both media, the oldest inflorescences were least desirable as an explant source.

Results of the ammonium:nitrate ratio study were inconclusive. The explant source seemed to determine whether or not callus was initiated, not the amount of nitrogen.

Although not histologically demonstrated, the origin of calli in the plant materials studied is most likely embryogenic. Embryogenic calli from inflorescence tissue have been recorded for Sorghum bicolor (L.) Moench [2], Pennisetum americanum (L.) K. Schum [13], Panicum maximum Jacq. [8], Triticum aestivum L. [9], and Oryza sativa L. [3]. Vasil and Vasil [14] suggest that somatic embryogenesis takes place in most cereal and grass cultures, but the degree of embryo organization may vary considerably. According to Heyser [6], cell lines which give rise to complete plantlets do so by embryogenesis as opposed to monopolar shoot or root primordia (organogenesis). Plants regenerated from a single Lolium multiflorum Lam. embryo originated both via differentiation of shoot-primordia and somatic embryos [1]. Research is currently underway to determine the precise origin of the embryogenic tissues from the inflorescences.

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Table I. Information and results from culture of young inflorescences of four Bothriochloa accessions.

	Accession			
	8793	8873b[a]	8894d	8911c
Date initiated (1984)	8-8	8-8,29,30	8-29	8-30
No. inoculations	50	46	11	19
Callus production (no. tubes)	30	28	11	18
Tubes producing plants	8	15	6	11
Plants produced #	155	229	99	39
Plants/tube avg.	19.4	15.3	16.5	3.5
Success rate[b] (%)	26.7	53.6	54.5	61.1

[a] data from the three dates of initiation were combined

[b] number of tubes after first transfer producing plants

Table II. Effect of media, accession and inflorescence maturity on callus initiation in Old World bluestem grasses (Bothriochloa spp.).

		inflorescence maturity [a]				
Medium	Accession	0	1	2	3	mean
1/2 MS	8793	.47±.09	.59±.09	.42±.10	.02±.08	.38
	8911c	.26±.12	.06±.12	.00±.09	.02±.07	.09
	mean	.37	.33	.21	.02	
LS	8793	.98±.08	.90±.09	.83±.10	.33±.08	.76
	8911c	.33±.12	.27±.13	.07±.09	.03±.07	.18
	mean	.66	.59	.45	.18	

[a] 0 = least and 3 = most mature

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