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Plant Regeneration from Seedling Meristems in Selected *Cenchrus* and *Pennisetum* Species

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Summary

Methods for producing embryos from nonreproductive plant tissues (somatic tissue) for plant regeneration are highly variable, even between closely related species. Somatic embryo generation media for three *Cenchrus* and *Pennisetum* species were studied in our lab. *Cenchrus ciliaris* L. Link. var. 'Common' and PI 409704 were highly embryogenic, whereas *Pennisetum orientale* var. 'Cowboy' and *Pennisetum flaccidum* var. 'Carostan' were not embryogenic within the 4-week study period. Overall, 2,4-dichlorophenoxyacetic acid (2,4-D) and α-naphthaleneacetic acid (NAA) allowed similar production of embryogenic tissue. For PI 409704, 0.5-μM 2,4-D provided the best response from apical meristem explants, while 30-μM NAA was the best for Common. Shoot and root formation, individually and in combination, on NAA was better than on 2,4-D. Plants were regenerated from white somatic embryos, which developed on a wide range of media.

Introduction

Buffelgrass is an apomictic, warm-season, perennial grass, having good forage characteristics and good drought tolerance (Bogdan 1977). Buffelgrass acreage in semiarid regions is limited by a lack of winterhardiness and salt tolerance. Several closely related species are known to be more winterhardy and to exhibit greater salt tolerance. Tissue culture systems can be used to select for desirable mutants (Negrutiu et al. 1984, Chaleff 1986) and, in transformation systems, to introduce desired genes (Hodges and Lorz 1987). Reliable in vitro systems must be developed before genotypic variations and transformed material can be accessed in this agamic complex.

Sankhla and Sankhla (1989) demonstrated the use of 2,4-D to initiate both friable and embryonic cell cultures from immature inflorescences of an apomictic tetraploid, *Cenchrus ciliaris* (Anjan Dhaman) cv. 75. No other publication exists describing the potential for regeneration in buffelgrass. We report here the development of embryogenic callus and plant regeneration from seedling apices of both tetraploid and pentaploid buffelgrass genotypes and two related forage grasses, *Pennisetum orientale* and *Pennisetum flaccidum* (Table 1).

Materials and Methods

Naked seeds, which were obtained by hand-rubbing inflorescences of each genotype, were sterilized in chlorine gas for the times indicated in Table 2. After dry sterilization, the seeds were germinated aseptically at 86 °F in the dark. Apical meristems from etiolated 5-day-old seedlings were microsurgically removed and transferred to 3.94- by 0.79-in. disposable petri dishes containing approximately 2 cu in. of callusing medium at a density of 10 apices per plate. The medium was solidified in agar (0.75% w/v agar) with MS basal salts (Murashige and Skoog 1965), B5 vitamins (Gamborg et al. 1968), sucrose (3% w/v), and either 2,4-D or NAA at concentrations of 0.5, 15, 30, or 45 μM. All treatments were replicated five times except on Cowboy, which was replicated only twice because of lack of seed. Plates were wrapped in plastic and maintained at 77 °F in the dark or under continuous light (14.86 microeinsteins/sq ft/s). Callus formation was typically evident within 1 week and was evaluated at 28 days for formation of organogenic and embryogenic callus.

Keywords: organogenesis / embryogenesis / tissue culture / buffelgrass / Laurisagrass / flaccidgrass.

Table 1. Plant material specifications for apical explant sources.

Genus species	Origin	Chromosomes (X = 9)
Cenchrus ciliaris 'Common'	Kenya	2N = 4X = 36
Cenchrus ciliaris PI409704	South Africa	2N = 5X = 45
Pennisetum orientale 'Cowboy'	Pakistan	2N = 4X = 36
Pennisetum flaccidum 'Carostan'	Czechoslovakia†	2N = 4X = 36

†Natural range throughout southern Asia – species center of diversity reported to be in Afghanistan.

Table 2. Chlorine gas sterilization exposure time by genotype.

Cultivar	Optimum sterilizing time	Seed germination	Plates contaminated
	hr	%	%
Common	1.5	18	13
PI 409704	3	54	5
Cowboy	5	32	14
Carostan	6	25	11

Embryogenic callus was transferred to hormone-free media and placed in a low-light location. Subsequently, germinating somatic embryos were transferred to hormone-free, half-strength MS media in magenta boxes and placed under photosynthetic photon flux density lights for a 16-hr photoperiod at 19.23 microeinsteins/sq ft/s.

When plants had outgrown the boxes, they were transferred to potting soil and grown in a growth chamber for 2 weeks. Regenerated plants were then moved to the greenhouse or field for evaluation.

Results and Discussion

Overall, 46.5% of the explants produced callus in the dark vs. 26.7% in the continuous-light treatment. Continuous light also detrimentally affected survival; 61.1% of the responding explants in continuous light became brown and died, while only 0.2% of responding dark-grown explants died. No embryogenic callus was produced in the continuous-light treatment (data not shown). Because continuous light inhibited the callusing response and the embryogenic callus production, only dark-grown explants will be discussed further.

Loose, friable callus was the predominate callus type formed in most hormone × genotype treatments. Embryogenic, or Type 1, callus was not produced in the *Pennisetum* species. In *Cenchrus*, Type 1 callus was produced at all hormone levels primarily because of the response of Common buffelgrass. The 0.5-µM 2,4-D concentration produced a high frequency of Type I callus; however, other levels produced very little callus in 409704, while Common buffelgrass had high frequencies at all levels. Five plants of Common buffelgrass and two plants of 409704 were regenerated from Type 1 callus. None of these plants showed obvious morphological variation. Subsequently, nine other plants were regenerated, and one displayed a dramatic reduction of growth habit. Many somatic embryos on Common buffelgrass germinated precociously and did not survive. Common buffelgrass was observed to have a high frequency of Type 1 callus production and should provide good material for establishing consistent regeneration protocols.

Shoot formation was similar on 2,4-D and NAA. On 2,4-D, all shoot formation was confined to the 0.5-µM rate, while shoots were formed at various NAA rates across species. Overall, root formation was dramatically better than shoot formation on NAA and occurred at most hormone concentrations, except for Carostan. Root formation on 2,4-D was not observed in the *Pennisetum* species and was limited to the two lowest concentrations in *Cenchrus*. Similarly, callus developing in both roots and shoots were more frequent on NAA, occurring at various concentrations (Table 3). Callus developing in both roots and shoots on 2,4-D was limited to PI 409704 at 0.5 µM.

From these four genotypes, we have regenerated 14 plants from somatic embryos. Plants have also been obtained in Common buffelgrass from calli where both root and shoot formation had occurred. While the conversion frequency of callus to somatic embryos was lower than that reported by Sankhla and Sankhla (1989), callus proliferation was faster (personal communication [Sankhla]). This suggests that seedling apices may be an alternative source of competent plant material for manipulations through tissue culture.

Table 3. Frequencies of organogenic and embryogenic responses to culture by *Cenchrus* and *Pennisetum* species.

	Experimental				*C. ciliaris*								*P. orientale*				*P. flaccidum*			
					Common				409704				Cowboy				Carostan			
Hormone	R†	S	B	E	R	S	B	E	R	S	B	E	R	S	B	E	R	S	B	E
NAA	19.4	5.5	5.5	17.5	16.5	4.7	5.9	32.9	48.2	7.4	11.1	3.7	10.3	5.1	2.6	0.0	7.1	7.1	0.0	0.0
0.5 µM	9.3	14.0	11.6	6.9	8.3	12.5	12.5	12.5	33.3	33.3	33.3	0.0	10.0	20.0	10.0	0.0	0.0	0.0	0.0	0.0
15 µM	26.2	4.8	4.8	11.9	23.5	0.0	11.8	23.5	27.3	9.1	0.0	9.1	37.5	0.0	0.0	0.0	16.7	16.7	0.0	0.0
30 µM	27.1	0.0	4.2	22.9	19.1	0.0	0.0	52.4	69.2	0.0	15.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
45 µM	12.5	3.1	0.0	31.3	17.4	4.4	0.0	43.5	NA	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2,4-D	2.1	3.4	1.0	16.1	0.7	2.1	0.0	25.5	5.7	6.8	3.4	11.4	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0
0.5 µM	5.7	11.4	3.4	28.4	2.3	7.0	0.0	39.5	12.9	19.5	39.7	25.8	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0
15 µM	1.4	0.0	0.0	12.7	0.0	0.0	0.0	28.6	5.3	0.0	0.0	5.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30 µM	0.0	0.0	0.0	7.5	0.0	0.0	0.0	11.1	0.0	0.0	0.0	5.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
45 µM	0.0	0.0	0.0	13.2	0.0	0.0	0.0	24.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	8.3	4.2	2.6	16.6	6.5	3.0	2.2	28.3	15.7	7.0	5.2	9.6	7.5	3.8	1.9	0.0	1.7	3.4	0.0	0.0

†Frequency of organogenic type; R - roots, S - shoots, B - both roots and shoots, E - Type 1 calli.

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