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Uniformity of plants regenerated from somatic embryos of *Panicum maximum* Jacq. (Guinea grass)

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Summary. Plants were regenerated by somatic embryogenesis from cultured leaf segments of *Panicum maximum* Jacq. (Guinea grass). All plants were phenotypically similar to the donor plant from which explants were obtained for culture. Examination of the cytological and morphological characteristics of the regenerated plants did not show any changes in mitotic (root tip) chromosome number, structural rearrangements of chromosomes, pollen stainability and morphological characteristics.

Key words: Cereals/grasses – Genetic variability – Guinea grass – *Panicum maximum* – Plant tissue culture – Somatic embryogenesis

Introduction

Clonal propagation of plants implies and requires absolute fidelity of the genotype during the period of in vitro growth and regeneration. Generation of variability during culture, therefore, can be a serious impediment to clonal propagation, but has been generally ignored in the enthusiasm for its professed uses in broadening of the genetic base for plant improvement.

Plant cells grown in culture exhibit evidence of karyotypic instability and variability as well as uniformity. A substantial amount of the variability is likely introduced during cell proliferation in vitro (Bayliss 1980), although some of it may also represent propagation of karyotypically diverse cells already present in the original explant (Matthews and Vasil 1975). Thus, plant tissue cultures have been described as rich sources of genetic variability (Larkin and Scowcroft 1981), but plants derived from the cultures often show less variability than the cultures themselves (Nishi et al. 1968; Shimada et al. 1969; Sacristan and Melchers 1969) because the euploid cells appear to have a selective advantage in morphogenetic competence.

In general, plants regenerated from pre-existing or axillary meristems tend to be similar to the parent plant. This fact is exploited widely in the in vitro propagation of many horticultural plants. On the other hand, de novo organization of shoot meristems in callus cultures often leads to regeneration of genetically diverse plants.

In the Gramineae, both relative uniformity (Cheng and Smith 1975; Gamborg et al. 1977; Gosch-Wackerle et al. 1979; Shimada and Yamada 1979; Nakamura and Keller 1982; McCoy and Phillips 1982) and extensive variability (Nishi and Mitsuoka 1969; Ahloowalia 1976; Heinz et al. 1977; Oono 1978; Kasperbauer et al. 1979; Orton 1980; McCoy et al. 1982) have been reported in plants regenerated from tissue cultures.

The appearance and extent of variability may depend on the explants used, the nature of tissue cultures, and the method of regeneration. For example, preliminary results have shown that plants obtained by somatic embryogenesis from tissue cultures of several species of the Gramineae are karyotypically and/or phenotypically similar to the donor plants (Haydu and Vasil 1981; Lu and Vasil 1981, 1982; Vasil and Vasil 1981a, b; Lu et al. 1982; Ozias-Akins and Vasil 1982; Wang and Vasil 1982; Vasil et al. 1982).

We now present further evidence showing the absence of meiotic chromosome abnormalities and phenotypic variability in a population of *Panicum maximum* Jacq. (Guinea grass) plants regenerated from somatic embryos obtained from cultured leaf segments.

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Materials and methods

A single plant of *Panicum maximum* Tift PM44 (an apomictic selection from PI 277946) growing in the field at Gainesville, Florida was used as the source of leaf explants. Leaves were obtained from six different tillers on the plant and cultured according to methods described earlier (Lu and Vasil 1981). Twenty randomly selected 30–45 cm high plants, PM44-1 to PM44-20, regenerated by somatic embryogenesis, were moved to Tifton, Georgia, for cytological and morphological analyses. Single plants of each of the 20 clones were transplanted to the field in April 1982, spaced 1 m apart, and fertilized with ammonium nitrate at a rate of 112 kg/ha N.

Plants were cut to a height of 15 cm after two months of growth to allow for a more uniform comparison of morphological characteristics in the regrowth. Twenty random post-anthesis inflorescences were collected from each clone. Inflorescence length, or the distance from the attachment of the lowest branch of the inflorescence to the tip of the inflorescence, and the length of the lowest inflorescence branch were measured to detect morphological variation.

Pollen was collected at 8 a.m. and placed in iodine-potassium iodide solution to determine pollen stainability. At least 500 pollen grains were counted in each clone.

Meiosis was studied in 30 pollen mother cells from seven of the clones in addition to the control. Inflorescences were fixed in Carnoy's solution. Pollen mother cells were squashed in 1% acetocarmine. Root tips from all twenty clones were pretreated for 2 h in a saturated solution of mono-bromonaphthalene, hydrolyzed in 5N HCl at room temperature for 8 min, rinsed in tap water and stained with Feulgen reagent for somatic chromosome counts.

Panicles were sealed in glassine bags to produce selfed seed. Embryo sacs were studied with a phase-contrast micro-

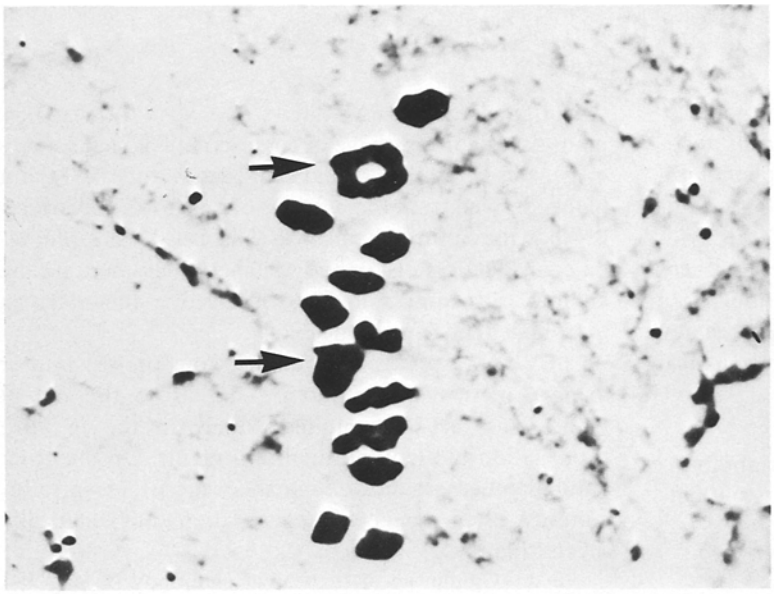

Fig. 1. Metaphase I chromosomes of PM44 showing 12 II and 2 IV (*arrows*). (×1,875)

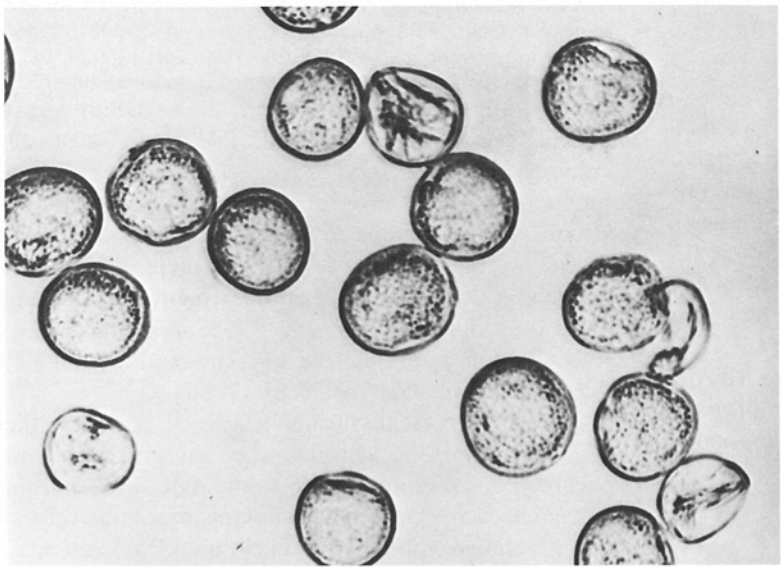

Fig. 2. Pollen of PM44 showing both stainable (iodine potassium iodide) and aborted grains. (×750)

Table 1. Metaphase I chromosome configuration, pollen stainability, and inflorescence and spikelet length characteristics of *Panicum maximum* plants derived by somatic embryogenesis

Plant	Chromosome configurations				Pollen stainability (%)	Length in cm	
	I	II	III	IV		Inflorescence	Lowest branch
PM44 (Control)	0.26	9.94	0.09	2.76	78	19.8±2.4	12.8±2.1
PM44 – 1	0.43	9.90	0.30	3.00	80	19.0±2.7	12.5±1.8
PM44 – 2					83	17.8±2.2	11.9±1.6
PM44 – 3	0.69	10.15	0.38	2.46	84	17.1±2.7	10.6±1.9
PM44 – 4	0.77	10.13	0.50	2.36	81	17.5±2.4	11.2±2.3
PM44 – 5	0.53	9.34	0.22	2.96	84	19.1±2.4	12.7±2.5
PM44 – 6	1.50	11.03	0.37	1.70	64	18.9±2.4	12.1±1.6
PM44 – 7					82	17.8±1.4	11.3±1.1
PM44 – 8					77	17.9±2.7	11.9±1.9
PM44 – 9					81	17.2±2.7	11.4±2.3
PM44 – 10	1.10	10.53	0.57	2.06	81	16.9±2.9	11.1±2.1
PM44 – 11					82	20.3±2.1	12.8±2.2
PM44 – 12					79	19.2±2.6	12.2±1.3
PM44 – 13	1.03	9.93	0.43	2.33	81	17.4±2.8	10.9±2.2
PM44 – 14					82	18.8±3.0	12.1±2.9
PM44 – 15					82	19.1±2.5	12.7±2.0
PM44 – 16					79	17.5±3.0	10.9±2.4
PM44 – 17					82	18.3±2.0	11.5±2.1
PM44 – 18					81	18.2±3.2	12.0±2.9
PM44 – 19					79	18.0±1.4	12.0±1.5
PM44 – 20					80	18.7±2.7	11.3±2.1
SE					1	0.2	0.2
CV (%)					5	5	6

scope in ovules cleared with methyl salicylate (Crane 1978). Reproduction in PM44 (Control) is by apomixis.

Results

Morphology of regenerated plants

Measurements of inflorescence length and the length of the lowest branch were similar in all clones to the control. Morphologically, all the twenty tissue culture-derived clones appeared to be similar to the control (Fig. 3).

All the plants flowered at the same time as the control, and reproduction was by apomixis as in the Tift PM44 control.

Cytology of regenerated plants

Somatic chromosome counts made on the control plant (donor plant from which leaf segments for culture were obtained) and all twenty tissue culture derived clones, PM44-1 to PM44-20, showed 2n = 32 chromosomes. No fragments, bridges or other abnormalities were observed in the plants regenerated in vitro or in the control.

Meiosis was observed in 7 of the 20 tissue culture-derived clones and the control. Frequencies of univalents, bivalents, trivalents and quadrivalents (Fig. 1) in each of the clones were very similar to the frequencies observed in the control (Table 1).

Pollen stainability (Table 1, Fig. 2) in all the clones, except PM 44-6, was similar to the control. PM 44-6 consistently showed about 15% less stainable pollen than the other clones. All clones, and the control, showed some pollen abortion resulting from the unequal distribution of chromosomes caused by the formation of univalents and multivalents at metaphase I.

Progeny of tissue culture regenerates

No abnormal, variable or chlorophyll deficient seedlings were observed among the 500 or more selfed but apomictic progenies obtained from each clone (owing to the apomictic development of Tift PM44 and its progeny, mainly dominant mutations would be expressed in the progeny of these plants. However, since obligate apomicts are highly heterozygous, a recessive mutation at a heterozygous locus could also be expressed).

Fig. 3. Morphological uniformity of plants regenerated from somatic embryos

Discussion

Within the limits of this study, which is based on the examination of a relatively small population, the regenerated plants all seem to be identical to the plant from which the original culture explants were obtained. The absence of any demonstrable chromosome abnormalities and phenotypic variants provides further support for the view that plants derived through somatic embryogenesis in the Gramineae are essentially free of genotypic variability (Vasil 1982 a, b, 1983). However, as pointed out in Introduction, tissue culture derived plants of the Gramineae have been reported to exhibit uniformity as well as variability. It would be very useful, therefore, to establish if there is any relationship between the mode of regeneration – organogenesis v/s somatic embryogenesis – and genetic uniformity/variability.

One clone, PM 44-6, consistently showed less stainable pollen grains than the other tissue culture-derived clones as well as the control. This reduced stainability can not be attributed to abnormal metaphase I chromosome behaviour since this clone had a slightly higher frequency of bivalents and a lower frequency of quadrivalents than the control and other clones, a fact that would actually favor improved pollen stainability. It is possible that the lower pollen stainability of PM44-6 could be due to some micro-environmental effects which were not eliminated since the clones were not replicated.

Conventional methods of detecting the appearance of genetic variability by meiotic segregation were not available in this study owing to the apomictic nature of the plants. However, in spite of the fact that no variability was revealed in the test population with the techniques used, the possibility remains that minor genetic changes or mutations did in fact take place. Only genetic analysis of selfed sexually produced progeny or the use of highly sensitive and discriminating techniques such as chromosome banding, restriction analysis of DNA, etc. may be able to detect such alterations. This is indeed indicated by the identification of a variety of mutations in tissue culture derived plants of *Zea mays* (Edallo et al. 1981), the isolation of mutant cell lines and plants of *Z. mays* resistant to *Helminthosporium maydis* (Gengenbach et al. 1977), and the appearance of spontaneous salt tolerant (Rangan and Vasil 1983) and S-2-aminoethylcysteine resistant cell lines of *Pennisetum americanum* (Boyes and Vasil, unpublished observations). The possibility that mutant cells that gave rise to the above mutant cell lines and plants were already present in the initial explants and proliferated preferentially in a culture environment should also be considered.

It is not known whether the genetic uniformity of plants regenerated from the embryogenic cultures of the Gramineae is because the cultures themselves are cytologically stable and homogeneous, or because of preferential selection and regeneration only from normal euploid cells. Only further comparative information on the cytology of the embryogenic callus cultures and the plants regenerated from them will provide answers to this question.

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