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REGENERATION OF GENETICALLY DIVERSE PLANTS FROM TISSUE CULTURES OF FORAGE GRASS – *PANICUM* SPS

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INDEX WORDS

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SUMMARY

Callus tissue cultures of 3 species of forage-grass (*Panicum*) were established from the excised embryos, shoot tips, and segments of young inflorescences, and induced to regenerate plants showing genetic diversity. These plants were transferred to the soil, and reared to maturity. The importance of in vitro methods for increasing the reservoirs of germplasm in forage-improvement programs is emphasized.

INTRODUCTION

The quality of animal stocks and their produce is dependent on the abundant availability, and nature of forages. However, there is a continuous depletion of the naturally available germplasm reservoirs of forages, and this has caused great concern. The routine methods of plant breeding for the induction of genetic variation are insufficient, and need to be supplemented with unconventional means. A multidisciplinary research approach has, therefore, been initiated with a view to assessing the feasibility of extending the technology of tissue culture (REINERT & BAJAJ, 1977) to induce genetic diversity in forage-improvement programs. This approach would enable, (i) to augment the available gene pool, (ii) quicken clonal propagation of a desirable germplam, and (iii) hasten the release of a variety. The present investigation is a general survey to study the regenerating potentials of various species of *Panicum*, a common forage grass, and deals with the production of genetically diverse plants from callus tissue cultures. The plants thus produced will be selected for desirable traits.

MATERIALS AND METHODS

The seeds of various forages i.e. *Panicum maximum* JACQ. (Guinea grass, cv. 59985), *P. miliaceum* L. (common millet, cvs. UP and Australian) and *P. antidotle*, were surface sterilized with chlorine water, the embryos were dissected under an AO stereobinocular and cultured on various media under aseptic conditions in a Laminar Flow Cabinet. The medium of MURASHIGE & SKOOG (1962), with minor modifications, and com-

binations of various growth regulators with 0.7% agar were used. The pH of the medium was adjusted at 5.6–5.8.

For the culture of segments of inflorescences (5 mm) and meristems (0.5 mm), the material was taken from field-grown plants. The cultures were raised in 150 × 25 mm Pyrex glass test tubes, and were incubated at 23–26°C in diffused light.

The regenerated plants were taken out of the tubes when an adequate root system had been developed. The agar was gently removed by keeping the plant under running tap-water, and then transferred to the field.

For cytological investigations, the callus cells and root tips were pretreated with α-bromonaphthalene, fixed in acetic alcohol, and stained with Feulgen and acetocarmine.

RESULTS AND DISCUSSION

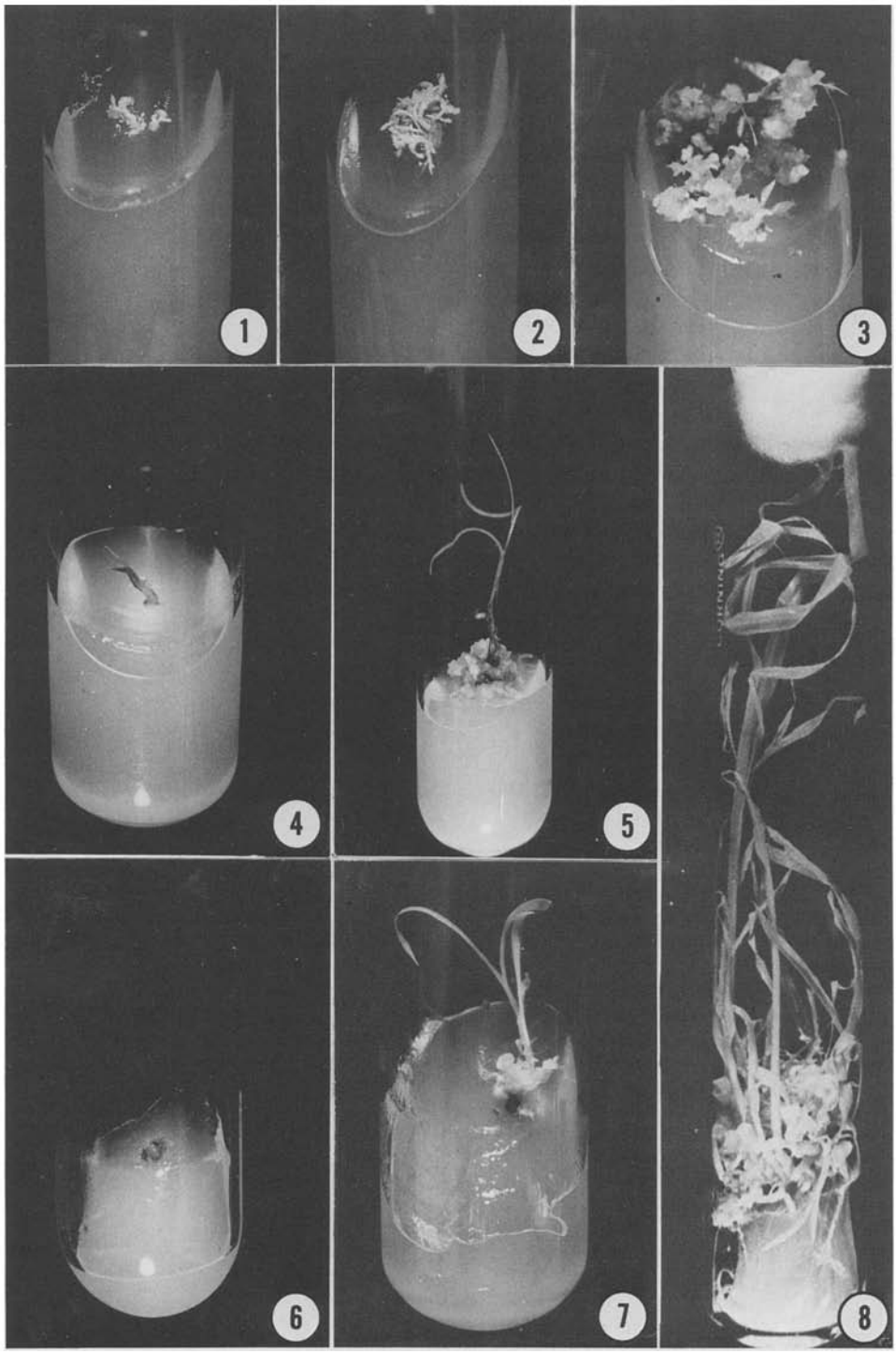
In previous studies, the successful use of different in vitro methods for the induction of genetic variability in various crops through the culturing of hybrid embryos (BAJAJ et al., 1978a), endosperm (BAJAJ et al., 1980), isolated protoplasts and pollen (BAJAJ et al., 1978b), in vitro fertilization (BAJAJ, 1979), and somatic hybridization (BAJAJ, 1974) was demonstrated. Although the differentiation of complete plants from callus cultures has been routinely obtained in a large number of plant species, the induction of reproducible regeneration in cereals and grasses is still a challenge (YAMADA, 1977; GREEN, 1978). The following are the observations on the growth of embryos, excised segments of inflorescence, and shoot tips from different species of *Panicum*, which are summarized in Table 1.

A. Inflorescence culture. Segments from the young inflorescence turned white, underwent quick elongation and almost doubled in size in 3 days (Fig. 1). They produced callus, roots and shoots in 3 weeks (Fig. 2 and 3). The growth response of *P. antidotle* was better than that of cv. UP and Australian.

B. Shoot meristem culture. Extreme tips of shoot meristem (varying from 0.5–1.5 mm) cultured on MS + 2,4-D (1.5 mg/l) + kinetin (0.2 mg/l) started to elongate, and showed 2–3 fold increase in size within a week (Fig. 4). They underwent occasional callusing, and complete plants were obtained in 3–5 weeks (Fig. 5). *P. maximum* showed a poorer response than *P. miliaceum*.

C. Embryo and seed culture. The seeds, and excised embryos reared on MS + 2,4-D (2 mg/l) began to proliferate within 5 days, and in two weeks, callus appeared all round (Fig. 6 and 7). The callus continued to grow to form a mass in about 5 weeks (Fig. 9). The cv. Australian showed faster and better proliferation than cv. UP, but was more sensitive to higher temperature (38°–40°C).

Fig. 1–8. Regeneration of plants from in vitro cultured segments of the inflorescence, shoot tips, embryos, and callus tissue cultures of various species of *Panicum*. Fig. 1–3. Cultures of segments of unopened inflorescence, 3, 15 and 23 days after inoculation. Fig. 4 and 5. Excised shoot tip after 3 and 21 days of culture on MS + 2,4-D; note the proliferation at the base of the regenerated plant in Fig. 5. Fig. 6 and 7. Culture of an excised embryo, 4 and 14 days after inoculation. Fig. 8. Differentiation of multiple shoots from embryo-derived callus on a medium devoid of 2.4-D and supplemented with casein hydrolysate.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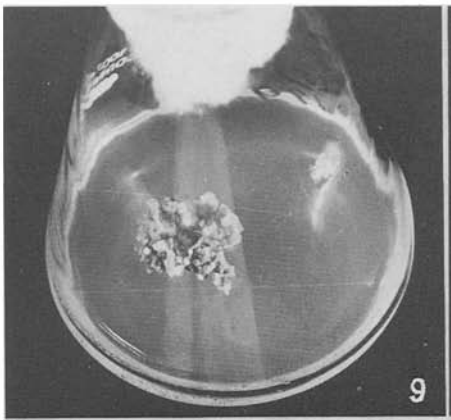
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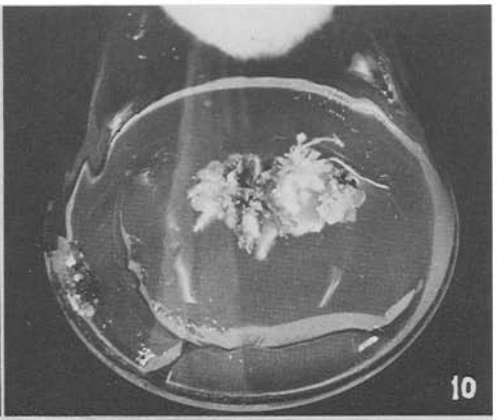
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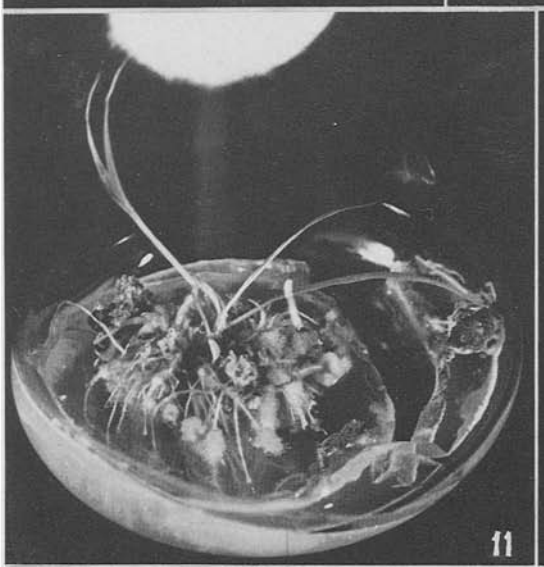
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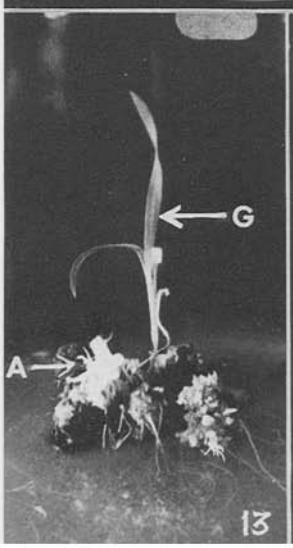
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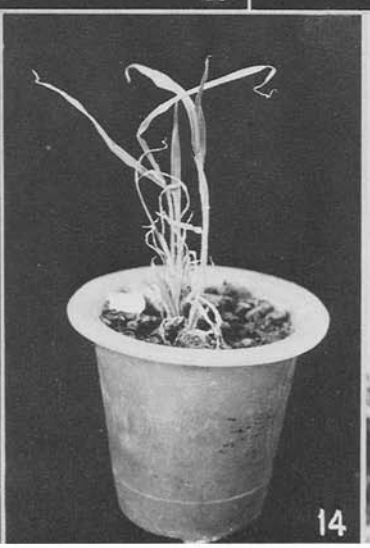
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Fig. 9–15. Establishment of callus cultures and the regeneration of plants. Fig. 9. A mass of callus 2 weeks after subculture on MS + 2,4-D (2 mg/l). Fig. 10–12. Various stages in the differentiation of plants from callus 1, 3 and 5 weeks after transfer of callus to 2.4-D-free medium respectively. Fig. 13. Differentiation of albino (A) and green (G) plants from callus cultures of *P. miliaceum*. Fig. 14. A callus-derived plant of *P. maximum* 10 days after transfer to pot. Fig. 15. Same after 6 weeks showing the formation of seeds.

D. Establishment of callus culture and regeneration of plants. The seeds and excised embryos produced a mass of callus (Fig. 9) in 5 weeks. The callus was periodically subcultured both on agar and in liquid cultures, and maintained on MS + 2,4-D (1 mg/l). The callus was cream to light brown, soft and friable, and produced a good cell suspension. The cells varied in size and shape, the round ones being most common.

When transferred to a medium devoid of 2,4-D (RANGAN, 1974), the callus underwent rhizogenesis (Fig. 10) within a week, and the shoots appeared after another week (Fig. 11). The cv. UP showed better differentiation than cv. Australian. In all, it took 6–8 weeks for the regeneration of plants from callus (Fig. 12).

The addition of casein hydrolysate to the medium resulted in the production of multiple shoots in some cultures (Fig. 8), and green and albino plants were regenerated in patches from the same mass of callus (Fig. 13). These segments of callus on isolation, either produced albino or green plants. The cultures producing albino and green plants were maintained separately for over six months. On transfer to pots (Fig. 14), and later to the field, these plants developed further, matured and set seeds (Fig. 15).

The response in vitro was genotypically oriented, and clear differences in their pattern of growth and differentiation were observed among species and cultivars. In general, *P. miliaceum* showed a better response than *P. maximum*. In the case of embryos, cv. Australian was better than cv. UP. However, the response was the reverse in the case of the inflorescence.

E. Induction of genetic diversity. The callus cells and root tip squashes showed a predominantly diploid chromosome number ($2n = 36$), however, aneuploids were very common. The chromosome number ranged from 29–36 in young cultures and polyploids in older cultures. The callus-derived plants showed a wide range of variation in morphological characters i.e. size, leaf shape, and tillering. Such plants are being screened for desirable traits.

The importance of the artificial induction and incorporation of genetic variation into plant breeding programs for increasing the production capacity of crops has been rightly stressed (WITTWER, 1974). In this respect plant tissue cultures are a rich source of variables (D'AMATO, 1977), and efforts are being focussed to exploit this tool.

The significance of the present investigation in the improvement of forages lies in the fact that in a relatively short time, a large number of genetically diverse plants can be produced. The gene pool can be further augmented by treating these cultures with various drugs and mutagens, especially to induce resistance to seed shattering, which is the most needed character (BURTON et al., 1973). Work on these lines is in progress.

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