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INDUCTION OF SOMATIC EMBRYOGENESIS IN SUSPENSION CULTURES DERIVED FROM SEED CALLUS IN *PANICUM MAXIMUM* (GUINEA GRASS)

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A regimen was estabilished to induce prolific embryogenesis in suspension cultures obtained from seed callus in *Panicum maximum* (Guinea grass). Creamish friable callus was obtained from seeds cultured on MS medium supplemented with various concentrations of 2, 4 - D (1.0 - 3.0 mg/1). Suspension culutres were raised by transferring 100 gm of callus in 40 ml liquid MS medium containing coconut water (CW) (5% V/V)+2, 4 - D (2.5 mg/1). A good homogeneous suspension was obtained after few sub cultures. Profuse embryogenesis was observed in MS medium supplemented with 1.0 mg/1 of 2, 4- D and 0.2 mg/1 Kn. Suspension cultures did not lose their embryo forming potential over a period of 30 months or so. No decrease in morphogenic competence was observed.

Keywords : *Panicum maximum;* Seed callus; Sommatic embryogenesis; Suspension culture.

Introduction

Regeneration of mature, fertile and genetically stable plants from single cells or protoplasts is an essential aspect of genetic engineering[1]. Previously, tissue culture of monocotyledonous plants, in general, and graminaceous taxa in particular was considered difficult. Potrykus *et al*[2]., reported that the basic pre-requisite for cell genetics plant regeneration from isolated single cells or protoplasts is difficult for a single plant species of this extrremely important group of crop plants. Although there are a few reports[3] of suspension cultures and protoplasts sustained regenerable suspension in some cereals and millets were established in the last two decades[4-7]. The morphogenic identification of embryogenic callus and its propagation and use as a source of suspension cultures and protoplasts was a crucial step in biotechnology.

Cereals and grasses constitute the most important group of plants. Members of this group are important source of food and fodder for mankind. Realising the importance of genetically pure plants for crop improvement, in the present study efforts were made to work out the protocol for inducing somatic embryogenesis in suspension cultures derived from seed callus in guinea grass, *Panicum maximum* and to plate these embryoids to obtain the plantlets.

Materials and Methods

Seeds of *Panicum maximum* Jacq. CV PGG 18 was obtained from Indian Grassland and Fodder Research Institute, Jhansi. Seeds were surface sterilized with 0.1% (W/V) $HgCl_2$ solution, for 5 minutes, washed thrice in sterile, distilled water and inoculated aseptically on MS basal medium supplemented with various concentrations of 2, 4-D(1.0-3.0 mg/l). The pH of the medium was adjusted to 5.8 before autoclaving at 1.06 kg. cm^2 for 15 minutes. Growth regulators were added before autoclaving. Cultures were incubated in continous light from florescent tubes and incandescent bulbs (1200 lux) at 26±2°C.

Suspension Culture : Suspension culture were obtained from seed callus by transferring it in to liquid medium and maintanined on a horizontal gyratory shaker at a speed of 100 rpm. Suspension was transferred with the help of syringe. Suspension culutres were also incubated in culture chamber (incubation chamber) where light, humidity and temperature are controlled.

Results and Discussion

Induction of Seed Callus : Seed of *Panicum maximum* cultured in MS medium supplemented with (1.0-3.0 mg/1) 2, 4-D developed in to the callus that was soft, creamish, friable, watery and shiny (Fig. 1). Some parts of the callus showed

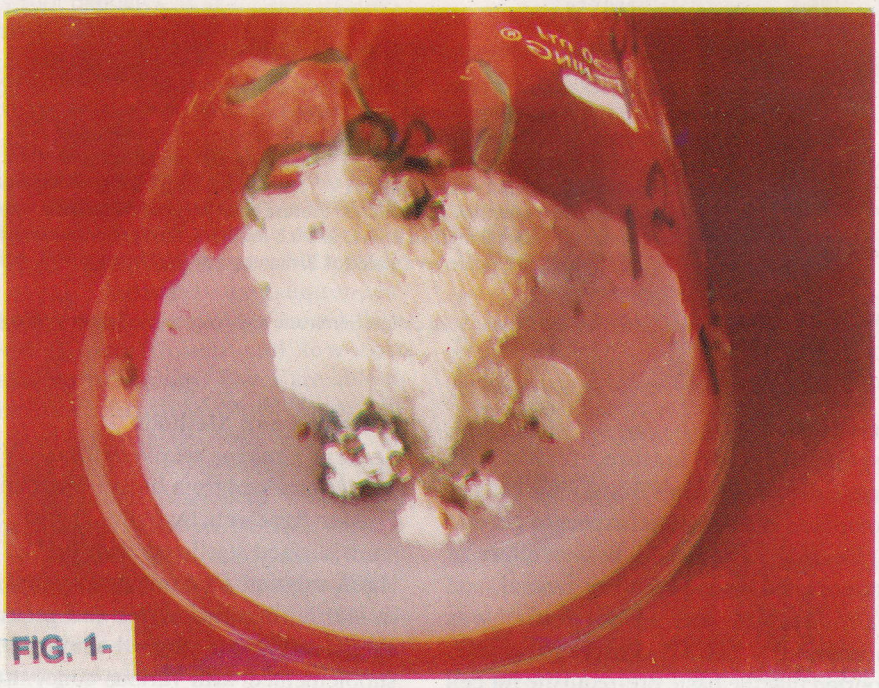

Fig. 1. Creamish, friable and fluffy callus formed on cultured seeds in MS+2, 4-D (1.0 mg/1-3.0 mg/l).

Fig. 2. Stock callus maintained on MS+2, 4-D (2.5 mg/l).

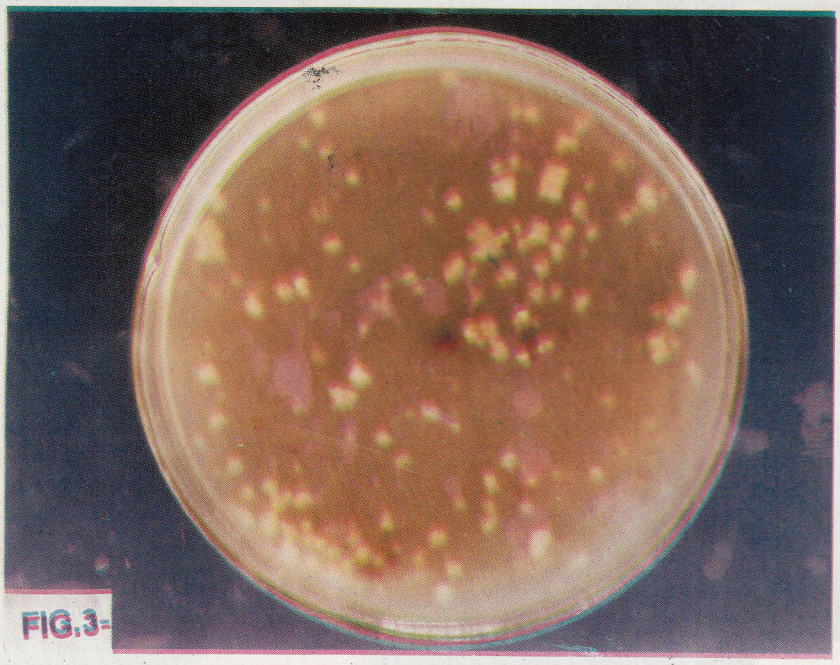

Fig. 3. Thick, mucilaginous suspension with small clums on MS+CW (5% v/v O+2, 4-D (2.5 mg/l).

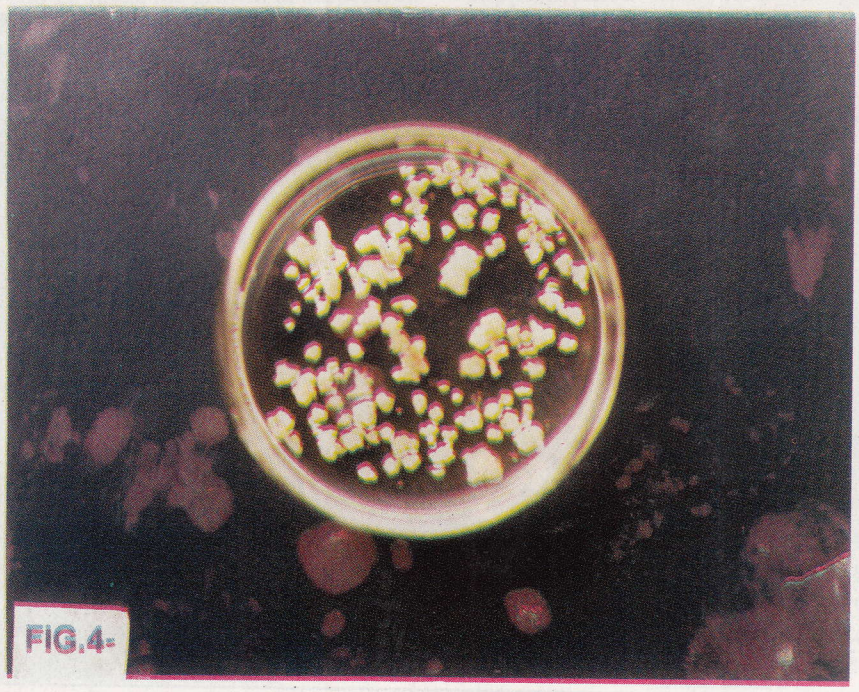

Fig. 4. Separated embryoid like structure from suspension which contained MS+2, 4-D (1.0 mg/1)+Kn (0.2 mg/l).

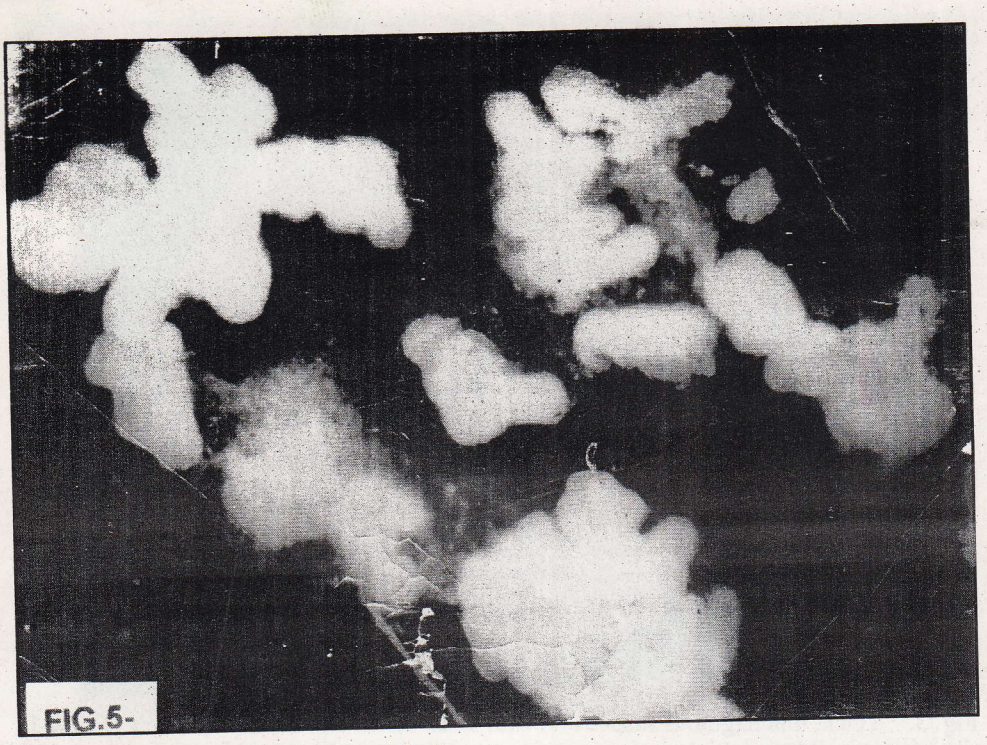

Fig. 5. Isolated embryoids (magnified).

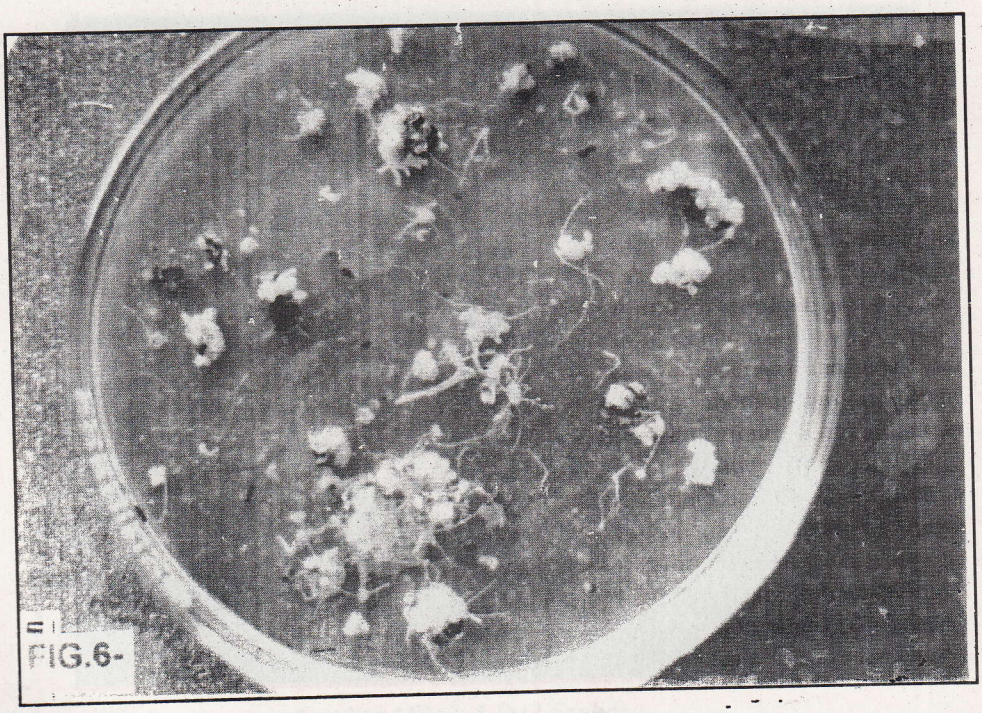

Fig. 6. Rhizogenesis on MS+2, 4-D (0.2 mg/l) +Kn (0.2 mg/1).

embryogenesis on incubation medium. Embryos were white, globular shaped and showed typical organization of a grass embryo. Many of the embryoids showed formation of secondary or even tertiary embryoid on their surface resulting in a cluster of embryoids. Callus surrounding the embryoids often turned black.

Raising of Suspension Culture : 100 mg of callus derived from the seed or seed-derived callus was inoculated in 40 ml MS liquid medium supplemented with CW (5% V/V)+ 2, 4-D (2.5 mg/l). A creamish homogeneous suspension was obtained after several sub-cultures (Fig. 3). On various levels of 2, 4-D, the growth of suspension was observed. Growth of suspension was enhanced in the media with 2, 4 - D in the range of 0.2 - 3.0 mg/l but on 5.0 mg/l of 2, 4-D, the growth declined. During the first few passages, the suspension cultures were grown in a medium with CW (5% V/V) + 2, 4 - D (2.5 mg/l) but subsequently maintained on MS medium supplemented with 2, 4 - D (2.5 mg/l) and sub-cultured every sixth week. In the first few sub-cultures, the growth of suspension was slow but in subsequent passages, the growth improved. The suspension had a lag phase of 10 days and then showed growth up to 45 days (Fig. 7). In the begining, larger cell clumps were frequently observed, but after several sub-cultures suspension contained single cells and smaller cell aggregates. The shape and the size of cells varied from small spherical with dense cytoplasm to large elongated cells.

Formation of Embryoids and other Organized Structure : Profuse embryogenesis was observed on MS liquid medium supplemented with 1.0 mg/l of 2, 4-D and Kn (0.2 ml/l). Embryos were formed in clusters often showed the formation of secondary or tertiary embryoids. One peculiar feature observed was that suspension which showed fully developed embryoids often turned black as was noticed on solid agarised media. Embryoids formed in creamish suspension were not fully developed and showed callusing and rooting on various conbination of 2, 4-D and Kn. Slow growth associated with rhizogenesis was observed on MS medium supplemented with 0.2 mg/l of 2, 4 - D and 0.2 mg/l of Kn. Few embryoids along with small cell clumps were observed in media containing 0.2 - 3.0 mg/l of Kn. Numerous white structures were seen in suspension which resembled root tips separated from roots. Similar observation was made when Kn was substituted by BAP.

However, the suspension did not lose its morphogenic potential over a period of 30 months on medium supplemented with CW (5% V/V) + 2, 4-D (2.5 mg/1).

Microscopic examination of suspension explained that the large elongated cells were non-embryogenic vacuolated and with sparse cytoplasm whereas, small spherical cells were densely cytoplasmic and had the capacity to divide.

Plating of Embryoids Formed in Suspension Cultures : Isolated embryoids (Fig. 4.5) formed on the medium containing 1.0 mg/1 of 2, 4- D + Kn (0.2 mg/l) and also on other media were plated on MS agar media supplemented with different growth substances. Only rhizogenesis (Fig. 6) could be observed.

Cell suspension cultures of *P. maximum* were raised transferring the self - creamish friable embryogenic callus derived from seeds on MS liquid + CW (5% V/V) + 2, 4 - D (2.5 mg/l). For the first few months, cultures were maintained on this medium but later transferred to MS+2, 4 - D (2.5 mg/l). Soft cream, friable embryogenic callus also proves very amenable for the establishment of embryogenic cell suspension cultures[8, 9] where as this type of callus proved favourable for the establishment of embryogenic cell suspension cultures of *Pennisetum americanum*[4, 10], *Panicum maximum*[11], *Saccharum officinarum*[12], *Pennisetum purpurenum*[5]. In these plants, compact. nodular, organized embryogenic callus was used to derive successful suspension cultures. In subsequent passages, the suspension turned thick, uniform, slimy and mucilaginous which are the

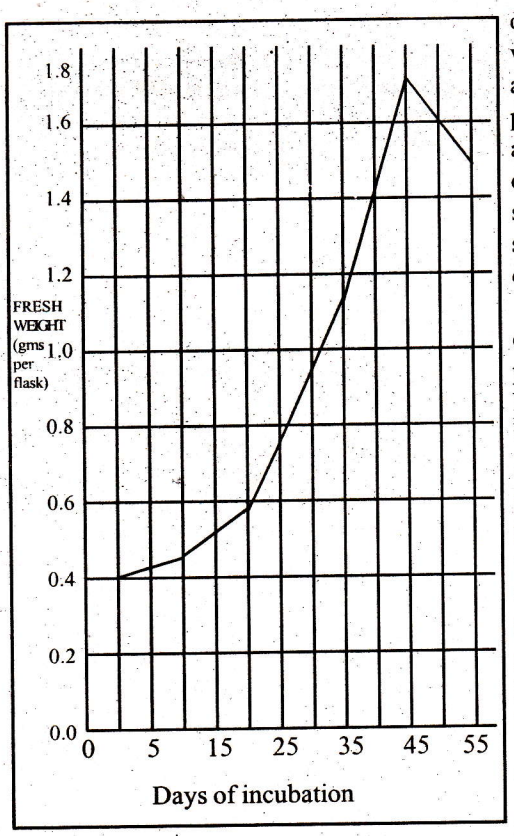

Fig. 7. Growth of stock suspension

characteristic features of grass suspension cultures and have also been found in several other grasses e.g. *Dactylis glomerata*[13] and *Pennisetum americamum*[1,10]. In all these grasses, embryogenesis was induced in mucilaginous suspension cultures but in a report on *Dactylis glomerata*[14], mucilaginous suspension was associated with a decrease or lack of embryogenic potential.

In the present study 2, 4- D has been found essential for induction of embryogenic suspension cultures and also for the formation of embryoids as reported by several workers. In suspension cultures, grown with either Kn or 2, 4-D (0.2 mg/l) + Kn (0.2 mg/l) small rounded structures were observed. Probably, these were root tips which had been sloughted off. But, when the medium was supplemented with high level of 2, 4 -D numerous embryoids were formed which were clustered and showed callusing around them. Incorporation of CW had a promotory effect on embryoid formation. In a few cultures where embryoids (fully developed) were formed when the suspension turned black. Darkening of the suspension caused by CW has also been observed in *Saccharum officinarum*[12].

In the present study, suspension cultures did not lose their embryo forming potential over a period of 30 months or so. No decrease in morphogenic competence was observed. Roots were hairy and brown. Efforts are being continued to induce shoot and full plantlet regenertion from these plated embryoids. These regenerated plants which will be of single cell origin would be extremely suitable for different genetic manipulation in crop improvement programme.

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