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EMBRYOGENESIS IN SUSPENSION CULTURES OF *PAPAVER SOMNIFERUM* L.

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Abstract

Papaver somniferum L. seedlings produced a light-green, soft and friable callus when cultured on ½ Murashige & Skoog (MS) medium containing 2, 4-D (0.5 mg/l) and Kinetin (0.1 mg/l). After 2-3 subcultures, callus pieces transferred to liquid medium and placed on orbital shaker showed active growth and formation of many cell clumps with loosely arranged cells. Structures resembling various stages of embryo development found in suspension cultures when transferred on solid MS medium containing Benzylaminopurine (0.5 mg/l), Adenosine Sulphate (25 mg/l) and Gibberellic acid (10 mg/l) gave rise to green globular embryoids.

Introduction

For maximum utilization of the potential of cell and tissue culture and for genetic improvement of plants through gene manipulation, *in vitro* embryogenesis provides the best solution. Somatic embryos may arise from a single cell through a series of organized divisions in a manner similar to the development of a zygotic embryo. They generally do not have vascular connection with the callus mass.

Plant regeneration from suspension cultures via somatic embryogenesis was first described in carrot (Steward *et al.*, 1958) and is now known in many dicotyledonous species (Konar & Nataraja, 1965; McWilliams *et al.*, 1974; Walker & Sluis, 1983; Ammirato, 1984). In monocotyledons somatic embryogenesis and regeneration of plants from suspension cultures have been reported in *Asparagus officinalis* (Wilmar & Hellendoorn, 1968) and 6 members of the Graminae (Vasil & Vasil, 1984). In tissue culture of cereals, plant regeneration generally occurs through the organization of multicellular shoot meristem as in *Oryza sativa* (Nakano & Maeda, 1979) and *Zea mays* (Springer *et al.*, 1979). Whereas embryogenesis has been reported in *Papaver bracteatum* (Staba *et al.*, 1982), in the present paper embryogenesis as observed in suspension cultures of *P. somniferum* is reported.

Materials and Methods

(i) *Callus and suspension culture establishment:* Fresh seeds of a high yielding *P. somniferum* L. were obtained from the Experimental Fields, Board of Narcotics, Bunair. The seeds were surface sterilized with 1% mercuric chloride for 2-3 minutes

washed in sterilized distilled water and inoculated on agar medium containing 5% sucrose. The seeds germinated in 7 days and unorganized callus tissue was established from cotyledon and hypocotyl on ½ strength Murashige & Skoog's (1962) medium supplemented with 0.5 mg/l 2, 4-D (2, 4-dichlorophenoxyacetic acid), 0.1 mg/l Kinetin (6-furylaminopurine) and 5% sucrose. Callus tissue was maintained for one year by frequent subculturings after 4-6 weeks in fresh medium supplemented with Benzlaminopurine (BAP) @ 0.5 mg/1, adenine sulphate (AS) @ 25 mg/1 and Gibberellic acid (GA_3) @ 10 mg/1. The callus cultures, about 0.5 cm^3 in size, were transferred into 250 ml Erlenmeyer flasks containing 50 ml of liquid medium supplemented with various growth hormones. Each treatment had 5 replicates. The cultures were placed on an orbital shaker with 80 rpm at 25 ± 1°C with 16 h illumination. The pH of the medium was adjusted at 5.8 and autoclaved at 15 psi for 15 min. The suspension cultures once established were subcultured every 7 days.

(ii) *Microscopical studies:* A drop of the suspension was placed on a slide and stained with safranin. Permanent mounts were made by using Haupt's adhesive. These slides were then studied under a Leitz Ortholux microscope.

Results

(i) *Callus initiation:* Callus from seedlings was induced on ½ MS medium supplemented with 0.5 mg/l of 2, 4-D and 0.1 mg/l of K within 4 weeks of culture. The callus when subcultured onto MS fortified with 0.75, 1.0 and 1.5 mg/l of 2, 4-D, although increased in bulk but there were no signs of embryogenesis. Addition of BAP and AS to the medium also had no effect on organogenesis, when 2, 4-D was eliminated either totally from the culture medium or decreased stepwise to zero level and/or replaced by 0.5 mg/l of BAP, 25 mg/l of AS and 10 mg/l of GA_3 to induce shoot formation (Jabbar, 1983), the calli first turned into a green mass followed by appearance of whitish beaded masses (Table 1). There were no signs of embryogenesis/or organogenesis and the calli at this stage were therefore transferred to the liquid medium to induce embryogenesis with the ultimate goal of plant-let formation.

(ii) *Embryogenesis:* The whitish beaded callus masses from the solid medium on transfer to the liquid medium, supplemented with either a) 0.5 mg/l BAP, b) 0.5 mg/l BAP and 25 mg/l AS and c) 0.5 mg/l of BAP, 25 mg/l AS and 10 mg/l of GA_3 readily formed suspensions by the cleavage of the callus masses. Growth in the suspensions was discernible after about one week of culture, because of numerous cell clump formation with loosely grouped cells. These cells could be easily separated from one another by gentle shaking or with a needle on a microscope slide. The number of these clumps further increased when a few of them were subcultured after 2 weeks on MS medium containing 0.5 mg/l BAP, 25 mg/l AS and 10 mg/l of GA_3. Moreover, these clumps had beaded structures on their surface which could easily break down during shaking and

Table 1. The effects of 2, 4-D, BAP, AS, K and GA_3 on callus induction and its further growth in *P. somniferum* seedlings. (Culture period lasted for 8 weeks).

Hormone Conc. mg/1					Calloge-	
2, 4-D	K	BAP	AS	GA_3	nesis	Remarks
0.1	0	0.0	00	0	+	Whitish callus
0.5	0	0.0	0	0	–	– –
1.0	0	0.0	0	0	–	Explant died after 2 weeks
0.5	0	0.1	0	0	++	White callus formation
0.0	0	0.1	0	0	+	Blackish brown friable callus
0.0	0	0.5	0	0	++	Callus blackish brown
0.0	0	1.0	0	0	+	Blackish callus
0.0	0	0.0	10	0	–	Moderate vegetative growth
0.0	0	0.0	25	0	–	Good vegetative growth
0.0	0	0.0	50	0	–	Vegetative growth inhibited
0.0	0	0.0	0	10	–	Shoot elongation
0.0	0	0.0	0	20	–	Prolific shoot elongation seedling getting week
0.1	0	0.5	25	10	++++	Friable yellowish white callus
0.0	0	0.5	25	10	++	Green beaded callus

– = no callus, + = Some callus, ++ = Moderate callus, +++ = Good callus, ++++ = Excellent callus.

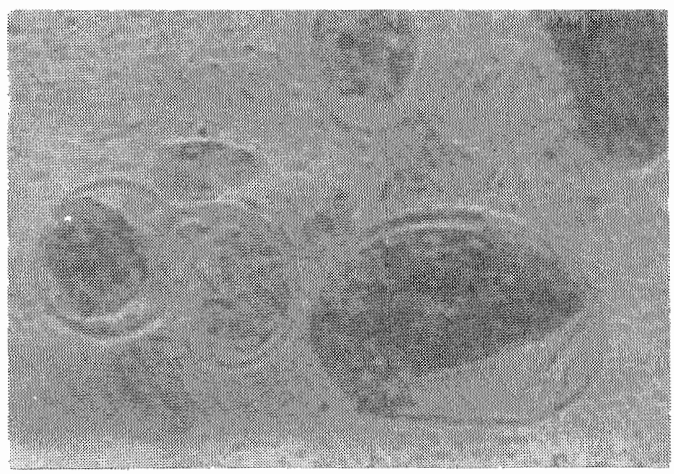

Fig. 1. Different developmental stages of embryo formation in suspension cultures of *Papaver somniferum*.

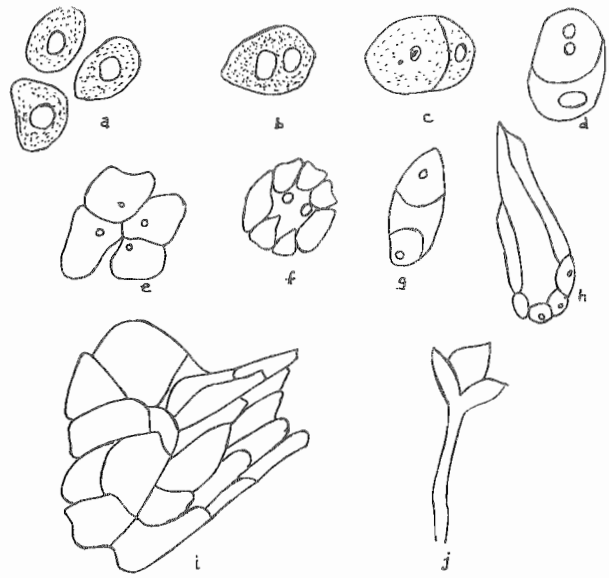

Fig. 2. Developmental stages of embryo formation in suspension cultures of *Papaver sommiferum.*
a-d Farly stages in the development of a proembryoid.
e-f Development of a globular embryoid.
g-h Cell aggregates from suspension cultures showing various stages of embryogeny (torpedo-shaped).
i-j Later stages in embryogeny (heart and cotyledonary-shaped).

either single or aggregates of a few cells could be detected when a drop was pippetted out and examined under the microoscope. These single cells or cell aggregates underwent serial changes resulting in embryoid-like structure formation. About 25% of the suspended cells gave rise to embryoids and these structures had a striking resemblance to the zygotic embryos.

A drop from the suspension cultures undergoing embryogenesis when mounted on a slide showed that embryoid development proceeded by the transverse division of a single densely cytoplasmic cell into two cells of unequal size (Fig. 1). The larger cell after further subdivisions gave rise to the suspensor, while the smaller cell, the embryoid initial formed a small pro-embryoidal mass which by further growth passed through the globular, torpedo, heart and cotyledonary stages (Figs. 2 and 3). These were similar to those observed in normal embryogeny. However, the early segmentation stages might not have been similar to those usually occurring during zygotiz embryogenesis.

The pro-embryoids were separated from the suspension cultures at a stage shown in Fig. 3 and inoculated on ½ MS solidified medium. In most cases these pro-embryoids callused. However addition of 10 or 20 mg/l of GA_3 suppressed callusing and approximately 5% normal looking seedlings developed from these embryos (Fig. 4). These seedlings possessed normal cotyledons and roots.

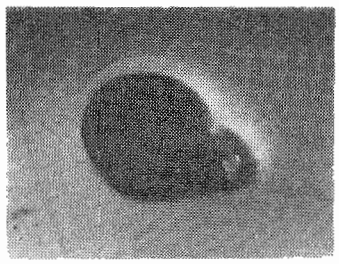

Fig. 3. Embryo formed in suspension cultures of *Papaver somniferum.*

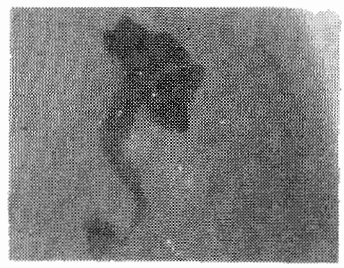

Fig. 4. Embryo after transfer to solid medium containing GA_3.

Discussion

Embryogenesis in *P. somniferum* was a two step process each requiring a different medium. The callus was initiated and bulked in the presence of 2, 4-D rich agar medium. The callus proliferated well in this medium with no signs of embryogenesis. Similar results were obtained by Sondahl & Sharp (1977) in obtaining embryogenesis in *Coffea arabica.* The callus on transfer to a medium containing AS, BAP and GA_3 developed into whitish beaded masses. These beaded masses formed suspension cultures when transferred to a liquid medium. Embryogenesis was observed in GA_3 rich medium. GA_3 has been reported to suppress embryogenesis in carrot (Fujimura & Komamine, 1975) and citrus (Tisserat & Murashige, 1977) but in our experiments GA_3 was found to be essential for embryo formation. Without GA_3 no embryogenesis occurred.

Acknowledgements

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