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In vitro Morphogenesis From Seeds of *Helianthus Annuus* L.

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Abstract

The objective of this study was to observe the *in vitro* morphogenetic response of a commercial hubrid SF-187 which is used in sunflower breeding programme. Basal MS media supplemented with NAA and BA was used. Cultures were incubated in complete obscurity at a temperature of 25°C. Morphogenesis and callogenesis were observed after 14 days of incubation. When exposed to light, shootlets turned green within two days. A maximum of fifty plants were regenerated from single explant when cultured on MS medium supplemented with 2.0 mg/l each of BAP and NAA. The plantlets were rooted when subcultured on MS media supplemented with 0.5 mg/l IAA.

Introduction

Sunflower is the second most important source of vegetable oil and protein in the world (Mayone, *et al.*, 1988). It contains 30-40 percent oil while its cake contains 35-50 percent proteins. Owing to its economic importance, there has been an increasing interest in devising genetic manipulation protocols for introducing novel agronomic traits into the cultivated sunflower.

Besides the classical methods of breeding, recent developments in biotechnology have opened new promising avenues for crop improvement. Somatic hybridization between wild and cultivated species, somaclonal variations and genetic transformation are new and useful techniques to introduce genes for better oil/protein ratios, stress tolerance, pest resistance and other interesting agronomic characters. Plant regeneration from diverse explants is a pre-requisite to gene manipulation, and it has been limiting the application of genetic manipulation techniques for sunflower improvement. Tissue culture techniques have been used for *in vitro* selection against disease resistance and propagation of valuable sunflower materials e.g male sterile female lines (Krauter and Friedt, 1991).

Plant regeneration has been achieved from cotyledons (Khalid *et al.*, 1992), from hypocotyl (Greco *et al.*, 1984), from protoplast derived calli (Monique *et al.*, 1991) and from immature embryos (Martine & Georage, 1988) using Murashige and Skoog (1962) medium supplemented with phytohormones at various levels. Direct adventitious shoots were achieved from cotyledons (Puglesi *et al.*, 1991). There are also reports of multiple shoot formation from apical meristem (Paterson and Everett, 1985). In this paper, we describe a protocol for indirect regeneration of clusters of fertile plants form calli derived directly from intact seeds.

Materials and Methods

Seeds of high yielding hybrid SF-187 were provided by the National Oilseed Development Project (NODP) of the National Agricultural Research Centre, Islamabad. These seeds were imbibed in distilled water for 24 hours. Seeds were surface disinfected by dipping in 0.1 percent aqueous mercuric chloride solution to which a few drops of tween 20 had been added, for fifteen minutes. Following several washings with sterile water, these seeds were dehulled for *in vitro* response.

For regeneration and callogenesis experiments, media used was Murashige and Skoog (1962) macro nutrient and micro nutrient salts and contained 0.5 mg/l each of nicotinic acid and pyridoxine, 0.1 mg/l thiamine, 100 mg/l myo-inositol, 3 percent sucrose nd 500 mg/l casein hydrolysate. The media tested for *in vitro* response, contained NAA and BAP at a concentration of 0, 0.1, 0.5, 1.0, 2.0 and 4.0 mg/l each as given in (Table 1). All the media were solidified with 5 g/l of agar and dispensed into 250 ml flasks @ 70 ml/ flask.

The pH of media was adjusted at 5.7 prior to autoclaving. Media were sterilized by autoclaving at 121°C for twenty minutes. Dehulled seeds were inoculated under aseptic conditions and were incubated in the dark at 25 ± 1°C. Twenty explants were cultured for each treatment and experiment was repeated at least three times. The regeneration ability was expressed as percentage of explants forming plantlets and percentage of explants forming calli was also scored.

Results and Discussion

The response of explants became visible within two weeks of *in vitro* culture, when signs of either regeneration or callogenesis became evident. The nature of response differed according to the medium used. When plain M. S. media (control) was used, seeds normally germinated with complete root and shoot system (Fig. 1) while abnormally swollen roots developed when the medium was supplemented with 0.10 mg/l PAB & NAA each (R_1 medium). At medium R_2 (0.5 mg/l BAP and NAA) explants underwent callogenesis after two weeks of incubation. When these calli were exposed to light, they turned green and many nodule like structures (0.3-5 mm diameter) started appearing on entire surface of the explants (Fig. 2). Similar types of calli were obtained by (Khalid *et al.*, 1992) from cotyledons using liquid media at similar concentration of NAA & BAP. These calli were subcultured after every 4 weeks on the same media but no regeneration was observed even after several subculturing.

Callogenesis became visible within ten days of inoculation on regeneration media, R_3 and R_4. After two weeks of incubation, morphogenic structures were evident on entire surface of the calli (Fig. 3) which turned green within 48 hours upon exposure to light. Subsequently, these globular

Table 1: Composition of six media* used for evaluating the *in vitro* morphogenesis response of sunflower hybrid SF-187.

Media Code	BAP mg/l	NAA mg/l
R_1	0.10	0.10
R_2	0.50	0.50
R_3	1.00	1.00
R_4	2.00	2.00
R_5	4.00	4.00

*Basal Medium: M.S. Salts and vitamins + 500 mg/l casamino acid + 3 percent sucrose

Table 2: Frequency of callogenesis and shoot regeneration from intact seeds on MS media supplemented with different levels of NAA & BAP.

Media	Percentage Regeneration	Percentage Callus
R_0	0.00	0.00
R_1	0.00	0.00
R_2	0.00	44.44
R_3	12.5	72.22
R_4	61.11	88.88
R_5	44.40	50.50

Fig. 1: A complete rooted plantlet obtained from seed germination

structures transformed into small shootlets very similar to those achieved by (Greco *et al.*, 1984) from hypocotyl derived calli. In this experiment, we were able to achieve fifty plantlets from a single explant while in other experiments, maximum number of shootlets achieved was twenty six (Greco *et al.*, 1984). These developing shoots were excised from the calli and transferred to tubes containing M.S. (1962) medium supplemented with IAA at a concentration of 0.5 mg/l. Regenerated plants were successfully rooted on M. S. medium and survival rate was

Fig. 2: Callus formation after four weeks when medi was supplemented with BAP and NAA (0.5 mg/

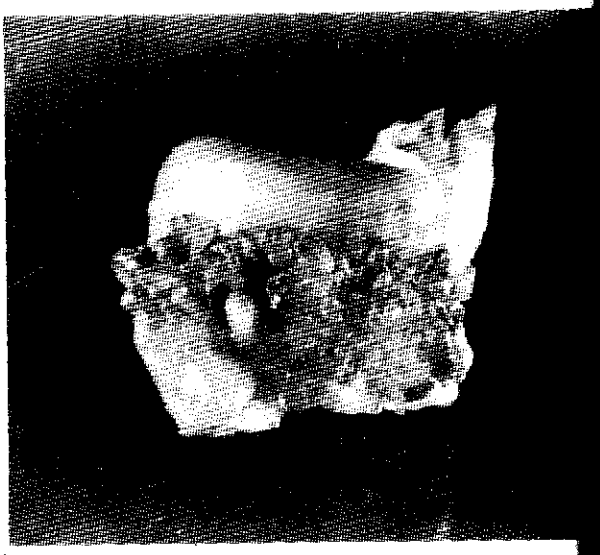

Fig. 3: A three week old culture showing sign morphogenesis

above 90 per cent. The regenerated plants started *in* flowering (Fig. 4) after 8 weeks in cultures. *in* flowering results in failure to obtain functional seeds. phenomenon is highly undesirable because it hi reading efforts and has been reported by several wor (Krauter and Freidt, 1991 and Nestares, *et al.*, 1 Thus,there is a need to study the physical and che factors which can reduce or eliminate early *in* flowering.

When concentration of BAP and NAA were h increased to 4 mg/l each (R_5 media), growth rate o

was also enhanced and more friable calli were achieved. But regeneration ability of such calli decreased (Table 2). When

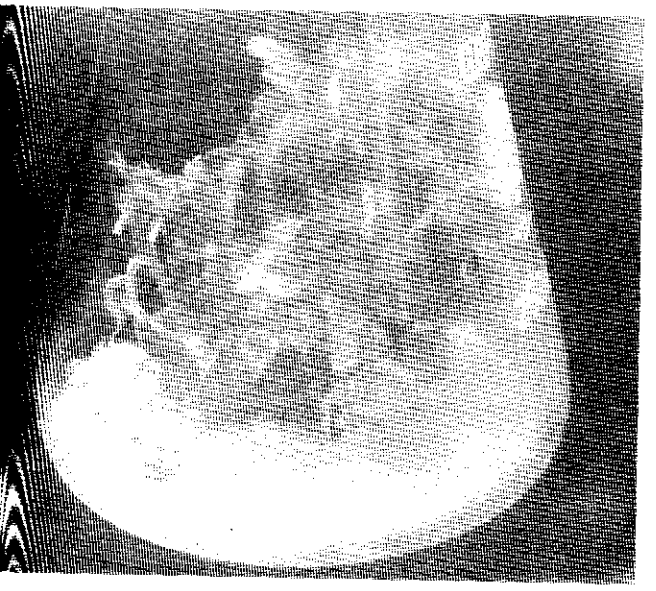

Fig. 4: *In vitro* flowering of *Helianthus annuus* L.

exposed to light, these calli turned brown and did not regenerate into shoots or roots when subcultured on the same medium. These findings indicate that calli obtained on medium with higher concentrations of NAA and BAP (> 2 mg/l each) are not capable to regenerate into plantlets.

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