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In vitro shoot regeneration and flowering of Sesame (*Sesamum indicum* L.) cv. SVPR - 1

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In vitro shoot regeneration and flowering were achieved from shoot tip and nodal explants of *Sesamum indicum* L. on MS basal medium containing different combinations and concentrations of cytokinins and auxins. The highest percentage (91.8) of shoot regeneration and number of shoots (25.9) were observed from shoot tip explants cultured on MS basal medium supplemented with 2.0 mg l^{-1} BAP and 0.3 mg l^{-1} NAA combination. Flower buds (8.12) were obtained in shoot tip explants cultures. *In vitro* flowers and root induction were achieved in MS medium containing 1.5 mg l^{-1} NAA and 0.03 mg l^{-1} BAP. Subsequently, the plants were transferred to red soil: sand: cowdung (2:1:1 -v/v/v). The survival rate was 78 % in the greenhouse conditions.

Key words : Sesame, *in vitro* flowering, multiple shoot regeneration, acclimatization.

Introduction

Sesame (*Sesamum indicum* L.) belongs to *Pedaliaceae* family and is considered as oldest oil seed crop. It is grown in India, China, Korea, Russia, Turkey, Mexico, South America and several countries of Africa. It is cultivated worldwide on a total area of over 7.7 million hectares with total production of 3.3 million tons (FAOSTAT data, 2008). Sesame seeds are an important source of edible oil and also widely used as spice. The seeds contain 50 - 60 % oil, which had excellent stability due to natural antioxidants such as sesamolin, sesamin and sesamol (Brar and Ahuja, 1979). Sesame oil is used in Ayurvedic system of medicine (Michael Murray *et al.,* 2005). Antioxidant and anticancer properties have been isolated from sesame seeds (Osawa *et al.,* 1990). The oil cake is rich in protein and it is used as cattle feed.

Sesame is highly recalcitrant to regenerate in *in vitro* conditions. However, many protocols for micropropagation (Rao and Vaidyanath, 1997;

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Gangopadhyay *et al.,* 1998; Sharma and Pareek, 1998), somatic embryos (Jeyamary and Jayabalan, 1997, Xu *et al.,* 1997) and indirect adventitious shoot regeneration (Taskin and Turgut, 1997, Younghee, 2001) have been achieved with low frequency. However, there is no report on high frequency *in vitro* shoot regeneration and flowering of *Sesamum indicum* L.

In vitro flowering has been reported as a rare process of importance in crop plants, mainly its high genetic purity (Stephen and Jayabalan, 1998). The *in vitro* flowering has been reported in many crop species (Vandana *et al.,* 1995, Jabeen *et al.,* 2005, Victorio and Lage, 2009). The present study presents findings of an experiment to work out a suitable protocol for efficient shoot regeneration and *in vitro* flowering in *Sesamum indicum* L. To our knowledge, this is the first report of the successful and established an efficient protocol for high frequency of shoot regeneration and *in vitro* flowering of in *Sesamum indicum* L.

Materials and methods

Plant material and disinfection

The seeds of *Sesamum indicum* L. cv. SVPR - 1 were used as a source of material. The seeds were obtained from Cotton Research Station, Srivilliputhur, Tamil Nadu, India. The seeds were washed with running tap water for 15 minutes, followed by soaking in 2 % of Teepol soap solution for 5 minutes and then seeds kept in running tap water for 15 minutes. Following repeated rinsing in distilled water. The seeds were disinfected with 70% ethanol for 45 seconds and washed with sterile distilled water for three times, followed by 0.1 % (w/v) aqueous mercuric chloride for 5 minutes and then washed three times with sterile distilled water. The disinfected seeds were germinated in 25 x 150 mm test tubes containing moistened cotton for seed germination. The cultures were maintained in dark for 48 h at 25 ± 2°C and then transferred to 16 h light and 8 h dark photoperiod condition with the light intensity of 30 $\mu mol\ m^{-2}\ s^{-1}$. Shoot tip and nodal segments were excised from seven-day-old aseptic seedlings and used as explants for shoot regeneration and *in vitro* flowering (Fig. 1a).

Culture conditions

MS basal medium (Murashige and Skoog, 1962) supplemented with 3 % (w/v) sucrose and 0.8 % (w/v) agar (Himedia, India) was used for subsequent experiments. The pH of the medium (supplemented with respective growth regulators) was adjusted to 5.8 with 1 N NaOH or 1 N HCl before gelled with

0.8 % (w/v) agar. The medium was dispensed into culture tubes (Borosil, India) and autoclaved at 105 kPa and 121ºC for 15 minutes. Shoot tip and nodal explants were implanted vertically on the culture medium (test tubes 150 × 25 mm, containing 10 ml medium) and plugged tightly with non-absorbent cotton. All the cultures were incubated at 25 ± 2ºC under 16/8 (light/dark) hours photoperiod of 30 µmol $m^{-2}s^{-1}$ irradiance provided by cool white fluorescent tubes (Philips, India). All subsequent subcultures were done at 20 days intervals.

In vitro shoot regeneration and flowering induction

Shoot tip and nodal explants were excised from seedlings (Fig. 1a) and were cultured on MS medium containing 3 % (w/v) sucrose, 0.8 % (w/v) agar, and supplemented with BAP and Kin (1.0 - 3.0 mg l^{-1}) alone or in combination with NAA (0.1 - 0.5 mg l^{-1}) tested for shoot regeneration. The proliferated shoots were transferred to [MS medium with 1.0 mg l^{-1} BAP + 0.2 mg l^{-1} GA_3 for shoot elongation. Data were recorded in all treatments after 3 weeks culture. Well-developed shoots (8 cm) were transferred to MS basal medium supplemented with 0.5 - 2.5 mg l^{-1} NAA and 0.01 - 0.05 mg l^{-1} BAP for flowers formation and root induction simultaneously. Plantlets with flowers were removed from the culture tubes. The roots were washed in tap water and then sterile distilled water followed by transferred to paper cups containing sterilized mixture of red soil: sand: cowdung (2:1:1 v/v/v) for hardening. These plantlets were placed for a month under environmental plant growth chamber (MRL-350H, Sanyo, Japan) for acclimatization. Acclimatized plantlets were transferred to the green house.

Statistical analysis

Mean values with standard errors were used for a parametric moods median test (Snedecor and Cochran, 1989). The data were analyzed for variance by Duncan's multiple range test (DMRT) using the SAS programme (SAS Institute, Cary, N.C.).

Results and discussion

Shoot tip explants were produced higher number of multiple shoots than nodal explants. The shoot induction was observed within 20 days of culture (Fig. 1b & c). This was in agreement with earlier report in sesame (Baskaran

and Jayabalan, 2006). The percentage of response varied with the type of growth regulator, concentration and explant type. The higher frequency (91.8) of shoot regeneration and maximum number of shoots (25.9) were observed in 2.0 mg l^{-1} BAP and 0.3 mg l^{-1} NAA combination (Table 1; Fig. 1d) than combinations of Kin and NAA. The superior activity of BAP compared to other cytokinins is reported in *Brassica campestris* (Hachey *et al.,* 1991). The shoots were transferred to elongation medium containing MS medium with BAP and GA_3 (Fig. 1e & f).

Induction of floral gradient was observed within 45 days of culture. Shoot tip explants were high response in producing flowers than nodal explants. *In vitro* flowering was achieved on MS medium supplemented with different combinations and concentrations of BAP and NAA. Higher number of flower buds (8.12) observed in shoot tip explant cultures than nodal explants (6.58) on medium containing 1.5 mg l^{-1} BAP and 0.2 mg l^{-1} NAA combination. This exogenous hormone has been added up to the endogenous contents, raising the hormonal level required for triggering the flowering (Jana and Shekhawat, 2010). Zimmerman *et al.,* (1985) opinion that interaction of carbohydrate and other nutritional factors with endogenous growth regulators can influence some biological parameters, which are altered when plant changes from juvenile to mature phase. *In vitro* flowering achieved from variety of hormone alone or in combinations (Vandana *et al.,* 1995, Sheeja and Mandal, 2003). Stephen and Jayabalan (1998) reported that NAA and GA_3 combinations highly inducted flower buds in *Coriandrum sativum*. On the other hand, auxins highly supported that *in vitro* flowering and fruit formation at low and high level of auxins (Sheeja and Mandal, 2003, Jabeen *et al.,* 2005).

The regenerated shoots were transferred to MS medium containing different concentrations and combinations of NAA (0.5 - 2.5 mg l^{-1}) and BAP (0.01 - 0.05 mg l^{-1}). Among the combinations used NAA (1.5 mg l^{-1}) and BAP (0.03 mg l^{-1}) combination was found to be best flowers and roots was also observed simultaneously (Table 2; Fig. 1g). Flowers and roots were observed within 45 days of culture. The percentage of rooting was increased with increasing concentration of NAA up to 2.0 mg l^{-1}. Rooting was declined vigorously when increasing (< 2.0 mg l^{-1}) further concentrations of NAA. Similar results were also noticed in *Helianthus annuus* (Patil *et al.,* 1993) and *Morus alba* (Naik and Lata, 1996).

The effects of cytokinins and the auxin on *in vitro* flowering were examined (Table 2). MS medium containing 1.5 mg l^{-1} NAA and 0.03 mg l^{-1} BAP resulted in uniform and synchronized flowering with 3 - 4 flowers buds per plant. The combination of NAA and BAP promoted flower buds in *Cymbidium ensifolium* (Chang and Chang, 2003) and *Vitex negundo* (Vadawale

et al., 2006). The rooted plants were transferred red soil: sand: cowdung (2:1:1v/v/v). The plants were acclimatized in the environmental plant growth chamber. The plants were established successfully in the greenhouse (Fig. 1h & i). The survival percentage was 78 %.

It concluded in this study that would be provided protocols for the high frequency of shoot regeneration from shoot tip explants and *In vitro* flowering of *S. indicum* L. This protocol can become a helpful tool for genetic transformation and reproductive growth and development studies. To our knowledge, this is the first report of the successful *in vitro* flowering via micropropagation in *S. indicum*.

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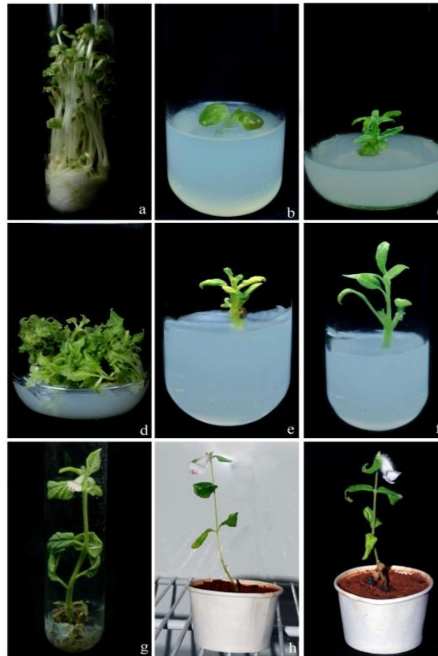

Figure 1. Multiple shoot regeneration and *in vitro* flowering from shoot tip explant of *Sesamum indicum* L. cv. SVPR - 1.
a. *In vitro* seedlings; **b.** Shoot initiation; **c.** Shoot regeneration after 2 weeks of culture; **d**. Multiple shoot after 5 weeks of culture; **e,** & f. Shoot elongation; **g.** Rooted plantlet with flower; **h.** Acclimatization in growth chamber; **i.** Hardened plant

Table 1. Effect of cytokinin and auxin combinations on multiple shoot regeneration of *Sesamum indicum* L.

MS basal medium + Growth hormones (mg l^{-1})	Shoot tip explant		Node explant	
	Percentage of response	**Mean shoot number / explant**	**Percentage of response**	**Mean shoot number / explant**
BAP + NAA				
1.0 + 0.1	70.2 ± 0.2^{de}	22.8 ± 0.5^{c}	55.8 ± 0.5^{bc}	13.8 ± 0.5^{f}
1.5 + 0.2	84.4 ± 0.4^{bc}	24.6 ± 0.6^{b}	57.8 ± 1.1^{b}	17.2 ± 0.3^{d}
2.0 + 0.3	91.8 ± 0.5^{a}	25.9 ± 0.3^{a}	49.5 ± 0.4^{d}	19.6 ± 1.4^{b}
2.5 + 0.4	86.3 ± 1.3^{b}	21.7 ± 1.0^{d}	60.3 ± 0.9^{a}	20.4 ± 0.5^{a}
3.0 + 0.5	75.4 ± 1.5^{d}	19.5 ± 1.3^{ef}	45.5 ± 0.2^{e}	19.4 ± 0.5^{bc}
Kin + NAA				
1.0 + 0.1	52.5 ± 0.4^{fg}	16.5 ± 0.4^{h}	41.5 ± 0.5^{f}	10.2 ± 0.5^{hi}
1.5 + 0.2	44.5 ± 0.3^{hi}	18.2 ± 1.2^{g}	33.2 ± 0.2^{hi}	13.4 ± 0.4^{fg}
2.0 + 0.3	55.9 ± 1.5^{f}	20.4 ± 0.5^{e}	47.4 ± 1.4^{de}	16.3 ± 0.9^{de}
2.5 + 0.4	46.5 ± 1.7^{h}	16.2 ± 1.0^{hi}	39.4 ± 0.4^{fg}	12.5 ± 0.5^{g}
3.0 + 0.5	39.4 ± 1.4^{i}	14.2 ± 1.4^{j}	35.1 ± 0.5^{h}	10.7 ± 0.6^{h}

Number of explants tested - 30, values are means ± SE of 5 replication of 5 repeated experiments.

Table 2. Influence of plant growth regulators on *in vitro* flowering maturation and root formation in *Sesamum indicum* L.

Plant growth regulators (mg l^{-1})	Percentage of rooting	Average number of roots / shoots	Root length / explants (cm)	Percentage of inflorescences/ shoot	Mean number of flower per shoot
NAA + BAP					
0.5 + 0.01	22.4 ± 1.5^{cd}	1.2 ± 0.3^{cd}	1.7 ± 0.3^{c}	06^{d}	4.56 ± 0.5^{c}
1.0 + 0.02	29.9 ± 2.0^{c}	2.9 ± 0.7^{b}	1.4 ± 0.2^{de}	28^{c}	6.97 ± 0.7^{b}
1.5 + 0.03	68.1 ± 3.0^{a}	4.8 ± 0.9^{a}	2.4 ± 0.5^{a}	83^{a}	9.48 ± 1.0^{a}
2.0 + 0.04	44.3 ± 1.5^{b}	2.2 ± 0.2^{bc}	1.9 ± 0.4^{b}	49^{b}	5.67 ± 0.4^{bc}
2.5 + 0.05	34.7 ± 1.0^{bc}	1.7 ± 0.4^{c}	1.5 ± 0.3^{d}	14^{cd}	2.65 ± 0.3^{d}

Number of explants tested - 30, values are means ± SE of 5 replication of 5 repeated experiments.