In vitro mass propagation and diverse callus orientation on *Sesamum indicum* L.-an important oil plant

P. Baskaran[*] and N. Jayabalan

Plant Biotechnology Unit, Department of Plant Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli-620 024, Tamil Nadu – India.

Baskaran, P. and Jayabalan, N. (2006). *In vitro* mass propagation and diverse callus orientation on *Sesamum indicum* L.-an important oil plant. . Journal of Agricultural Technology 2(2): 259-269.

Effect of various plant growth regulators on shoot proliferation from shoot tips and callus induction in hypocotyls and cotyledon explants of *Sesamum indicum* L. VRI1 was investigated. Cultures were maintained on the MS medium supplemented with different concentrations of growth regulators. The combination of BAP and Kin increased shoot proliferation. Proliferated shoots were rooted in NAA (8.0 μM). Rooted plants were successfully acclimatized under cool and reduced light intensity, with the survival rate reaching almost 80%. A callus was derived from hypocotyls and cotyledon segments. Hypocotyl has shown a best response in callus induction. The range between 2.2–22.6 μM 2, 4-D and 2.6–26.8 μM NAA was effective for callus formation and NAA was more effective than 2, 4-D. Root was easily differentiated when the calli were rapidly declined. BAP (8.8–44.4 μM) and rigid concentration of auxins (2.6 μM NAA, 2.8 μM IAA, 2.4 μM IBA and 2.2 μM 2, 4- D) were assessed. Cytokinins at high concentration inhibited the root development but promoted the green part formation.

Key words: cotyledon, growth regulators, hypocotyl, MS medium and shoot tip

Abbreviations: Ads - adenine sulphate, BAP - 6- benzylaminopurine, 2, 4-dichlorophenoxy acetic acid, GA_3 – gibberellic acid, IAA - indole-3-acetic acid, IBA – indole-3-butyric acid, Kin – Kinetin, MS – Murashige and Skoog's medium, NAA – α-naphthaleneacetic acid.

Introduction

Sesame (*Sesamum indicum* L.) belongs to the family *Pedaliaceae* and it is considered to be the oldest oil seed crop (Brar and Ahuja, 1979). It is widely grown in many parts of the world including India. Sesame seed is an important source of edible oil and it is also widely used as a spice. The seed contains 50-60% oil, which has excellent stability due to the presence of natural antioxidants such as sesamolin, sesamin and sesamol (Brar and Ahuja, 1979). Sesame oil is also used medicinally in Ayurvedic system of medicine.

[*]Corresponding author: P. Baskaran; e-mail: baskaran_research@yahoo.com.in

Recently, several compounds with anti-oxidant and anti-cancer properties have been isolated (Osawa, 1992; Mimura *et al*., 1994). In Sri Lanka, the leaves and roots are made into a preparation for dyeing hair and the leaf paste is used as emollient plaster (Wesis, 1971). The oil cake is rich in protein and is used as cattle feed.

The fatty acid composition of sesame oil varies considerably among the different cultivars worldwide (Yermanos, 1972; Brar, 1982). The cultivation of this crop is much restricted to poor soil therefore the yield is relatively low compared to that of other oil crops. Selection through conventional methods has not been very successful. Attempts therefore were made to obtain variants through tissue culture, since in recent years cell culture techniques have been successfully utilized to obtain useful variants in several species of economic importance (Evans *et al*., 1984).

Plant tissue culture technology has been available to the plant breeders for nearly four decades and has been extensively employed for crop improvement in several oil seed crops. However, very little information is available on sesame. It is found to be highly recalcitrant in nature. The first reported study on tissue culture in *Sesamum* was that of Lee *et al*. (1985) and George *et al*. (1987) have established tissue cultures from different parts of sesame. Kim *et al*. (1987) studied on the effect of explants and hormone combination on callus induction. Chae and Park (1987) have established herbicide tolerant cell lines without achieving the plant regeneration. Lee *et al*. (1988) and Kim and Byeon (1991) investigated the effect of growth regulators on callus induction and organogenesis from different explants of *Sesamum*. The present communication describes as an immediate and subsidiary objective we report have standardization of a reproducible micropropagation, callus induction protocol and morphogenesis in cultivated varieties of sesame like VRI1.

Materials and methods

Plant material and disinfection

The seeds of *Sesamum indicum* L.VRI1 were used as a source of material. The seeds were obtained from Oil Seed Research Station, Viruthachalam, Tamil Nadu, India. The seeds were washed with running tap water for 30 minutes. Following repeated rinsing in distilled water, liquid soap solution and distilled water the seeds were rinsed in 70% (v/v) ethanol for 30 seconds and washed three times with sterile distilled water. Surface sterilization was done with freshly prepared 0.1% (w/v) aqueous mercuric chloride for ten minutes and then rinsed five times for 10 minutes in sterile

distilled water. The seeds were then aseptically placed on MS medium supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar (Hi media, Mumbai, India) or moistened cotton in culture vessels. After 10 day old aseptic plants for explants like shoot tip for organogenesis and cotyledon and hypocotyl for callus induction.

Culture conditions

MS basal medium supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar 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. In all the experiments, the chemicals used were of analytical grade (Himedia and Sigma). The medium was dispensed into culture vessels (Borosil, India) and autoclaved at 105 kPa and 121ºC for 15 minutes. The shoot tip explants were implanted vertically and cotyledon and hypocotyl explants were implanted horizontally on the culture medium (test tubes 150 × 25 mm, containing 15 ml medium) and plugged tightly with non-absorbent cotton. All the cultures were incubated at 25±2ºC under 16 hours photoperiod of 45-50 µmol m^{-2} s^{-1} irradiance provided by cool white fluorescent tubes (Philips, India). All subsequent subcultures were done at 4 week intervals.

Shoot tip cultures

Shoot tip explants were cultured on MS medium containing 3% (w/v) sucrose and with 0.8% (w/v) agar and supplemented with different concentrations of BAP (8.8-44.4 µM) were used in combination with fixed concentration of Kin (4.6 µM) and Ads (2.7 µM). The proliferated shoots after 4 weeks were transferred to low BAP containing medium (MS + BAP (4.4 µM) + GA_3 (1.4 µM) + L-Ascorbic acid (5.6 µM) and data were recorded after 4 week in each individual treatment. Well-developed shoots from the above cultures were transferred to MS medium supplemented with NAA (8.0 µM) for root induction. Plantlets with well-developed roots were removed from the culture tubes. After washing the roots in tap water and sterile distilled water and they were transferred to plastic pots containing sterilized mixture of garden soil: sand: cowdung (2:1:1). These pots were placed for a month under cool and reduced light intensity, for hardening and acclimatization (Roussos *et al*., 1999). Experiments were set up in a Randomized Block Design (RBD) and each experiment usually had 10 replications and repeated at least three times. The treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5% probability level according to Gomez and Gomez (1976).

Hypocotyl and cotyledon culture

The hypocotyl and cotyledon explants were cultured in MS medium consisting of 3% (w/v) sucrose, 0.8% (w/v) agar and with various concentrations of 2.2-22.6 µM 2, 4-D and 2.6-26.8 µM NAA alone or in combination with BAP (8.8-44.4 µM) and NAA (2.6 µM), IAA (2.8 µM), IBA (2.4 µM) and 2, 4-D (2.2 µM) for callus induction and shoot regeneration. The percentage of callus induction and morphogenetic nature of calli were observed and statistically recorded.

Results

Shoot multiplication and regeneration

To enhance the rate of multiplication, MS basal medium was supplemented with increasing concentration of BAP (8.8-44.4 µM) in combination with rigid concentration of Kin (4.6 µM) and Ads (2.7 µM). An each concentration and combination of media was marked and set up individually. In each combination with different concentrations of BAP and Kin or Ads induced the multiple shoots. Higher concentration of BAP (17.7-44.4 µM) with Kin or Ads in the medium exposed an increase in the number of axillary and adventitious shoots as well as shoot length (Table 1). On the other hand, BAP (8.8 µM) and Kin (4.6 µM) in the medium gave a significantly lower number of shoots per explant. The number of axillary shoots also was reduced when the concentration of BAP was raised. Whitish-green compact callus was formed directly at cut ends of shoot tip segments in each medium. This callus was transpired dark brown and black and the shoots declined in long-tenure culture. Callus was removed from the shoots and then cultured on same media. The cultures were refreshed every 3 week for reduction of basal callus and phenolic exudation. Vitrification of shoots with swelled leaves and stunted internodes were produced in MS medium containing BAP (26.6-44.4) with Kin (4.6 µM) or Ads (2.7 µM) after 4 weeks culture. All cultures were transferred to low BAP containing medium [MS + BAP (4.4 µM) + GA_3 (1.4 µM) + L-Ascorbic acid (5.6 µM)] and were marked same set up concentrations and maintained for 4 weeks. From this culture, a significant shoot number and beneficial shoot length was obtained in MS medium fortified with BAP (35.5 µM) and Kin (4.6 µM). However, unvitrified, healthy (non-swelling) leaves and long internodes were produced.

At all concentrations measured, BAP (8.8 µM) and Kin (4.6 µM) gave the highest shoot length, reaching almost 6.1 cm, while the inclusion of BAP (44.4 µM), Kin (4.6 µM) and Ads (2.7 µM) in the growth medium suppressed

shoot length (Table 1). Consequently, multiple shoot was high BAP containing media followed by transfer to low BAP containing media resulted for number of desirable plantlets to rooting. All *in vitro* developed shoots were rooted in MS medium with NAA (8 µM). Well-developed shoots with roots were transferred to pots containing sterile soil and established in green house conditions. There was no detectable variation among the acclimatized plants with respect to morphological and growth characteristics. All the micropropagated plants were free from external defects.

Table 1. Effect of cytokinin combination on multiple shoot production of *Sesamum indicum* L.

MS + Growth hormones (µM)	Percentage of response	Number of shoots/ Explant (mean±SE)	Shoot length (cm) (mean±SE)
Kin (4.6) + BAP			
8.8	98.3^{b}	2.6 ± 1.26^{de}	6.1 ± 0.12^{a}
17.7	100^{a}	4.5 ± 1.44^{d}	5.7 ± 0.14^{ab}
26.6	100^{a}	6.9 ± 1.18^{bc}	5.2 ± 0.11^{b}
35.5	100^{a}	11.5 ± 1.41^{a}	4.9 ± 0.19^{bc}
44.4	95.0^{c}	7.2 ± 2.96^{b}	3.8 ± 0.16^{d}
Ads (2.7) + BAP			
8.8	91.6^{c}	3.5 ± 1.44^{e}	5.6 ± 0.20^{a}
17.7	99.3^{ab}	5.4 ± 1.05^{d}	5.3 ± 0.18^{ab}
26.6	100^{a}	7.0 ± 2.20^{bc}	5.0 ± 0.24^{b}
35.5	100^{a}	8.4 ± 2.54^{a}	4.3 ± 0.17^{c}
44.4	98.6^{b}	7.4 ± 2.12^{b}	3.4 ± 0.12^{d}
Kin (4.6)+Ads (2.7) + BAP			
8.8	100^{a}	7.0 ± 0.94^{b}	5.2 ± 0.27^{a}
17.7	100^{a}	7.6 ± 1.41^{a}	4.6 ± 0.30^{b}
26.6	100^{a}	6.7 ± 2.76^{bc}	4.0 ± 0.28^{c}
35.5	100^{a}	6.5 ± 1.56^{c}	3.6 ± 0.24^{cd}
44.4	99.3^{ab}	6.3 ± 1.71^{cd}	3.0 ± 0.18^{d}

Data correspond to average results of three independent repeated experiments; each with 10 cultures and data was recorded after 8-wk of culture.
In each column, the mean values with different alphabetical letters are significantly different from each other ($P<0.05$); comparison by DMRT.

Culture of hypocotyl and cotyledon

The hypocotyl and cotyledon explants of *Sesamum indicum* L.VRI1 cultured on MS basal medium alone did not show morphogenetic response and eventually necrosed. Profuse callus growth was observed from cut ends of the explants after 10 days of culturing on MS media supplemented with 2,4-D and

NAA. NAA was more effective than 2, 4-D. The hypocotyl explants were more sensitive and highly responsive in producing callus than the cotyledon explants (Table 2). Considerable variation was observed in the callusing response of explants, when different growth hormones were used. In the present study, all the explants produced callus with different concentration and combinations of growth regulator except NAA where root induction occurred in long-term culture. All the explants proliferated profusely with whitish friable callus on MS medium augmented with 2, 4-D and NAA (Table 2). The rate of calli was not varied in 4.5–9 µM 2, 4-D and 5.3-10.7 µM NAA. The nature of the callus differed in the medium containing 8.8–44.4 µM BAP and 2.6 µM NAA or 2.8 µM IAA or 2.4 µM IBA or 2.2 µM 2, 4-D. In the present study, hypocotyl and cotyledon explants when cultured on MS medium fortified with BAP (8.8–26.6 µM), NAA (2.6 µM), IAA (2.8 µM), IBA (2.4 µM) and 2,4-D (2.2 µM) was maximum percentage and enhanced the different nature of the callus and did not show shoot bud formation (Table 3).

Discussion

Shoot regeneration

Multiple shoot induction was attained with increase in concentration of BAP in the medium. George *et al*. (1987, 1989), Rajender Rao and Vaidyanath (1998a, b) and Baskaran and Jayabalan (2003) reported that the sesame found to be high concentration of cytokinins induced axillary bud proliferation. A combination of cytokinin has been enhanced the shoot number as well as shoot length. Similar response was observed in *Eclipta alba* (Baskaran and Jayabalan, 2005a) and *Sorghum bicolor* (Baskaran and Jayabalan, 2005b). Multiple shoot induction of sesame was attained due to the application of high concentration of BAP in culture medium but a detrimental effect in the form of vitrification with swelled leaves and stunted internodes were noticed. Similar observations were also made by Mondal *et al*. (1993) and Gaurab Gangopadhyay *et al*. (1998) in Tea, Papay and sesame micropropagation respectively. The addition of ascorbic acid to the culture medium reduced phenolic browning and prolonged survival. Similar result was reported by Scott *et al*. (1988) and Linington (1991). However, BAP was most effective synthetic cytokinin for stimulation of axillary shoot proliferation. This result was agreed in different plant systems (Bhojwani, 1980; Welander *et al*., 1989; Devi *et al*., 1994; Baskaran and Jayabalan, 2003; Baskaran and Jayabalan, 2005b). The auxin of NAA was effective for rooting in sesame. Similar result was reported by Baskaran and Jayabalan (2003) in sesame.

Table 2. Effect of auxins on callus induction and morphogenesis in *Sesamum indicum* L.

MS + Growth hormones (μM)	Callus response (%) (mean±SE)		Nature of callus
	Hypocotyl	Cotyledon	
NAA			
2.6	76.6 ± 1.18	56.7 ± 1.61	WFC
5.3	83.3 ± 1.20	59.0 ± 0.51	WFC
8.0	85.0 ± 0.72	66.0 ± 1.24	WFC
10.7	90.7 ± 1.20	70.3 ± 0.84	WFC
13.4	89.0 ± 1.10	69.7 ± 0.71	WFC
16.1	84.0 ± 1.41	67.0 ± 1.24	WFC
18.7	78.6 ± 1.31	65.3 ± 1.44	WFC
21.4	75.3 ± 1.51	57.0 ± 0.47	WFC
24.1	68.0 ± 1.60	55.5 ± 1.28	WFC
26.8	58.0 ± 1.23	48.6 ± 0.72	WFC
2,4-D			
2.2	69.7 ± 0.10	54.0 ± 1.88	WFC
4.5	71.6 ± 0.71	56.0 ± 1.69	WFC
6.7	72.0± 1.41	59.0 ± 0.51	WFC
9.0	74.5 ± 1.70	63.0 ± 1.92	WFC
11.3	73.0 ± 0.94	61.0 ± 1.41	WFC
13.5	70.3 ± 0.98	58.6 ± 2.12	WFC
15.8	66.8 ± 1.18	58.0 ± 1.24	WFC
18.0	62.6 ± 1.50	55.5 ± 2.20	WFC
20.3	59.7 ± 0.10	54.1 ± 1.92	WFC
22.6	52.0 ± 1.24	50.6 ± 1.18	WFC

WFC – Whitish Friable Callus

Data correspond to average results of three independent repeated experiments; each with 10 cultures and data was recorded after 4-wk of culture.

Callus cultures

In this study, higher rate of callus was produced in the hypocotyl explants. Similar results were reported by George *et al*. (1987), Lee *et al*. (1988) and Rajender Rao *et al*. (1997). The different concentrations of 2, 4-D and combination of 2, 4-D and BAP produced various nature of callus but the callus of NAA promoted roots in long term culture. Similar result was observed by George *et al*. (1987) and Rajender Rao *et al*. (1997). There were

Table 3. Effect of auxins and BAP on callus induction and morphogenesis in *Sesamum indicum* L.

MS + Growth hormones (μM)	Callus response (%) (mean±SE)		Nature of callus
	Hypocotyl	Cotyledon	
NAA (2.6) + BAP			
8.8	80.0 ± 0.47	74.3 ± 0.73	WGF
17.6	83.0 ± 0.72	71.6 ± 1.44	GCC
26.6	81.0 ± 0.94	73.0 ± 0.94	GCC
35.5	77.6 ± 0.72	69.3 ± 1.90	GFC
44.4	70.6 ± 1.18	65.7 ± 1.90	GFC
2,4-D (2.2) + BAP			
8.8	80.0 ± 0.94	77.3 ± 2.22	WFC
17.6	82.6 ± 0.80	73.0 ± 2.05	WFC
26.6	79.3 ± 1.44	73.6 ± 1.90	WGF
35.5	72.0 ± 1.41	71.7 ± 1.10	GFC
44.4	68.7 ± 1.90	64.6 ± 2.12	GFC
IBA (2.4) + BAP			
8.8	73.6 ± 1.10	67.0 ± 1.69	WFC
17.6	75.7 ± 2.12	64.0 ± 1.90	GCC
26.6	67.5 ± 2.88	62.3 ± 2.76	GCC
35.5	63.0 ± 1.95	57.3 ± 2.68	WGC
44.4	60.0 ± 3.85	53.7 ± 1.90	WGC
IAA (2.8) + BAP			
8.8	71.0 ± 2.62	67.1 ± 3.90	WFC
17.6	73.0 ± 2.16	69.7 ± 3.78	WGC
26.6	69.3 ± 3.31	62.6 ± 2.68	WGC
35.5	61.7 ± 2.22	58.6 ± 4.00	GCC
44.4	55.0 ± 2.94	51.7 ± 1.78	GCC

WGC – Whitish Green Compact, GCC – Green Compact Callus, WFC – Whitish Friable Callus, WGF – Whitish Green Friable, GFC - Greenish Friable Callus.
Data correspond to average results of three independent repeated experiments; each with 10 cultures and data was recorded after 4-wk of culture.

no differences in callus induction between 4.5–9 μM 2, 4-D and 5.3–10.7 μM NAA. Similar result was observed by Kim *et al*. (1987). The nature of the callus maintained same when subcultured on the same media but differed when the combination was altered during subculture. Similar work was done by Varisai Mohamed and Jayabalan, (1996). In each combination, auxins and cytokinin stated various nature of callus. However, they are not gave shoot bud

formation. The combination of different auxins and cytokinin was produced greenish compact globular callus (Rajender Rao *et al*., 1997). Taskin *et al*. (1997) reported that in sesame where produced only on BAP and NAA combination.

In conclusion, in the present investigation, we report that the *S. indicum* shoot tip explants cultured for 2 weeks in MS medium supplemented with high BAP then cultured in MS medium without growth regulators for 8 weeks, could regenerate shoots at healthy, unvitrified shoots/explant. This protocol can provide an alternative one for the rapid micropropagation of *S. indicum*. The present study on callus induction is a highly repeatable protocol and is relatively efficient and forms the basis for regeneration of plant, somatic embryogenesis, and somaclonal variant and suitable for further biotechnological applications like genetic transformation leading to the production of transgenic plants with a desired trait.

Acknowledgements

The first author is thankful to Dr.V.T. Sridharan and Dr.Patrick Gomez for their critical appraisal of the paper and to N. Ponnusamy and N. Kamaraj for giving the financial support. The author wishes to thank Dr. Bhupathi and G.G. Gideon for their helpful discussion and critical reading of the manuscript.

References

Baskaran, P. and Jayabalan, N. (2005a). An efficient micropropagation system for *Eclipta alba* – a valuable medicinal herb. In Vitro Cellular Developmental Biology Plant 41: 532-539.

Baskaran, P. and Jayabalan, N. (2005b). An efficient plant regeneration system for *Sorghum bicolor* – a valuable major cereal crop. Plant Biotechnology 7: 247-257.

Baskaran, P. and Jayabalan, N. (2003). Effect of growth regulators on multiple shoot production in *Sesamum indicum* L. Indian Journal of Plant Physiology (special issue) : 639-642.

Bhojwani, S.S. (1980). Micropropagation method for a hybrid willow (*Salix matsudana x alba* NZ-1002). New Zealand Journal of Botany 18: 209-214.

Brar, G.S. and Ahuja, K.L. (1979). Sesame. In: Its culture, genetics, breeding and biochemistry. Eds. C.P. Malik. Annual Review of Plant Science, Kalyani publishers, New Delhi, India: 245–313.

Brar, G.S. (1982). Variations and correlations in oil content and fatty acid composition of sesame. Indian Journal of Agriculture Science 52: 434-439.

Chae, Y.A., Park, S.K. and Anand, I.J. (1987). Selection *in vitro* for herbicide tolerant cell lines of *Sesamum indicum* 2: Selection of herbicide tolerant calli and plant regeneration. Korean Journal of Plant Breeding 19: 75-80.

Devi, Y.S., Mukherjee, B.B. and Gupta, S. (1994). Rapid cloning of elite teak (*Tectona grandis* Linn.) by *in vitro* multiple shoot production. Indian Journal of Experimental Biology 32: 668-671.

Evans, D.A., Sharp, W.R. and Bravo, J.E. (1984). Cell culture methods for crop improvement. In: In Handbook of plant cell culture: crop species. Eds. W.R. Sharp, D.A. Evans, P.V. Ammirato and Yamada, Y. Macmillan Publishing Co, New York. 47-68.

Gaurab Gangopadhyay, Poddar, R. and Gupta, S. (1998). Micropropagation of sesame (*Sesamum indicum* L.) by *in vitro* multiple shoot production for nodal explant. Phytomorphology 48: 83-90.

George, L., Bapat, V.A. and Rao, P.S. (1987). *In vitro* multiplication of sesame (*Sesamum indicum* L.) through Tissue culture. Annals of Botany 60: 17-21.

George, L., Bapat, V.A. and Rao, P.S. (1989). Plant regeneration *in vitro* in different cultivars of sesame (*Sesamum indicum* L.). Proceeding of Indian Academy Science 99: 135-137.

Gomez, K.A. and Gomez, K.A. (1976). Statistical procedures for agricultural research with emphasis on rice. International Rice Research Institute. Los Banos, Philippines. Iddagoda, N., Kateva, N. and Butenko, R.G. (1988). *In vitro* clonal micropropagation of tea (*Camellia sinensis* L.). Indian Journal of Plant Physiology 31: 1-10.

Kim, H.Y. and Byeon, G.H. (1991). Effect of growth regulators on organ cultures of sesame. Sub Tropical Agriculture 8: 93-103.

Kim, M.K., Park, S.K. and Chae, Y.A. (1987). Selection *in vitro* for herbicide tolerant cell lines of *Sesamum indicum*: Effects of explants and hormone combinations on callus induction. Korean Journal of Plant Breeding 19: 70-94.

Lee, J.I., Park, Y.H., Park, Y.S. and Kim, B.G. (1985). Propagation of sesame (*S. indicum* L.) through shoot tip culture. Korean Journal of Breeding 17: 367-372.

Lee, S.Y., Kim, H.S., Lee, Y.T. and Park, C.H. (1988). Effect of growth regulators, cold pretreatment and genotype in anther culture of sesame (*Sesamum indicum* L.). Research reports of the rural development administration biotechnology 30: 74-79.

Linington, I.M. (1991). In vitro propagation of *Dipterocarpus alatus* and *Dipterocarpus intricatus.* Plant Cell Tissue and Organ Culture 27: 89-93.

Mimura, A. (1994). Antioxidative and anticancer components produced by cell culture of sesame. ACS symposium – series (USA): 547.

Mondal, M., Gupta, S. and Mukherjee, B.B. (1993). Effects of 6-Benzyl aminopurine (BAP) and sucrose on chlorophyll content in leaves of *in vitro* cloned papaya, *Carica papaya* var. Honey dew. Indian Journal of Experimental Biology 31: 338-341.

Osawa, T. (1992). Phenolic antioxidants in dietary plants as anti mutangenes. ACS symposium-series (USA): 507.

Rajender Rao, K. and Vaidyanath, K. (1997). Callus induction and morphogenesis in sesame (*Sesamum indicum* L.). Advances in Plant Sciences 10: 21-26

Rajender Rao, K., Vaidyanath, K. (1998a.). Somatic embryogenesis and regeneration from different explants of *Sesamum indicum* L. National Symp. Commercial aspects of plant tissue culture, Molecular Biology and Medicinal Plant Biotechnology, Jamia Hamdard, New Delhi.

Rajender Rao, K. and Vaidyanath, K. (1998b). Synseed and micropropagation in Sesamum. National Symposium on “Perspectives in biotechnology” held at the Department of botany, Kakatiya University Warangal, India.

Roussos, P.A., Tolia-Morioli, A., Pontikis, C.A. and Kotsias, D. (1999) Rapid multiplication of Jojoba seedlings by *in vitro* culture. Plant Cell Tissue and Organ Culture 57: 133-137.

Taskin, Kemal Melih, Turgut and Kenan (1997). *In vitro* regeneration of Sesame (*Sesamum indicum* L.). Turkish Journal of Botany 21: 15-18.

Varisai Mohamed, S. and Jayabalan, N. (1996). A protocol for horsegram (*Macrotyloma uniflorum* Lam. Verdc) callus induction. Israel Journal of Plant Science 44: 142-145.

Welander, M., Welander, N.T. and Brackman, A.S. (1989). Regulation of *in vitro* shoot multiplication in syringa, Alnus and Malus by different carbon sources. Horticulture Science 64: 361-366.

Wesis, E.A. (1971). Castor, Sesame and Sunflower. Leonard Hill, London: 31-525.

Yermanos, D.M. (1972). Oil content and composition of the seed in the world collection of sesame introductions. Journal of American Oil Chemical Society 49: 20-23.

(Received 23 July 2006; accepted 15 October 2006)