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MICROPROPAGATION OF *SESAMUM INDICUM* VARIETY GUJARAT-1

ANUPAMA GOYAL, SHIKHA ROY and ASHWANI KUMAR
Department of Botony, University of Rajasthan, Jaipur-302004, India.

Excise plant parts like hypocotyl, cotyledon and shoot apex when cultured on MS medium containing auxins, IAA, IBA, NAA, 2,4-D developed callus. Embryogenic callus was also obtained. Shoot apex resulted in bud proliferation when it was cultured on MS alongwith BAP.

Keywords : Embryos; Multiple leafy shoots; Murashige and Skoog's medium; *S. indicum.*

Introduction

India is the largest Sesame growing country. The chief Sesame growing countries lie in the tropics as well as in the temperates. They are China, India, Burma, African (Sudan, Nigeria, Uganda) and Latin American countries (particularly Mexico). In India Sesame is cultivated in Uttar Pradesh, Madhya Pradesh, Rajasthan, Maharashtra, Andhra Pradesh and Tamil Nadu.

Materials and Methods

Certified seeds of *Sesamum indicum* variety Gujarat-1 were obtained. They were surface sterilized with 0.1% mercuric chloride for 1-2 min. The seeds were then thoroughly washed with sterile distilled water for 5 minutes and aseptically germinated on half strength MS medium or on filter paper bridges in test tubes. Explants like cotyledon, hypocotyl, shoot apex of around 0.8-1 cm size were excised from 7 days old aseptically grown seedlings. Explants were inoculated on MS basal medium[1] containing 30mg/l sucrose and 8mg/l of agar. Different growth regulators were added to MS medium on the requirement of the experiment. The pH of the medium was adjusted to 5.8 before autoclaving. All the cultures were incubated at 25±2°C under 18:6 hr light and dark period. During embryo formation cultures were kept in continuous dark. Experiments were repeated at least twice to confirm the results. The callus obtained on combination of NAA and BAP was subcultured after 2 weeks.

Results and Discussion

Explants did not show callusing or rooting in the absence of growth regulators on MS medium. Callus was initiated from hypocotyl, cotyledon, shoot apex within 14 days on MS medium supplemented with different growth regulators at various concentrations (Table 1). Maximum nodulated callus was recorded on MS medium containing NAA (6mg/1) + bAP (2mg/1) from hypocotyl region (Fig.1). Auxins like IAA, NAA, IBA and 2,4-D were less favourable for callus initiation and its growth. 2,4-D induced fragile callus from the hypocotyl region and shoot apex. *In vitro* multiplication of Sesame (*Sesamum indicum*) through tissue culture has been achieved[2]. Induction of the callus from the mesophyll protoplasts of *Brassica campestris* an oilseed crop has been reported.[3] Callus obtained on NAA (6mg/1) when subcultured on BAP (5 mg/1) and coconut milk (10%) showed formation of embryogenic buds and also

Table 1. Responses of auxins and cytokinins on different explants of *Sesamum indicum* Variety Gujarat-1.

Medium/growth regulators (mg/l)	Hypocotyl	Cotyledon	Shoot apex
MS	NR	NR	NR
MS+IAA (0.5)	R	R	R
MS+IAA (3.0)	$C^{+}R^{+}$	$C^{+}R$	$C^{+}R$
MS+IBA (5.0)	$C^{+}R$	$C^{+}R$	C^{+}
MS+NAA (0.5)	C^{++}	C^{++}	C^{++}
MS+NAA (5.0)	C^{++}	C^{++}	C^{+++}
MS+2, 4-D (1.0)	C^{++}	C^{++}	C^{++}
MS+BAP (1.0)	C^{+}	C^{+}	C^{++}
MS+BAP (4.0)	Swelling	C^{++}	C^{++}
MS+BAP (5.0)+Coconut milk (10%)	C^{+++} embryogenic	C^{+++}	C^{+++} embryogenic+ leafy shoots
MS+Kn (1.0)	Swelling	Enlargement	C^{+}
MS+BAP (5.0)+IBA (0.5)	R^{++}	C^{+}	C^{++} R^{++}
MS+NAA (6.0)+BAP(2.0)	C^{++++}	-	C^{++}
MS+BAP (1.0)+Kn(1.0)	C^{+++}	increase in size of the cotyledonary explants	C^{+++}
MS+NAA (6.0)+BAP (2.0)+ coconut milk (1%)	C^{++++}	-	C^{+++}

NR - No response
C^{+} - Callus very poor
C^{++} - Callus poor
C^{+++} - Callus good
C^{++++} - Callus excellent.

Table 2. Regeneration of shoot apex of *Sesamum indicum* variety Gujarat-1.

Medium/growth regulators(mg/1)	No. of shoot buds proliferation
MS+BAP (5)	3-4
MS+BAP(9)+adenine sulphate(40)	10-15
MS+BAP(10-12)+adenine sulphate(40)	2-3
MS+BAP(9)+coconut milk (1.5%)	3

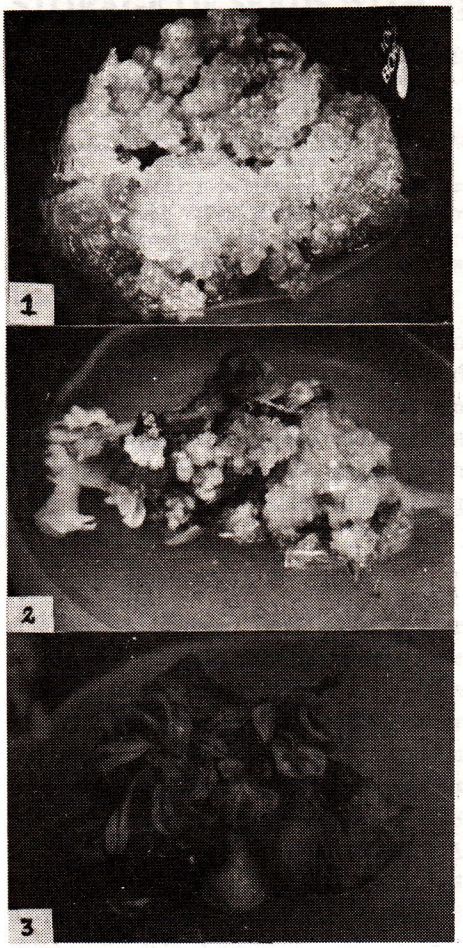

Fig. 1 Callus of *Sesamum indicum* derived from hypocotyl.

Fig. 2 Formation of embryoids and leafy shoots in the callus.

Fig. 3 Formation of multiple shoot buds from apical shoot explant.

formation of leafy shoots (Fig.2). Somatic embryogenesis and plant regeneration from cultured zygotic embryos of Soybean *(Glycine max* L. Merr.) has been reported.[4] Media supplemented with K (1.0mg/1) singly or in combination with BAP (1.0 mg/1) suppressed callus formation from cotyledonary explants, whereas shoot apex and hypocotyl explants induced callusing. On MS supplemented with various auxins and auxins in combination with cytokinins in the right amounts callusing was induced. This is due to the stimulation of all division and subsequent callus growth with auxin present appear to be characteristics of all cytokinins. Medium supplemented with NAA (6.0 mg/1) & BAP (2.0 mg/1) resulted in callus formation only and it suppressed bud proliferation.This is due to the inhibiting effect of auxin on bud proliferation as auxin promotes apical dominance and thus causes the lateral buds to become inactive. On transfer of the callus on an auxin free MS medium, lateral buds become active and so embryogenic buds and leafy shoots differentiated from the callus.

The best callus obtained on NAA (6mg/1) and BAP (2mg/1) was brownish and nodulated with root primordia. This brown calllus becomes green when subcultured on MS+NAA (6mg/1)+BAP (2.0 mg/1)+coconut milk (1%).

Apical shoot explants showed multiple bud formation. The number of bud proliferation depended on the amount of BAP of the culture medium MS+BAP (9mg/1)+adenine sulfate(40mg/1) resulted in maximum shoot bud induction (10-15 or more in some cases) (Fig.3). The number of buds decreased on high dose of BAP (10-12 mg/1). Regeneration of Soybean *(Glycine*

max L. Merr.) from cultured primary leaf tissue has been studied.[5] Formation of shoot buds on BAP took place as cytokinins have stimulating influence on lateral bud growth. However,the medium MS+BAP (9mg/1) which induced maximum shoot bud proliferation showed a negative response when coconut milk was added to it (Table 2).

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