

DIRECT SHOOT BUD DIFFERENTIATION FROM DIFFERENT EXPLANTS OF *IN VITRO* REGENERATED SHOOTS IN SESAME

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Explant taken from *in vitro* regenerated shoots of *Sesamum indicum* cv. pratap showed regeneration potential. When they were separated and kept on different concentrations of BAP (1.0, 2.0, 5.0 and 10.0 mg/l) induced number of shoot buds which developed into well developed plantlets.

Keywords : Regeneration, *in vitro* explants.

Sesame oil is extensively used in southern part of India for cooking purposes. This oil also possess medicinal value so it is applied to skin of the infants¹. It is also used to prevent falling of hair after mixing with some other contents². There are very few reports on tissue culture of this crop. Direct regeneration was reported in sesame by George *et al.*³ using pulse treatment of seeds.

Source of *in vitro* explants : Seeds of *Sesamum indicum* cv. pratap were sterilized with 0.1% aqueous solution of Mercuric chloride for 4-5 minute and thoroughly washed with sterilized distilled water. These seeds were soaked in MS liquid medium supplemented with BAP (100 mg/l) for 72 hrs. Seeds were germinated on BAP (10.0 mg/l) containing medium agarified with 0.8% of Agar. Cotyledonary node with shoot tip were excised after 7-8 days of germination and transferred on same concentration of the BAP for shoot bud induction. Well developed shoots were observed at cotyledonary region of the explant. Which were used as a source of *in vitro* explants viz., leaf, node and shoot tip for further culture work.

First, Second and Third leaf, node and shoot tip were excised from regenerated shoots and kept on various concentration of BAP and Kn (1.0, 2.0, 5.0, 10.0 mg/l). Kinetin supplemented medium was totally found incapable to induce any response. While BAP (1.0-10.0 mg/l) supplemented medium

induced various number of shoot buds with different explants. Mid rib region of leaf explant gave rise about 1-3 shoots on BAP (2.0 and 10.0 mg/l) containing medium. The shoot buds were poor and small on 2.0 mg/l of BAP while more number of healthy shoots were recorded on 10.0 mg/l of BAP containing medium (Table-1) in 20% cultured explants. In other cases instead of mid rib portion shoot could be induced from basal segments of immature as well as mature leaves⁴ by using MS medium supplemented with BAP and NAA. Van Altvorst *et al.*⁵ also reported shoot regeneration from leaf segments of *Dianthus caryophyllus*. In present study irrespective to basal part of the leaf segment entire lamina was found highly responsive to induce shoots on medium supplemented with BAP (10.0 mg/l) alongwith amino acids (1.0-5.0 mg/l).

About 5-8 shoots were observed with L-glutamine (5.0 mg/l) + BAP (10.0 mg/l) and about 5-6 shoots with L-serine (5.0 mg/l) + BAP (10.0 mg/l) in 25-35% cultures respectively (Table-1). Jethwani and Kothari⁶ also reported shoot regeneration from leaf derived callus of *Dianthus chinensis* by using PAA in combination of BAP supplemented medium. In our studies only BAP gave satisfactory response with various concentrations of amino acids.

Nodal segments and shoot tip explants were also reported good for direct shoot bud

Table : 1 Response of different *explants of *Sesamum indicum* cv. pratap cultured on MS medium supplemented with various concentrations of BAP alone or in combination of different amino acids after 6 weeks of culture.

BAP with AA mg/l	Leaf		Nodal segment		Shoot tip	
	% Response	Ave no. of shoots/ explant ±SD	% Response	Ave no. of shoots/ explant ±SD	% Response	Ave no. of shoots/ explant ±SD
1.0	-	-	50	1.00±0.00	30	1.00±0.00
2.0	20	1.00±0.00	40	1.00±0.00	45	1.00±0.00
5.0	-	-	25	3.50±0.83	40	1.00±0.00
10.0	20	2.50±0.70	25	3.75±0.95	20	3.50±0.57
BAPL-Glu						
10.0 1.0	-	-	20	1.00±0.00	30	2.20±0.83
10.0 3.0	25	3.24±1.25	25	2.00±0.00	25	4.00±1.58
10.0 5.0	35	6.00±1.50	30	2.00±0.00	25	7.25±0.95
BAPL-Ser						
10.0 1.0	30	2.00±0.00	10	1.00±0.00	20	1.00±0.00
10.0 3.1	20	5.64±2.54	30	3.05±0.89	30	3.83±1.47
10.0 5.0	25	5.75±0.95	20	3.75±0.95	30	4.60±0.89

* Explants excised from 7-8 week old *in vitro* regenerated shoots growing on media containing BAP (10.0 mg/l) in combination of glutamine (5.0mg/l).

AA = Amino acid

L-Glu = L-Glutamine

L-Ser = L-Serine

SD = Standard deviation

induction. In nodes and shoot tips a differentiated meristem zone is already present, which gets induced by BAP and new vegetative shoot buds are formed. These results are similar to the observations made by earlier workers⁷⁻¹¹. Here in case of sesame both of these explants showed regeneration potential on different concentration of BAP alone or in combination of amino acids (Table-1). In incorporation of L-glutamine did not improve regeneration response in case of nodal segments. While L-serine (5.0 mg/l) in combination of BAP (10.0 mg/l) gave rise about 3-5 shoots after 8 weeks of culture. Incorporation of L-glutamine and L-serine found good to induce regeneration with shoot tip explants about 7-8 shoots were obtained in 25% cultured explant (Table-1). *In vitro* nodal segments and shoot tip explants were also used as an explant in different trees¹², but shoot tip explant was found less responsive than nodal explants. Similar observations were made by Pattnaik *et al*¹³, but in our case shoot tip explants were found more responsive than nodal explants. *In vitro* shoot tips were also reported more competent than nodal and internodal segments in terms of number of shoots in *B.alboglabra*¹⁴. Same were observed in *Brassica juncea*¹⁵. Probably the reason may be that apical meristem of these plants were more responsive than axillary one.

In this experiment incorporation of amino acids like L-glutamine and L-serine in regeneration medium improved regeneration potential. According to earlier reports amino acids are known to be stimulatory as well as inhibitory for growth of various systems out of these proline and L-serine are known as inducer of mitosis being a part of cell glucoprotein, extension¹⁶⁻¹⁷ and perform specific action on somatic embryogenesis in carrot¹⁸.

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