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IN VITRO CALLUS CULTURE AND PLANT REGENERATION FROM DIFFERENT EXPLANTS OF *LYCOPERSICON ESCULENTUM* MILL.

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Callus induction from hypocotyl and cotyledon explants of *L.esculentum* Mill. cv PKM 1 were cultured on MS medium supplemented with different concentrations of IAA, NAA, in combination with BAP and KIN (1.0 mg/1). The Frequency of callus induction increased with increasing concentrations of auxins, the optimal levels being 2.0 mg/l of IAA/NAA and 1.0 mg/l of BAP/KIN. Among the explants hypocotyl was found to be more efficient in producing callus. Shoots were induced from callus cultures of these explants on the same medium. Maximum number of multiple shoots were obtained from hypocotyl derived callus. The regenerated shoots were rooted on MS medium containing 2.0 mg/l IBA, and were successfully hardened and transferred to the field.

Keywords : Callus; Cotyledon; Culture; Hypocotyl; *Lycopersicon esculentum* Mill.

Introduction

Tomato is an important vegetable crop grown all over the world. It belongs to the family *solanaceae.* Crop improvement through *in vitro* techniques provide unique possibilities for over coming the barriers of incompatibility existing between remote species. Earlier works on *in vitro* culture have been carried out in tomato by several workers[2-5]. This paper describes an efficient protocol for callus induction and plant regeneration from hypocotyl and cotyledon cultures of *L. esculentum* Mill.

Materials and Method

Seeds of *L. esculentum* Mill. cv PKM 1 were obtained from Anna farm, Department of Agriculture, Government of Tamil Nadu, Kudumianmalai, Pudukkottai. Seeds were washed in running tap water to remove surface adhered particles and then with soap solution for 5 minutes and in 70% (w/v) ethanol for 20 seconds. Seeds were rinsed in distilled water 3 times and surface sterlised with 0.1% (w/v) mercuric chloride for 5 minutes and then rinsed in sterile distilled water. These seeds were inoculated in to test tubes containing moistened cotton for germination under dark. The tubes were then transferred to a growth chamber at 25 ± 2°C with 16h/8h photoperiod for further growth. The explants were selected from 21 days old seedlings and cultured on MS medium[6] containing 3% (w/v) sucrose, 0.7% (w/v) agar, and supplimented with different concentrations of auxins (IAA, NAA) in combination with BAP/KIN, 1.0 mg/1. For rooting IBA (0.5 - 3.0 mg/1) were experimented on MS Basal medium. The pH of the medium was adjusted to 5.8 before it was autoclaved at 121°C for 15 minutes. The cultures were kept in alternating 16h (2500 lux) light and 8h dark at 25 ± 2°C. Observations were recorded after three weeks of culture.

Results and Discussion

Callus induction : Hypocotyl and cotyledon explants produced callus efficiently on the MS medium supplimented with IAA/NAA (0.5 - 3.0 mg/l) and BAP/KIN (1.0 mg/1). Significant callus formation was observed with in a week at the cut ends and surface of the explants. Higher frequency of callus induction was achieved from hypocotyl explants followed by cotyledonary explants. Compact green nodular callus was observed

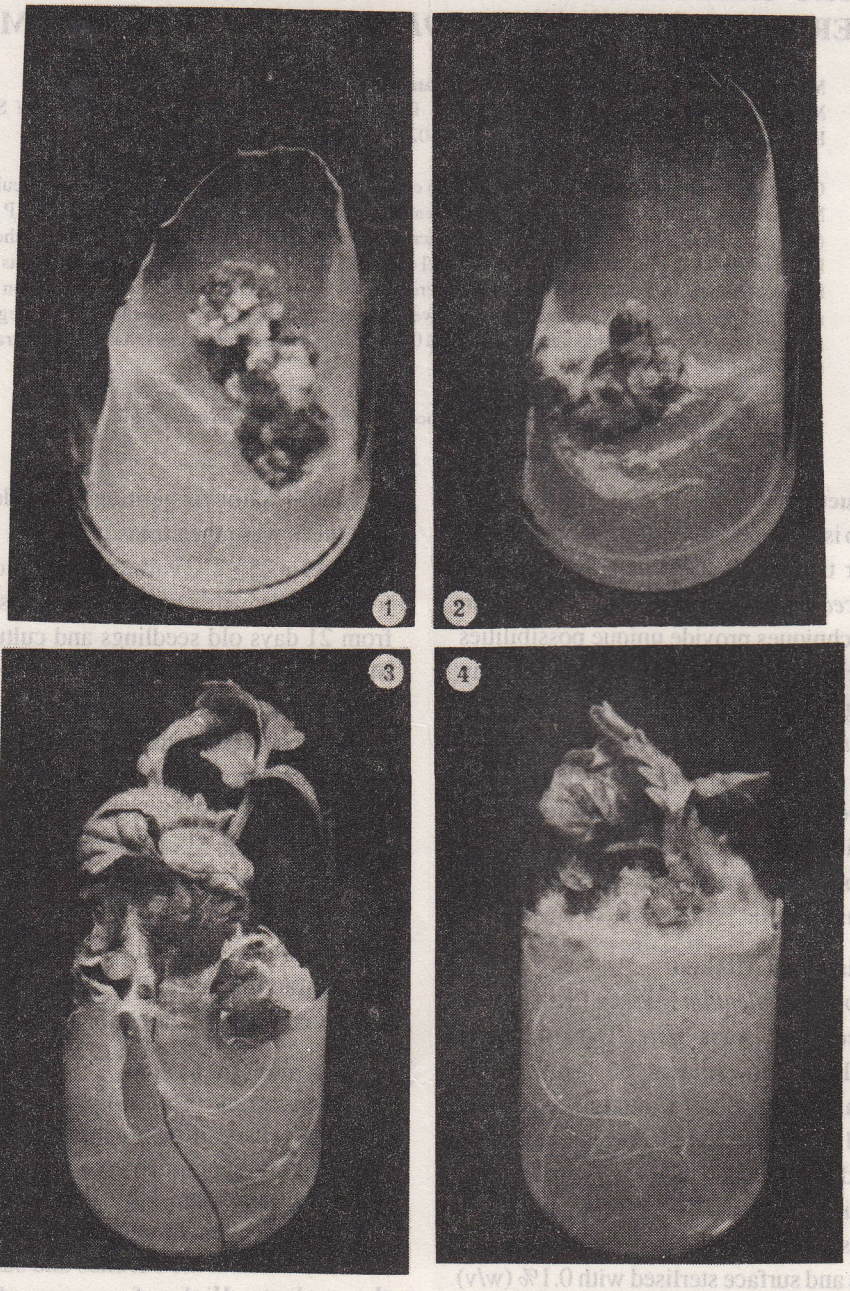

Fig.1. Callus Proliferation from Hypocotyl explants; Fig.2. Callus Proliferation from Cotyledonary explants; Fig.3. Plant regeneration from Hypocotyl derived callus; Fig.4. Plant regeneration from Cotyledonary derived callus.

Table 1. Comparative effect of various concentrations of IAA and NAA in combination with 1.0 mg/l BAP on shoot regeneration cotyledon explants of *L.esculentum* Mill. (Mean ± SD)

Growth Hormones (mg/l)	Hypocotyl				Cotyledon			
	% of callusing	% of shoot Proliferation	No. of multiple shoots	Shoot length	% of callusing	% of Shoot Proliferation	No. of multiple Shoots	Shoot length
IAA								
0.5	40.0	18.3	1.33 ± 0.57	4.06 ± 0.55	36.0	15.0	1.00 ± 0.00	3.80 ± 0.10
1.0	56.6	20.0	3.66 ± 0.57	5.13 ± 0.20	55.0	18.3	2.6 6± 0.57	4.86 ± 0.35
1.5	68.3	31.6	5.66 ± 0.57	6.33 ± 0.56	65.0	30.0	4.66 ± 0.57	5.93 ± 0.25
2.0	91.6	66.6	10.3 ± 0.57	9.40 ± 0.52	86.6	58.3	9.00 ± 0.00	8.36 ± 0.47
2.5	85.0	58.3	7.00 ± 1.00	7.03 ± 0.75	73.3	53.3	6.00 ± 1.00	6.66 ± 0.61
3.0	55.0	35.0	4.33 ± 0.57	5.86 ± 0.30	48.3	33.3	3.33 ± 0.57	5.03 ± 0.15
NAA								
0.5	38.3	13.3	1.00 ± 1.00	3.83 ± 0.47	35.0	11.6	0.33 ± 0.57	3.47 ± 0.25
1.0	55.0	15.0	2.60 ± 0.57	4.86 ± 0.35	50.0	13.3	1.66 ± 0.57	4.06 ± 0.37
1.5	66.6	30.0	5.00 ± 1.00	6.06 ± 0.20	63.3	26.6	3.33 ± 0.57	5.76 ± 0.15
2.0	86.6	61.6	8.66 ± 0.57	8.70 ± 0.52	81.6	56.6	7.66 ± 0.57	7.80 ± 0.10
2.5	63.3	48.3	5.66 ± 0.57	6.66 ± 0.41	58.3	48.3	5.66 ± 0.57	5.50 ± 0.26
3.0	40.0	33.3	2.33 0.57	5.53 ± 0.58	40.0	30.3	1.33 ± 0.57	4.70 ± 0.36

Each value represents 20 replicates and each experiment was repeated atleast thrice.

Table 2. Comparative effect of various concentrations of IAA and NAA in combination with 1.0 mg/l KN on shoot regeneration cotyledon explants of *L.esculentum* Mill. (Mean ± SD)

Growth Hormones (mg/l)	Hypocotyl				Cotyledon			
	% of callusing	% of shoot Proliferation	No. of multiple shoots	Shoot length	% of callusing	% of Shoot Poliferation	No. of multiple shoot	Shoot length
IAA								
0.5	38.3	16.6	1.00 ± 1.00	3.86 ± 0.55	40.0	18.3	1.33 ± 0.57	3.90 ± 0.10
1.0	55.0	18.3	3.33 ± 0.57	4.86 ± 0.32	56.6	20.0	3.66 ± 0.57	4.96 ± 0.25
1.5	66.6	30.0	5.00 ± 1.00	6.20 ± 0.36	68.3	31.6	5.33 ± 0.57	6.13 ± 0.32
2.0	90.0	65.0	10.0 ± 1.00	9.16 ± 0.65	88.3	66.6	10.00 ± 1.00	8.76 ± 0.25
2.5	81.6	56.6	7.00 ± 1.00	6.96 ± 0.61	78.3	55.0	7.33 ± 1.15	6.90 ± 0.26
3.0	51.6	31.6	4.33 ± 0.57	5.70 ± 0.43	50.0	33.3	4.66 ± 0.57	5.03 ± 0.15
NAA								
0.5	40.0	11.6	0.33 ± 0.57	3.80 ± 0.43	41.6	15.0	1.33 ± 0.57	3.56 ± 0.25
1.0	53.3	16.6	2.33 ± 0.57	4.66 ± 0.37	55.0	20.0	2.66 ± 0.57	4.23 ± 0.32
1.5	66.6	26.6	4.66 ± 1.52	5.96 ± 0.25	68.3	30.0	5.00 ± 1.00	6.06 ± 0.15
2.0	85.0	60.0	8.33 ± 0.57	8.50 ± 0.50	88.3	63.3	10.33 ± 1.52	9.63 ± 0.47
2.5	61.6	43.3	5.33 ± 0.57	6.46 ± 0.45	60.0	45.0	5.66 ± 0.57	5.86 ± 0.81
3.0	35.0	31.6	2.00 ± 1.00	5.36 ± 0.55	38.3	33.3	2.66 ± 1.15	4.66 ± 0.32

Each value represents 20 replicates and each experiment was repeated atleast thrice.

Table 3. Effect of IBA on root induction in regenerated plantlets.

Growth Hormones mg/l	% of Root induction from shoots		Average No. of Roots/Shoot (Mean ± SD)	
	Hypocotyl	Cotyledon	Hypocotyl	Cotyledon
0.5	28.3	26.6	5.00 ± 1.00	4.66 ± 0.57
1.0	35.0	30.0	6.33 ± 0.57	5.66 ± 0.57
1.5	46.6	45.0	7.00 ± 1.00	7.00 ± 1.00
2.0	78.3	75.0	11.60 ± 0.57	11.33 ± 0.57
2.5	58.3	53.3	9.00 ± 0.57	8.66 ± 0.57
3.0	46.6	43.3	6.66 ± 0.57	6.00 ± 1.00

Each value represents 20 replicates and each experiment was repeated atleast thrice.

in hypocotyl and cotyledon explants with in three weeks (Fig. 1 & 2). The combination of IAA 2.0 mg/l and BAP/KIN 1.0 mg/l produced higher frequency of callus than the NAA 2.0 mg/1 and BAP/KIN 1.0 mg/1 combination (Tables 1 & 2). Among the different concentrations of auxins used for callus induction, 2.0 mg/1 was found to be optimum, while increasing the auxin concentrations above this level reduced the callusing frequency and induced rhizogenesis. Moreover the combination of higher auxin and lower cytokinin was found to be more effective for callus induction. Similar results were obtained in tomato by Gunay and Rao[7].

Multiple shoot regeneration: Multiple shoot regeneration occured from hypocotyl and cotyledon explants on the same medium within 5 weeks. Maximum frequency of multiple shoots were achieved from hypocotyl derived callus followed by cotyledonary callus on media containing IAA/NAA (2.0 mg/l) and BAP 1.0 mg/l, (Fig. 3) whereas higher frequency of multiple shoot induction was achieved from cotyledonary callus (Fig. 4) on the media containing IAA/NAA 2.0 mg/1 and KIN 1.0 mg/1 (Tables 1 & 2) produced. Gunay and Rao[7] reported that the optimum plant regeneration was observed on MS medium with IAA 0.5 mg/1 and BAP 2.0 mg/1 in hypocotyl and cotyledon explants, where as Padmanabhan *et al.*,[1] obtained shoots on MS medium containing 0.5 mg/1 IAA and 3.0 mg/l KIN. Higher frequency of multiple shoot proliferation was observed on MS medium containing IAA/NAA 2.0 mg/l and BAP/KIN 1.0 mg/1.

Root induction: Well developed shoots were cultured on MS medium containing IBA (0.5 -3.0 mg/l) for root induction (Table 3). Rooting percentage was observed high from hypocotyl followed by cotyledon derived shoots. Roots emerged from the cut end of the shoots within 7 days. Maximum frequency of root induction was observed on MS medium containing 2.0 mg/l IBA. The roots were white, long and slender with hairs. The rooted plantlets were first transferred to pots having vermiculate and garden soil (3:1). The pots were kept in covered glass trays for a week in an incubator at 25° C under 16h photoperiod. After 8-10 days these plantlets were transferred to the field.

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