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DIRECT REGENERATION FROM COTYLEDON CULTURE OF *LYCOPERSICON ESCULENTUM* MILL.

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Cotyledonexplants of tomato *(Lycopersicon esculentum Mill. cv. Pusaruby)* cultured on MS medium supplemented with different auxins like IAA, NAA, and 2,4-D produced callus whereas callusing was more pronounced in IAA followed by NAA, while 2,4,-D gave little response. Cytokinin alone produced callus with shoot primordia developed from cut edges. BAP alone showed better response in shoot differentiation than Kn. Among various combinations of auxins and cytokinins IAA+BAP and IAA + Kn produced green nodular callus with shoots whereas, NAA+Kn and NAA + BAP produced only callus. The percentage of responding cultures, and mean number of shoots per explant produced on IAA (0.5mg/l) + BAP (4.5 and 5.0 mg/l) were higher than all the concentrations and combinations of IAA+Kn used. However, maximum number of shoots were observed at 0.5 mg/l IAA + 5.0 mg/l BAP. Microshoots were rooted on MS basal medium supplemented with 0.5 mg/l IAA.

Keywords : Cotyledon culture; *In vitro* regeneration; *Lycopersicon esculentum.*

Introduction

Tomato *(Lycopersicon esculentum Mill.)* is a major vegetable crop that has achieved tremendous popularity over the last century. It is grown in almost every country of the world in the field, green houses and net houses. The tomato crop is very versatile and is grown either for fresh market or processing.Tomato production and consumption has grown quite rapidly over the past 25 years. Tomato by its nature is perennial plant, but is commercially cultivated as an annual crop. *In vitro* regeneration of cultivated tomato has been a subject of research because of the commercial value of the crop and its amenability for further improvement via genetic manipulation[1]. Consequently numerous studies on plant regeneration from a wide range of tissue and organs of wild and cultivated tomato germplasm have been conducted[2-6].

Material and Methods

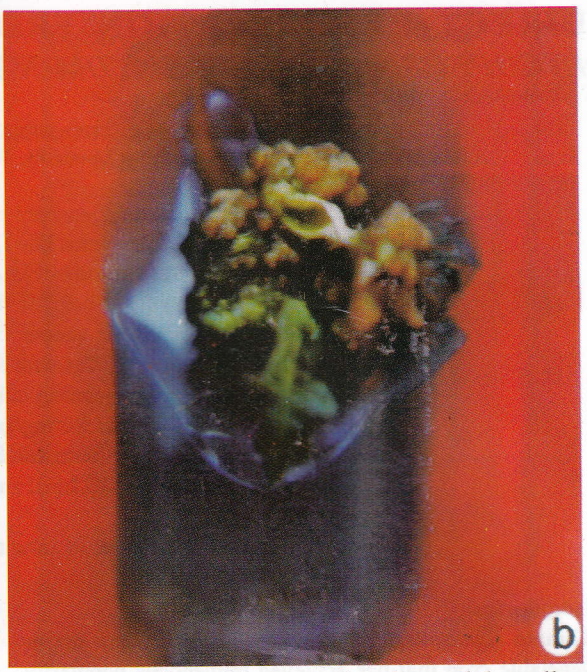
The seeds of *L. esculentum* cv. Pusa Ruby were obtained from Maharashtra Hybrid Seeds Co. Seeds were surface sterilized with ethonol (70%) for 30 seconds followed by mercuric chloride (0.2%) for 5 minutes and then rinsed several times with distilled water. Seeds were germinated aseptically on MS basal medium. The medium was gelled with 0.8% agar and cultures were incubated at 25±2°C under 8/16 hrs photo period. The cotyledons were excised from *in vitro* seedlings and cut into 1.0cm² pieces and inoculated on MS medium supplemented with various auxins, cytokinins and different concentrations and combinations of auxins + cytokinins (Table 1and 2). The cultures were incubated under 2000 lux for 16 hours at 25±2°C.

Results and Discussion

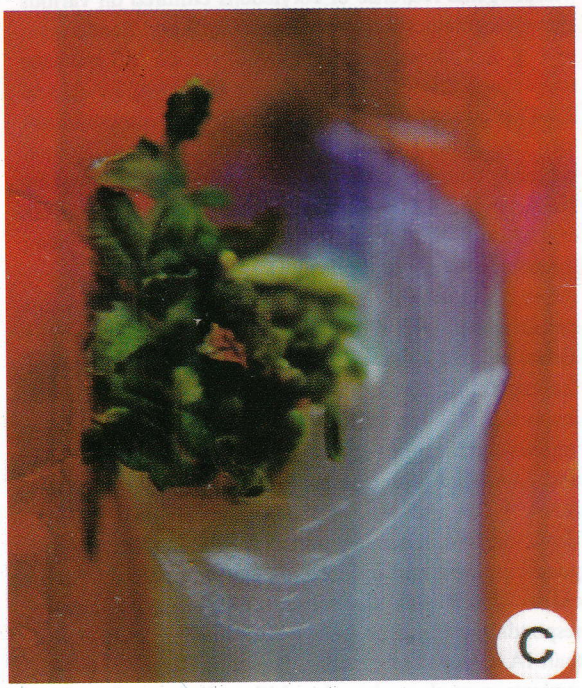
Morphogenic response of cotyledons cultured on various growth regulators is presented in Tables 1 and 2 and shown in Fig. 1. Cotyledon explants cultured on MS medium supplemented with different auxins like IAA, NAA, and 2,4-D produced whitish friable callus; which could not turn into green and not able to induce shoots. Whereas, callusing was more pronounced in MS+IAA followed by NAA, while 2,4-D gave less response. However, low levels of IAA and NAA were found to be stimulated rooting from the cut ends of the explant.

When cotyledons cultured on MS medium supplemented with different concentrations of cytokinins alone, induced callus + shoot buds from the cut ends of the explant. It was found that BAP alone showed better response in shoot differentiation than kinetin. Among various combinations of auxins and cytokinins, IAA+BAP and IAA +Kn produced green nodular callus, whereas NAA + Kn and NAA+BAP produced only callus. However, IAA in combination with BAP and kinetin gave good response and induced shoots with green nodular callus and it was also observed that percentage of responding cultures on IAA + BAP medium were higher than IAA +Kn combinations. Based on these results, the explants were also cultured on MS medium supplemented with different concentrations of BAP/Kn+ 0.5 mg/l IAA,

(a) Shoot formation on M S + IAA (1.0 mg/l) + BAP (5.0 mg/l).



(b) Enlarged view of the same showing nodular callus with shoot formation.



(c) Direct regeneration on MS + IAA (0.5 mg/l) + BAP (5.0 mg/l) showing adventitious shoot formation.

(d) Root formation on M S medium supplemented with IAA (1 mg/l).

Fig.1. a-d : *In vitro* regeneration from cotyledon explants.

Table 1. Morphogenic response of cotyledon cultures of *L. esculentum cv.* Pusa Ruby on MS medium supplemented with auxins and cytokinins and auxin + cytokinin combinations.

Growth regulator concentration (mg/l)	% of cultures responding	Morphogenic response
IAA 1.0	48	callus
NAA 1.0	38	callus
2,4-D 1.0	28	callus
BAP 1.0	53	Green callus + shoot buds
Kinetin 1.0	45	Green callus + shoot buds
NAA1.0+BAP 0.5	60	Callus
NAA 1.0 + BAP 1.0	62	Callus
NAA 1.0 + BAP 2.0	63	Callus
NAA 1.0 + Kinetin 0.5	60	Callus
NAA 1.0 + Kinetin 1.0	65	Callus
NAA 1.0 + Kinetin 2.0	68	Callus
IAA 1.0 + BAP 0.5	50	Nodular callus + shoots
IAA 1.0 + BAP 1.0	55	Green nodular callus + shoots
IAA 1.0 + BAP 2.0	64	Green nodular callus + shoots
IAA 1.0 + Kinetin 0.5	40	Nodular callus + shoots
IAA 1.0 + Kinetin 1.0	45	Green nodular callus + shoots
IAA 1.0 + Kinetin 2.0	50	Green nodular callus + shoots

Data scored after six weeks of culture based on 10 replicates.

and the data were presented in Table 2. All the concentrations of IAA + BAP and IAA + Kn combinatins produced shoots. The percentage of responding cultures and mean number of shoots per explant produced on IAA (0.5 mg/l) + BAP (4.5 and 5.0 mg/l) medium were higher than all the concentrations and combinations of IAA + Kn (Fig.1). But, among the various concentrations of IAA+Kn tested 0.5 mg/l IAA + 2.0 mg/l Kn produced more shoots/explant. From these results it is evident that IAA in combination with BAP was most suitable for plant regeneration. Maximum number of shoots was observed at 0.5 mg/l IAA +5.0 mg/l BAP compared to IAA + Kn combination. Early response from the explant was also found in the medium containing MS + BAP compared to Kn.

Yang[7] found the regeneration of plantlets from cotyledon explants cultured on modified MS or B_5 medium in tomato. Plantlet regeneration from cotyledon cultures of tomato was also observed by Dai *et al.*[8]. They noted the difference in morphogenic response depending upon the age of seedlings. Kozyreva *et al.*[9] have found the intraspecific variation in callus formation from cotyledon cultures of 7 cultivars and 2 accessions of tomato. Jawahar *et al.*[10] have also induced *in vitro* induction of plantlet regeneration from cotyledon callus of tomato cv. PKM1. They have observed shoot regeneration only in the presence of combinations of NAA and kinetin in contrast to our present results. Thus, for *in vitro* regeneration of tomato more number of shoots was found on MS + 0.5mg/l IAA+5.0 mg/IBAP in the present findings.

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Table 2. Direct regeneration from cotyledon cultures of *L. esculentum cv.* Pusa Ruby on MS medium supplemented with IAA in combination of BAP/Kinetin.

Growth regulator concentration (mg/l)			Morphogenic response	
IAA	BAP	Kinetin	% of cultures with shoots	Mean No. of shoots per explant
0.5	1.0	-	32	1.2±0.10
0.5	1.5	-	30	2.6±0.12
0.5	2.0	-	48	2.8±0.32
0.5	2.5	-	49	2.5±0.24
0.5	3.0	-	55	2.3±0.21
0.5	3.5	-	51	3.2±0.20
0.5	4.0	-	65	5.3±0.14
0.5	4.5	-	60	7.2±0.22
0.5	5.0	-	74	9.3±0.33
1.0	0.5	-	50	0.2±0.21
1.0	1.0	-	56	0.5±0.20
1.0	1.5	-	58	0.8±0.12
1.0	2.0	-	62	1.2±0.20
1.0	2.5	-	64	1.1±0.21
1.0	3.0	-	65	2.2±0.23
1.0	3.5	-	66	2.5±0.12
1.0	4.0	-	66	3.4±0.14
1.0	4.5	-	67	5.3±0.12
1.0	5.0	-	69	6.4±0.31
0.5	-	1.0	35	1.2±0.14
0.5	-	1.5	33	3.6±0.21
0.5	-	2.0	58	4.8±0.13
0.5	-	2.5	50	3.2±0.14
1.0	-	0.5	49	1.2±0.13
1.0	-	1.0	51	2.3±0.23
1.0	-	1.5	52	2.2±0.03
1.0	-	2.0	54	2.8±0.22
1.0	-	2.5	49	2.2±0.21

Data scored after six weeks of culture based on 10 replicates.

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