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TISSUE CULTURE STUDIES IN TOMATTO (LYCOPERSICON ESCULANTUM, PKM1) FROM COTYLEDONARY LEAF EXPLANTS

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ABSTRACT

A protocol was developed for callus induction and regeneration in tomato (*Lycopersicon esculentum*) var. PKM1. This, study was done as a pre-requisite for the genetic manipulation studies to enrich nutrient material in tomato. Cotyledon explants of tomato were used for callus induction. Callus induction was observed within seven days of culture from the cut surface of the cotyledon explants on MS medium supplemented with various concentrations of BAP and KIN (0.5 to 2.0 mg/l) for multiple shoot induction. Most Calli were cultured on MS medium having concentrations of 1.0 mg/l for regeneration. Among the four levels of BAP employed in Murashige and Skoog (MS) media 1.5mg/l BAP was found superior in growth traits. Highest regeneration frequency (87.5%) and number of shoots per callus (11.1 shoots/explants) were observed on MS media supplemented with 1.5 mg/l BAP. Tomato shoots were shifted to half MS medium containing IBA 0.5 mg/l for rooting and all responded positively to rooting. In future, these results will be very useful for all the tomato genetic engineering studies.

Keywords: *Lycopersicon esculentum*, Callus induction, Cotyledon leaf, BAP, KIN, IBA.

1. INTRODUCTION

Tomato belongs to the family Solanaceae by its nature of a perennial plant but is commercially cultivated as an annual crop. Tomato (*Lycopersicon esculentum* Mill) is the second most popular vegetable crop next to potato in the world [1]. Tomato is planted in almost 4 million ha worldwide. Lycopene is part of the family of pigments known as carotenoids which are natural compounds that create colours of fruits and vegetables. Lycopene is the most powerful antioxidant in the carotenoid family and it protects humans from free radicals that degrade many parts of the body; lycopene is also known to prevent cancer [2]. At present, tomatoes are consumed at a higher rate in the developed countries than in the developing countries and hence it may be referred to as a luxury crop. It is grown in tropical, sub-tropical and temperate areas [3]. It is one of the most important protective foods as it possesses appreciable quantities of vitamins and minerals and sometime rightly referred to as poor man's orange (4). Plant tissue culture method use for maximum callus induction and increased plantlet regeneration. This would help to achieve prolific *in vitro* propagation in tomato. *In vitro* regeneration of cultivated tomato has been a subject of research because of the commercial values of the cropand its amenability for further improvement via genetic manipulation (5). Consequently, numerous studies on plant regeneration from a wide range of tissues and organs of wild and cultivated tomato germplasm have been conducted (6, 7, 8, 9 and 10)

Although, there are many reports on tomato tissue culture, but only limited studies have been reported for Indian cultivars and particular there are no reports especially for the PKM1 a popular cultivar from Tamilnadu. Plant regeneration protocol is a pre-requisite for the genetic manipulation studies to enrich the nutritional value in local tomato variety PKM1.The objective of the present study was to develop an efficient protocol for callus induction and plant regeneration in tomato (*Lycopersicon esculentum*) var.

2. MATERIALS AND METHODS

2.1. Plant Material

Field grown plants were used as source of explants. Cotyledons of seven day old seedlings were selected as explants for plant regeneration. The explants were surface-disinfected in few drops of Tween-20 for 10 minutes, and rinsed three times with sterile distilled water. Then they were treated with 0.1% (w/v) mercuric chloride for five minutes. Finally, the materials were thoroughly rinsed with sterile distilled water for five times to remove the traces of mercuric chloride. All the explants were cut into small pieces before culture.

2.2. Sterilization of Explants

The explants were washed thoroughly in running tap water, (20 min) then shoot tips were wiped thoroughly using 70% ethanol. Explants were then treated with 0.1% Tween20 (10 min) with constant shaking then the explants were washed 3-4 times with sterile distilled water. The explants were then washed in 0.25% sodium hypochlorite (5-7 min) and it was washed thoroughly with sterile distilled water. Followed by treatment with 0.1% $HgC1_2$ (7- 10 min) with constant shaking during the sterilization process inside the laminar hood. And finally rinsed thoroughly (3-5 times) with sterile distilled water to remove any traces of mercuric chloride and then it was dried.

2.3. Callus Induction

The explants were cultured on MS medium supplemented with various concentrations and combinations of plant growth regulators including 0.5, 1.0, 1.5 and 2.0 mg/l of BAP and KIN for callus induction. Callus induction was noticed in all media formulations. A significant increase in callus proliferation was observed over the culture period. The frequency and percentage of callus induction was observed maximum in cotyledon.

2.4. Shoot Regeneration

Cotyledon derived calluses were cultured on MS medium supplemented with various concentration and combinations of plant growth regulators including 0.5, 1.0, 1.5 and 2.0 mg/l of BAP and KIN for shoot initiation and proliferation. The shoot clumps were initiated on the calli within 12-15 days of culture. The cytokinins BAP and KIN promotes cell division, shoot multiplication and auxiliary bud formation.

2.5. Root Induction

Well developed elongated shoots (2 to 3 cm) were excised and transferred on to MS medium supplemented with different concentrations (0.5, 1.0, 1.5 and 2.0 mg/l) of IBA for root induction.

2.6. Acclimatization

Rooted plantlets were gently washed with running tap water and followed by sterile distilled water and then successfully transferred to plastic cups containing sterile garden soil. The hardened plantlets were maintained in the culture room for a week. The polyethylene bags were removed after 2 weeks and then the hardened plantlets were successfully transferred to normal room temperature. These plants were lucratively transferred to net house for further growth and development.

3. RESULT AND DISCUSSION

The seeds of PKM1 were germinated in soil under green house condition. Cotyledon explants from 8-12 days field grown seedling were cultured on MS medium supplemented with various concentrations of BAP and KIN (0.5 to 2.0 mg/l) for multiple shoot induction. The effect of various concentrations of cytokinins (BAP and KIN) on shoot regeneration from the cotyledon explants of PKM1 was studied. Obtained highest callus in the MS medium supplemented with 3.0 mg/l BAP and 2.5 mg/l IAA. Callus induction was observed within seven days of culture from the cut surface of the cotyledon explants (Fig. A).

Cotyledon derived callus was cultured on MS medium fortified with different concentrations of BAP and KIN (0.5 to 2.0 mg/l) for shoot initiation and proliferation. The shoot clumps were started appearing on the calli after 2 weeks of culture (Fig. B). Green adventitious shoot buds developed on the compact masses was subcultured to on the same media for 2 to 3 weeks interval for shoot bud proliferation. The average number of shoots per explants was recorded after 3 weeks of culture. Highest regeneration frequency (87.5%) and number of shoots per callus (11.1 shoots/explants) were observed on MS media supplemented with 1.5 mg/l BAP (Table 1) (11). The mean number of shoots decreased with increase in the concentrations of BAP up to optimal level (1.5 mg/l).

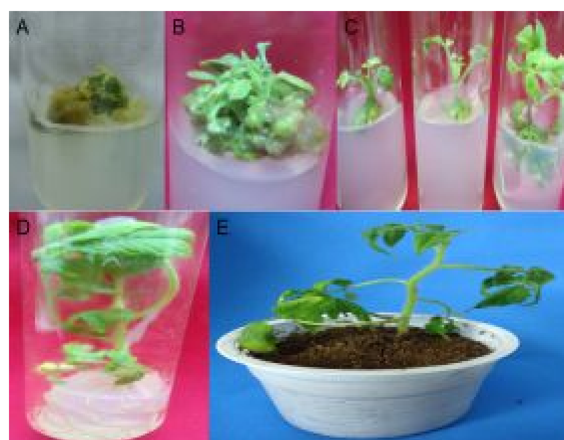

Figure A: Callus induction of PKM1 variety of tomato.

Figure B: Indirect organogenesis and multiple shoot buds induction.

Figure C: Different stages of shoot buds elongation of PKM1 variety of tomato.

Figure D: Root induction of PKM1 variety of tomato.

Figure E: Hardening of rooted plantlets of PKM1 variety of tomato.

Subculture of regenerated shoots into same medium exhibited further shoot proliferation with minimal basal callus. Subculture of regenerated shoots into the same medium

exhibited further shoot proliferation with minimal basal callus. In the present study, low regeneration frequency of shoot buds was observed in the medium supplemented with 2.0 mg/l KIN. The shoots were subcultured on the same medium and were allowed to grow till they attained 2-3 cm height within 4 weeks (Fig.C). The regenerated shoots were subcultured on to the same medium and were allowed to grow till they attained height of approximately 2-3 cm. The combination of 0.5 mg/l KIN and 0.5 mg/l BAP was found optimum for inducing shoot regeneration(12).

Table 1: Effect of cytokinins on shoot induction from cotyledon Explants of tomato cultivar PKM1 after **3** week

Cytokinins Conc. (mg/l)	Percent of regeneration (Mean ± SE)	Mean No. of Shoots/ explant (Mean ± SE)
BAP		
0.5	62.49±5.89	4.8 ±2.33
1.0	79.16±5.89	9.3 ±6.65
1.5	87.50±17.68	11.1 ±5.87
2.0	70.83±5.90	7.9 ±1.98
KIN		
0.5	50.00±0.00	0.0 ±0.00(callusing)
1.0	62.50±17.68	2.2 ±0.35
1.5	79.15±5.87	3.3 ±0.07
2.0	58.33±11.78	2.2 ±0.35

Well developed elongated shoots (2 to 3 cm) were excised and cultured on half strength MS medium supplemented with different concentrations of IBA (0.5-2.0 mg/l) for root induction. The best rooting was found to be in half-strength medium supplemented with 0.2 mg/l IBA (4). Among the concentrations tested, maximum number of root induction (22.25 roots/shoot) was observed in half strength MS medium containing 0.5 mg/l IBA (Table 2) followed by 5.5 roots/shoot found in the media having 1.0 mg/l IBA, whereas, (3.5 roots/shoot) root induction was observed in the media having 1.5 mg/l IBA(Fig. D).

Table 2: Effect of IBA on rooting of *in vitro* derived shoots of local tomato variety PKM1

IBA Conc. (mg/l)	Percent of root formation	No of root/soot	Root length
0.5	100	22.25	6.45
1.0	100	5.5	4.2
1.5	100	3.5	1.2
2.0	0	0.00	0.00

When the plantlets were attained 6-8 cm long then they had developed a good root system subsequently they were gradually transferred from growth room to the plastic cups and kept for fifteen days (Fig. E). Then the plantlets were regularly sprayed with water, covered with polythene sheet to maintain high humidity around juvenile plants. Plantlets were subsequently transferred into small pots and gradually acclimatized to the field condition. The survival rate of the transferred plantlets to soil was 60%.

4. CONCLUSION

This study has shown that 1.5 mg/l BAP is better for shoots induction in tomato cultivars and the tomato cultivars varied in response to shoots formation and shoots length. The best rooting was found to be in half-strength medium supplemented with 0.2 mg/l IBA. Also, higher concentrations of with 3.0 mg/l BAP and 2.5 mg/l IAA encouraged the formation of callus. This study is a baseline to carry further research on this tomato variety for improvement by using gene transfer technology.

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