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Original Research Article

In vitro callus induction, regeneration and micropropagation of *Solanum lycopersicum*

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A B S T R A C T

Solanum lycopersicum belonging to family Solanaceae is used mainly as vegetable. The plant has also different medicinal values. Attempts have been taken to standardize the micropropagation protocol from *in vitro* regenerated plant . The hypocotyle and cotyledons from germinated seedlings were used as explants for callus induction.The basal medium was MS with 3.0% sucrose and 0.8% agar..The PGRs used were different concentrations of 2,4-D,BAP, IBA. Callus induction was found both in 2,4-D and BAP. Both rhizogenesis and caulogenesis were obtained from callus.Highest shoot bud regeneration was noted in 2.0 mg/l BAP. Rooting was observed in 0.5mg/l IBA. The plantlets were then hardened and transferred to pot.

Keywords

Micro-propagation; Rhizogenesis; Caulogenesis; 2,4-D; BAP;

Introduction

Solanum lycopersicum belonging to family Solanaceae has a much lower sugar content than other edible fruits and is considered as vegetable for most culinary uses. Medicinally the plant is used as mild aperients, blood purifier, cholagogue and digestive. The plant is used in homoeopathy for treating rheumatic conditions, colds, chills, digestive disorders, diabetes, obesity, leucorrhoea, metrorrhagia. It promotes flow of bile; mildly laxative, especially when taken raw. Tomato is recommended for diabetics. It is a major dietary source of carotenoidlycopene. Tomato juice inhibits carcinogenic N-nitroso compound formation chiefly in the stomach. Tomato can decrease the risk condition such as cancer, osteoporosis and cardiovascular diseases (Bhowmik *et. al.*,2012). Callus induction and regeneration was previously reported by several workers(Gubis *et.al*,2004; Chen *et.al.*,1999; Devi *et.al*.,2008; Ishag *et.al*.,2009: Lima *et.al.*, 2009; Chaudhury *et.al*,2010; Osman *et.al*., 2010).The present objective of research is to standardize the protocol for micropropagation of *in vitro* regenerated tomato plant.

Materials and Methods

Seeds of *Solanum lycopersicum* were collected from nursery and used as source

of explant. The sterilization of seed was done by dipping them in 70% ethanol for 10 seconds followed by continuous shaking. Then the explants were washed with detergent Tween-20 for 5 mins and after that explants were surface sterilized by 0.1% mercuric chloride ($HgCl_2$) for 1 min then finally rinsed for 3 times with sterilized distilled water. All the process of sterilization and transfer were carried out inside the laminar air flow with proper sterilization techniques. Axenic culture of seeds were cultured individually on MS medium (Murashige and Skoog,1962).

The hypocotyle and cotyledons from germinated seedlings were then transferred to the callus induction media with different concentrations of PGRs (2,4-D & BAP). After 30 days of callus induction when huge mass of callus was observed with small shoot buds, it was taken out and cut into small 2 pieces having shoot buds and then again transferred to the shoot induction media i.e., MS with BAP(1.0-3.0 mg/l)for further growth and multiplication of shoot buds. After 45 days, the multiple shoots were transferred to the root induction medium of different concentrations (0.5-1.0 mg/l) of IBA . After 45 days, when the plantlets were found well matured and adequate amount of roots were observed, they were then transferred to sterilized sand and soil (1:1) for hardening.

Results and Discussion

Seeds were cultured individually on MS medium and the number and the rate of seed germination was recorded after 15 and 30 days of transfer (Fig. 1). Observations were recorded as mentioned in Table 1

The seedlings derived from axenic culture were used as source of explants for callus induction. The hypocotyle and cotyledon part of the plantlet were used for callus induction. Different types of PGRs (2,4-D & BAP) were used for callus induction and their response was recorded after 3 weeks as given in Table 2. Callus induction was noted both in BAP &2,4-D (Fig.2a & 2b) but in BAP, rhizogenesis and caulogenesis were found along with callus(Table 2, Fig.2b). Callus induction was reported in only BAP by Osman *et al*., (2010) but no report was found regarding rhizogenesis or caulogenesis in BAP. After 30 days of callus induction when huge mass of callus was observed with small shoot buds, it was taken out and cut into small pieces having shoot bud and then again transferred to the shoot induction media having different concentrations of BAP for further growth and multiplication of shoot bud. The observations were recorded as mentioned in Table 2. It is evident from Table 3 that 2.0 mg/l BAP showed better response in regeneration with larger leaves and better shoot growth (Fig. 3) .After 30 days, when the multiple shoots were observed, they were transferred to the root induction medium with different concentrations of IBA (0.5- 1.0 mg/l) . In this step, shoots were excised and transferred into root induction media. Best rooting was noted in 0.5mg/l IBA (Fig.4) .Chaudhury *et. al* ., (2010) reported that 0.1 mg/l IBA in combination with 0.0025mg/l BAP promoted root induction.Highest rooting was also reported in IBA by Osman *et. al*.,(2010) After 45 days, the plantlets were transferred to sterilized sand and soil (1:1) for hardening(Fig.5)

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Table.1 Rate of seed germination of *Solanum lycopersicum*

No. of given seed	No. of days	No. of germinated seed	Rate of germination (%)
50	15	30	60
50	30	45	90

Table.2 Effect of different PGRs on callus induction of *Solanum lycopersicum* in MS medium

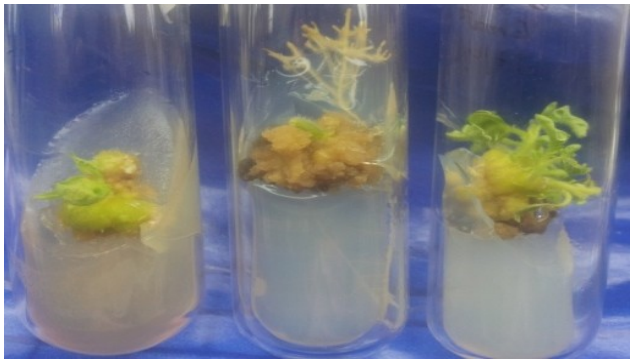
Name of PGR(s)	Concentration of PGR(s) (mg/l)	Response after 3 weeks	Morphogenetic nature
BAP	1.0	Callus induction	Green and yellow coloured callus with caulogenesis
	2.0	Callus induction	Brown coloured callus with rhizogenesis
	3.0	Callus induction	Brown and yellow coloured callus with caulogenesis
2,4-D	1.0	Callus induction	White coloured and friable callus
	2.0	Callus induction	Brownish black coloured friable callus
	3.0	Callus induction	Yellow coloured callus

Table.3 Effect of different concentrations of BAP on regeneration of callus of *Solanum lycopersicum* in MS media

Different concentrations of BAP	Response	Morphogenetic nature
	30 days	
	No. of shoot (M±SE)	
1.0 mg/l	4.0±0.00	Few shoots with smaller leaves
2.0 mg/l	8.0±0.70	Shoots with larger leaves and better growth
3.0 mg/l	6.5±0.88	few shoots with retarded growth

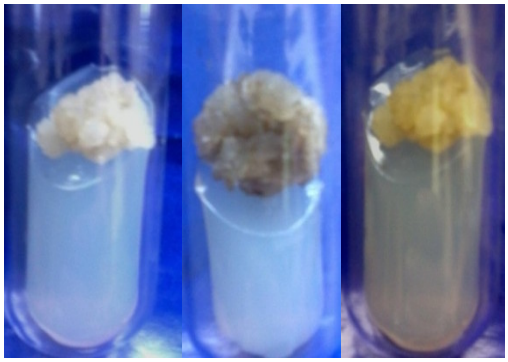
Figure.1 Axenic culture of *Solanum lycopersicum*

Figure.**2a** Callus induction of *Solanum lycopersicum* at different concentrations of BAP



1.0 mg/l	2.0mg/l	3.0mg/l

Figure.**2b** Callus induction of *Solanum lycopersicum* at different concentrations of 2,4-D



1.0 mg/l ,	2.0mg/l ,	3.0mg/l

Figure.**3** Regeneration of *Solanum lycopersicum* from its callus at different conc. of BAP

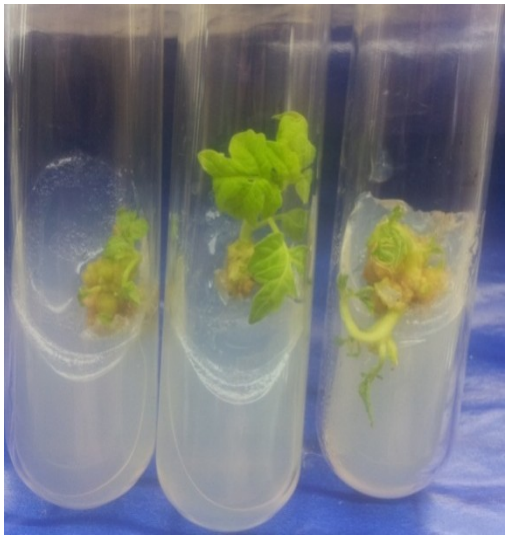


Figure.**4** Root induction at 0.5 mg/l IBA

Figure.5 Hardening of *in vitro* cultured *Solanum lycopersicum*

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