Science Research Reporter, 4(1): 89-93, (April - 2014)

ISSN: 2249-2321 (Print); ISSN: 2249-7846 (Online)
Received: 15-01-2014, Revised: 15-02-2014, Accepted: 17-03-2014

Full length Article

Studies on callus induction and shoot regeneration in Tomato

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ABSTRACT

The studies on callus induction using different explants and regeneration of plants from callus were carried out in tomato (*Lycopercicum esculentum*) cv. Pusa ruby. Cotyledonanry, nodal and internodal explants were cultured on MS media having different concentrations of BAP and NAA to induce callus. MS medium containing 2.0 mgs BAP and 0.2 mgs/l NAA shows best callusing and shoot regeneration response using internode explants. The shoots regenerated were multiplied *In vitro* on 0.1mg/l of BAP and different concentrations of IAA (0.0, 1.0, 1.5, 2.0, and 2.5). Shoots developed were rooted on full and half strength MS media containing IBA. Half strength MS medium was found to be the best rooting medium, however addition of IBA (2.0 mg/l) was found essential to induce profuse roots.

Key words: Callus induction, 2,4-D, BAP, TDZ, Shoot Regeneration, Tomato, Explants

INTRODUCTION

Tomato is commercially cultivated solanaceae vegetable crop grown all over the world. The tomato crop is very versatile and is grown either for fresh market or processing. Tomato is reach in Vit-A and Vit-C and fiber and is also cholesterol (Hobson and Davies, 1971). Its production is affected by various stresses such as diseases high temp draught, salinity and its vulnerability to frequent insects and pest attack. Jones *et al.,* (1991) presented major diseases of tomato caused by fungi, bacteria, viruses and various nematodes. Tissue culture is an important tool of biotechnology which can be used to improve productively of crop via rapid availability of superior planting material (Bhatia and Ashwath, 2004) invitro techniques are important tools for modern plant improvement program to develop suitable cultivars in a minimum time (Tagi *et al.,* 2002)

Several researchers have reported about adventitious regeneration in tomato deal with induction of shoots on hypocotyls apical meristems, cotyledons, stems, petioles, leaves, anthers and inflorescence (Moghaleb *et al.,* 1999, Raziuddil *et al.,* 2004, Brichkova *et al.,* 2002, Young *et al.,* 1987, Branca *et al.,* 1990, Compton and Veilleux 1991) however the improvement of adventitious shoot regeneration system using tissue culture method of tomato plants is still important due to the diverse morphogenic potential of different genotypes (Tomsone *et al*., 2004)

The regeneration of shoots from callus has been found to depend on factors such as genotype, explant type, composition of media, quantity of media, plant growth regulators used in media gelling agent, light intensity and quality, photoperiod provided, temperature, culture container (Reed, 1999; Bhatia *et al.,* 1994).

Shoot apex nodal segments and root segments were successfully used for callus induction and regeneration (Jatoi *et al.,* 2001). Various hormonal combinations are used to induce callus and regeneration like BAP and IAA, IAA and Kin (Chen *et al.,* 1999).

The present investigation was carried out to develop protocol for efficient callus induction and shoot regeneration using different explants of tomato.

Callus induction and regeneration of shoots protocol was established in many laboratories but still there is very little information regarding the studies on secondary metabolites in regenerated plants. Therefore, the present study was carried out to find best concentration of hormone and shoot regeneration of tomato.

MATERIAL AND METHOD

The present study was carried out in the plant tissue culture laboratory, H.U.Gugle Biotech, Jamkhed, Dist Ahmednagar in 2011. The Tomato seeds cv. Pusa Ruby were collected from local market.

MS media preparation

A half strength MS medium without sugar, without hormone was prepared and used for invitro germination of tomato seeds. MS medium having all major, minor elements with Fe EDTA and organic constituents, vitamin and cytokinin and auxins was prepared in advance for callus induction and regeneration experiments. The Hi-media AR grade chemicals were used for preparing media stock solution. Media was prepared using distilled water. Agar agar used as gelling agent. The pH of medium was adjusted to 5.8 and then media was autoclaved at 121^0C for 20 min.

Surface sterilization of seeds

Seeds were washed with running tap water to remove dust particles and then treated with 0.2% bavistin to avoid the load of microbe for half an hour and carried to laminar air flow for further treatment. The seeds were again rinsed using sterilized distilled water and then surface sterilized first with 70% alcohol for 30sec and then with sodium hypochlorite (05 ,10,15,and 20%) (Table-1) for 10 min and then rinsed five times with sterile distilled water to remove all the traces of alcohol and sodium hypochlorite.

Explant growing and preparation

To establish invitro cultures the tomato seeds were inoculated on ½ strength MS medium without sucrose and without hormone. After surface sterilization procedure the cultures were allowed to germinate in growth room and incubated at 28 ±2˚c in dark for 6 days. The cultures were observed for germination, growth of explants and microbial contamination. The cultures after germination incubated at 28±2˚c with 8 hours photoperiod under 1500 lux cool light.

Callus induction and regeneration

One month old invitro grown tomato seedling explants were used as a source of explants for callus induction and regeneration study. The cotyledon discs (4mm²), hypocotyle (10mm) and internodes segments (10mm) were used and cultured on basal MS media supplemented with different concentration of BAP in mgs per liter (0.0, 1.0, 2.0, 3.0 and 4.0) incombination with NAA in mgs per liter (0.2) for 6 weeks (Table-2). The data were recorded for percent of explant formed callus, degree of callus formation, Shoot regeneration response % and av.no of shoots/explant. Six explants were placed in each treatment.

Invitro propagation of shoots

The shoots regenerated from different calli was selected as an explant for shoot multiplication study. The shoots were sub cultured and inoculated on basal MS media. The basal MS media was supplemented with different concentrations of IAA (0.0, 1.0, 1.5, 2.0, and 2.5 mgs/l) and BAP (0.1mgs/l) (Table 3) for shoot regeneration. The cultures were incubated at 28±2˚c with 8 hours photoperiod. The data were recorded after 60 days of incubation for av. Ht. of plantlets, av. No of leaf/ plantlets, av. No of roots/plantlets.

Rooting of shoots

The shoots developed were rooted invitro on full strength and half strength MS media containing different concentration of rooting hormone IBA (0.5,1.0, 2.0, 3.0 mgs/l) table-4. The data were recorded after 60 days of incubation for plant rooting %, av. height of shoot, av. no of roots.

RESULTS AND DISCUSSION

In vitro plant development

To develop explant source the tomato seed were surface sterilized by using sodium hypochlorite at different concentration. Use of sodium hypochlorite has already been proved to be essential in tomato tissue culture and in vitro seed germination (Chaudhary *et al.,* 2007). The seed germination was observed after 7-10 days in germinating media by treating seeds with different concentrations of sodium hypochlorite.

Table 1: Effect of sodium hypochlorite v/v (4% bleach) on survival and explant culture contamination of tomato cv. Pusa ruby.

Na hypochlorite	No of cultures (10 seed/jar)	Germination %	Non germination%	Contamination %
05%	10 jar	---	---	100%
10%	10 jar	82%	18%	60%
15%	10 jar	93%	07%	11%
20%	10 jar	00%	100%	00%

(Data for germination was recorded after 10 days of inoculation)

The highest rate of germination and low contamination was observed in seeds treated with 15% sodium hypochlorite. The germination rate was 93%, 7% seeds were not germinated and culture contamination was 11 % (Table-1).

Callus induction and shoot regeneration-In the present study we have used cotyledon discs, hypocotyle, and internode segments for callus induction and direct shoot regeneration on medium containing different concentrations of BAP and NAA. The highest percent of callus formation was observed in cotyledon explants but unable to regenerate shoot. Jatoi *et al.,* (2001) observed an increase in callus formation in two tomato hybrids with increase in BAP concentration. The highest number of explants formed callus (100%) and shoot regeneration (100%) and av. No of shoots per explant (1.3) was observed in media containing BAP 2 mgs and NAA 0.2 mgs by using internode segments as explants (Table-2). One cut end of internode segments was inoculated in media. The cut end above media shows direct shoot formation. Same observation was recorded when hypocotyle explants and inoculated on media. The media without hormone do not neither show any callus formation nor shoot regeneration in all the explant types used.

Table 2: Effect of BAP on callus induction and shoot regeneration using hypocotyle, Cotyledon and internodes as explants in tomato

Explant type	Conc. Of BAP	Conc. Of NAA	% of explants induced callus	Degree of callus formation	Shoot Regeneration response %	Av.no of shoots/ explant
Hypocotyle	0.0	0.2	00.0%	---	00.0%	0.0
	1.0	0.2	85%	+	66.0%	1.0
	2.0	0.2	95%	+	73.0%	1.0
	3.0	0.2	90%	++	77.0%	1.2
	4.0	0.2	90%	++	60.0%	1.0
cotyledon	0.0	0.2	00.0%	---	00.0%	0.0
	1.0	0.2	100%	++	00.0%	0.0
	2.0	0.2	100%	++	00.0%	0.0
	3.0	0.2	100%	++	00.0%	0.0
	4.0	0.2	100%	++	00.0%	0.0
Internodes Segments	0.0	0.2	00.0%	---	00.0%	0.0
	1.0	0.2	75%	+	87.0%	1.0
	2.0	*0.2*	*100%*	*+*	*100%*	*1.3*
	3.0	0.2	90%	+	63.0%	1.2
	4.0	0.2	90%	++	28.0%	1.0

---=no callus, +=slight callus, ++=moderate callus, +++=massive callus

Table 3: Effects of IAA and BAP on invitro shoot multiplication of Tomato (av. of 10 plantlets in each treatment)

Conc. Of IAA (mgs/l)	Conc. Of BAP (mgs/l)	Av. Ht. of plantlets after 60 days after inoculation(mm)	Av. No of leaf/ plantlets after 60 days after inoculation	Av. No of roots/plantlets after 60 days after inoculation
0.0	0.1	16	3	1.0
1.0	0.1	23	3	2.0
1.5	0.1	44	5	2.0
2.0	*0.1*	*47*	*5*	*2.5*
2.5	0.1	37	4	2.0

Table-4 Effects of IBA and media strength on invitro rooting of Tomato

Media strength	Conc. of IBA(mgs/l)	Rooting response %	Av. Height of shoot (mm)	Av. no of roots
Full MS Media	0.0	100%	26	3
	0.5	100%	34	3
	1.0	100%	54	6
	2.0	100%	48	8
	3.0	100%	43	8
Half MS Media	0.0	100%	39	5
	0.5	100%	42	9
	1.0	100%	35	14
	2.0	*100%*	*47*	*17*
	3.0	100%	33	8

(Av. of 10 plantlets in each treatment) after 60 days of inoculation.

Shoot multiplication & rooting-The media containing 2.0 mg/l IAA and 0.1 mgs/l BAP shows average height 47mm, having 5 leaves with average 2.5 roots/shoots followed by media containing 1.5 mg/l IAA and 0.1 mgs/l BAP. The media having only BAP induce lowest root number (1) and dwarf growth (16mm) (Table-3). Regenerated shoots were transferred for rooting on full strength and half strength MS media containing different concentrations of IBA. There was 100% rooting response in all the media combinations as well as media without IBA (Table-4). However, half strength MS media containing 2.0mgs/l IBA resulted maximum longer profuse rooting (17). The beneficial effect of using half strength MS media for rooting of invitro induced shoots has already been reported for tomato. Devi *et al.,* 2008 reported that the best rooting was found to be in half strength medium supplemented with 0.2mg/l IBA. Well developed shoots having profuse roots were transplanted in portrays having cocopeat for hardening. After one month of hardening plantlets were potted in earthen pots.

Conclusion

The callus formation and direct shoot regeneration was observed in both hypocotyle and internode explants. The maximum shoot regeneration was observed on medium containing 2mgs/l BAP and 0.2mgs/l NAA.

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How to Cite this Article:

Sherkar H D and Chavan A M, 2014. Studies on callus induction and shoot regeneration in Tomato. *Sci. Res. Rept.,* **4**(1):89-93.