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Adventitious shoot regeneration in different explants of six genotypes of tomato

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ABSTRACT

A protocol has been standardized for adventitious shoot regeneration from different explants without intervening callus formation in six genotypes of tomato. Maximum shoot bud formation was obtained on a medium supplemented with 2.0mgl^{-1} BAP. Tomato hybrid TH802 has the highest organogenetic potential as compared to other genotypes. Sub culturing of shoots buds on the same medium led to continuous production of multiple shoots. Regenerated shoots were rooted on hormone free MS basal medium. Plantlets were transferred to the field after hardening in the pots containing sand, soil and farmyard manure (1:1:1) in a green house. The regenerated plants were identical to the *in vivo* raised plants in agro biological features.

Key words: *Lycopersicon esculentum*, adventitious shoot regeneration, plantlets, tomato hybrids

INTRODUCTION

Tomato is one of the foremost vegetable crops grown in tropical, sub-tropical and temperate regions of the world. The conventional method for growing and propagating the tomato is by seed. For practical breeding purposes the tomato is considered a self-pollinated crop and most improvement programmes continue on a pedigree basis and has been concentrated on obtaining increased yield, improved fruit quality, altered plant growth, disease and pest resistance. The incorporation of such desirable traits into cultivated tomato is usually attempted by crossing with wild species. However, inter-specific incongruity between many of these species limits the value of sexual hybridization as a tool for the introduction of important traits from wild species. Genetic engineering technique could be useful in the creation of new breeding approaches to produce plant varieties with novel characteristics. However, adventitious shoot or embryo regeneration system is a pre requisite for these techniques. Extensive work on tissue culture has been done covering various aspects (Sink & Reynold, 10). Particularly, *in vitro* regeneration of shoot from different explants of tomato has been the main pursuit but in all the cases regeneration of shoots has been obtained through callus. In the present study, we describe the regeneration potential of different explants of various genotypes including hybrids under *in vitro* conditions without callus formation. The system developed for adventitious shoot regeneration was found to be both efficient and suitable for all six commercial cultivars studied, and is therefore expected to pave way for molecular biology based breeding of tomato.

MATERIALS AND METHODS

The seeds of six genotypes namely Haelani, Accession-2, TH802 (Haelani x Accession-2), VFN8, Punjab chuhara, TH2312 (VFN8 x Punjab chuhara) of *Lycopersicon esculentum* were collected from Department of Vegetable Crops, PAU, Ludhiana (India). They were surface sterilized in 70% ethanol for 30 seconds followed by sodium hypochlorite (4%) for 2 minutes and rinsed 4-5 times with sterile distilled water. The seeds were inoculated on half strength MS (Murashige & Skoog, 7) medium for germination. Different explants were excised from 14 days old in vitro raised seedlings for shoot bud induction. Cotyledon, hypocotyls and shoot tip explants were cultured on MS medium supplemented with BAP or Kinetin (0.5-2.5 mgl-1) alone or in combination with auxins (NAA, IBA, IAA : 0.1-0.5 mgl-1). All media were supplemented with sucrose 3% and agar 0.8% (Ranbaxy India Ltd.). The pH of medium was adjusted to 5.7 before autoclaving at 121^{o}c at 15 lb^{-2} for 15 minutes. Molten medium (40 ml) was poured into 100 ml Erlenmeyer conical flasks. The cultures were incubated in a controlled environmental chamber having 16h photoperiod (3500 lux), 25±1^{o}C temperature and 60% relative humidity. The regenerated

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shoots were transferred to media supplemented with different concentrations of auxins (0.5-2.0 mgl^{-1} IBA, NAA, IAA) for rooting. Plantlets were transplanted in plastic pots (6 cm diameter) containing sterilized mixture of sand, soil and farmyard manure (1:1:1) and transferred to green house maintained at 25±2°C and 80±5% relative humidity for hardening.

Data was taken as percentage of survival plants after 4 weeks. All experiments were of completely randomized design and repeated at least twice. Each treatment consists of 4 replicates (5 explants per replication). The percent explants forming shoot buds and the mean number of shoot buds per explant was recorded after 4 weeks of culture. Percentage data was subjected to ascertain transformation for proportions before analysis by ANOVA (analysis of variance) and then converted back to percentages for presentation in tables (Snedecor and Cochran, 11). Treatment means were statistically compared by least significant difference (LSD).

RESULTS AND DISCUSSION

The shoot induction was significantly influenced by cytokinin type and concentration. BAP was found to be superior to Kn (Data not given). The effectiveness of BAP can be due to the abilities of plant tissue to metabolize the natural hormones more readily than artificial growth regulators or due to the abilities of BAP to induce natural hormones such as Zeatin within tissue and thus works through natural hormones (Zaerr & Mapes, 13). Data on shoot induction in different explants on different concentration of BAP showed significant variation (Table 1). Maximum percentage of explants showing shoot induction was achieved on 2.0 mgl^{-1} BAP. Similar results were also achieved by Sharma & Wakhlu, 8; Dwivedi *et al.* 3; Compton & Veillux, 1; El-Farash *et al.* 4 and Titok *et al.*12. The genotypic differences were also observed in all the genotypes studied so far. This may be due to the variation in specific level of endogenous hormones as influenced by genotypes and environmental factors. Similar results have also been reported in tomato (El-Farash *et al*. 4 and Kurtz and Linebeyer, 6).

The shoot buds initiated on cotyledonary explants on MS medium containing 2.0 mgl^{-1} BAP were sub cultured on medium supplemented with different concentrations of BAP for shoot multiplication and elongation (Table 2). The results revealed best responses on MS medium fortified with 2 mgl^{-1} BAP. The maximum number of shoots per explant was observed in hybrid TH802 (5.17) as compared to its parents, Accession-2 (5.05) and Haelani (4.22). Similarly, hybrid TH2312 showed maximum number of shoots per explant (5.11) as compared to its parents VFN8 (5.01) and Punjab chuhara (4.94). Better performance of hybrids over its parents could be attributed to the heterotic type effect as observed in tomato (Titok *et al*., 12).

Table 1. Effect of BAP on different explants of various genotypes of *Lycopersicon esculentum* Mill. on adventitious bud induction after 4 weeks of culture.

Genotypes explants	Percent response BAP ($mg1^{-1}$)				
	0.5	1.0	1.5	2.0	2.5
Haelani					
Shoot tip	-	-	-	66	80
Cotyledon					
Hypocotyl					
Accession-2					
Shoot tip	-	-	25	50	100
Cotyledon	-	-	25	80	100
Hypocotyl	-	50	66	60	80
TH802					
Shoot tip	25	66	60	100	50
Cotyledon	-	50	60	100	60
Hypocotyl	-	75	40	100	75
VFN8					
Shoot tip	75	80	80	100	80
Cotyledon	17	50	80	100	80
Hypocotyl	-	40	60	60	60
Punjab chuhara					
Shoot tip	20	40	80	100	60
Cotyledon	20	40	60	100	80
Hypocotyl	-	-	40	60	70
TH2312					
Shoot tip	-	17	67	83	33
Cotyledon	-	17	67	67	50
Hypocotyl	-	-	67	75	40

The regenerated shoots (4-5 cm) were rooted on MS basal medium. The maximum number of roots per shoot (17.55) and root length (8.62 cm) was observed in TH802 genotype (Table 3). Addition of auxins in the medium induced callus formation. Similar results reported by Davis *et al*., 2 and Dwivedi *et al*., 3 can be attributed due to the higher level of endogenous auxins in tomato. Genotypic differences in rooting response were also observed with hybrid TH802 gave best results followed by Punjab chuhara.

The regenerated plants were treated with different concentration of glycerol (an antitranspirant) to increase the percent survival rate and were transplanted in pots containing sand, soil and farmyard manure (1:1:1) in the green house). Maximum survival (71%) was achieved with 0.5% glycerol whereas the plantlets transplanted without glycerol treatment showed 50% survival rate

Table 2. Effect of BAP on shoot multiplication and elongation from cotyledon explants of various genotypes of *Lycopersicon esculentum* Mill. 4 weeks of culture.

Genotypes	BAP ($mg1^{-1}$)				
	0.5	1.0	1.5	2.0	2.5
Haelani					
No.of shoots/explant	1.12^{a}	2.45^{b}	2.94^{c}	4.22^{c}	3.33^{d}
Shoot length (cm)	3.28	3.31	3.33	3.30	3.30
Accession-2					
No.ofshoots/explant	1.05^{a}	2.61^{b}	3.34^{c}	5.05^{d}	3.67^{c}
Shoot length (cm)	3.52	3.35	3.37	3.35	3.40
TH802					
No.of shoots/explant	1.50^{a}	2.89^{b}	3.44^{c}	5.17^{d}	3.67^{c}
Shoot length (cm)	3.37	3.35	3.40	3.35	3.37
VFN8					
No.of shoots/explant	0.22^{a}	1.87^{b}	2.83^{c}	5.01^{c}	3.72^{d}
Shoot length (cm)	3.36	3.23	3.25	3.14	3.10
Punjab Chuhara					
No.of shoots/explant	1.16^{a}	2.39^{b}	3.00^{c}	4.94^{c}	4.11^{d}
Shoot length (cm)	3.43	3.34	3.37	3.30	3.22
TH2312					
No.of shoots/explant	0.22^{a}	2.72^{b}	3.89^{c}	5.11^{d}	4.11^{c}
Shoot length (cm)	3.45	3.41	3.36	3.22	3.36

Values are mean ± s.e. for 4 replications (each replication consists of 5 explants)
Mean with the same superscript nare not significantly different from each other within rows at 5% level.

Table 3. Response of different genotypes of *Lycopersicon esculentum* Mill. For rooting of *in vitro* raised shoots on MS basal medium after 15 days of culture.

Genotype	No. of roots/plantlet	Root length (cm)
Haelani	10.44^{a}	5.89^{a}
Accession-2	14.92^{b}	7.31^{a}
TH802	17.55^{c}	8.62^{a}
VFN8	11.32^{a}	5.33^{a}
Punjab chuhara	15.08^{b}	6.58^{a}
TH2312	13.36^{a}	6.44^{a}

Values are mean ± s.e. for 4 replications (each replication consists of 5 explants)
Mean with the same superscript are not significantly different from each other within rows at 5% level.

(Data not presented). The hardened plants after one month were transplanted into the field. All the regenerates exhibited normal morphological characteristics when compared with *in vivo* plants (Table 4). The above protocol can be used for large-scale colonel propagation of different genotypes.

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Table 4. Morphological characteristics of different genotypes of *Lycopersicon esculentum* Mill. In field after 6 weeks of transplantation.

Genotype	*In vivo* raised plants		*In vitro* raised plants	
	Plant height (cm)	No. of branches/plant	Plant height (cm)	No. of branches/plant
Haelani	11.2±0.8	2.9±0.2	11.4±0.5	2.8±0.1
Accession-2	13.8± 0.4	3.6±0.2	14.5±0.2	3.5±0.2
TH802	15.9±0.5	3.4±0.3	16.5±0.6	3.6±0.1
VFN8	11.8±0.4	2.7±0.2	12.2±0.5	2.8±0.2
Punjab Chuhara	14.2±0.2	3.2±0.2	15.4±0.4	3.2±0.2
TH2312		2.5±0.2	13.2±0.4	2.6±0.2

Values are mean ± s.e. of 30 plants for each genotype

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