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ADVENTITIOUS SHOOT FORMATION IN SIX CULTIVARS OF TOMATO (*LYCOPERSICON ESCULENTUM* Mill.)

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A protocol has been developed for adventitious shoot regeneration from different explants without intervening callus formation in six cultivars of *Lycopersicon esculentum*. Maximum shoot buds regeneration was obtained on a medium supplemented with 2.0 mgl^{-1} BAP. Hybrid TH 802 has the highest regeneration potential as compared to other cultivars. Sub culturing of shoot bud on a medium led to continuous production of multiple shoots. Regenerated shoots were rooted on a hormone-free MS medium. Plantlets were transferred to field after hardening in the pots containing sand 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.

Keywords : Hybrids; *Lycopersicon esculentum*; Plant regeneration; Vegetable crop.

Introduction

Tomato is one of the foremost vegetable crop grown in tropical, sub-tropical and temperate region 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- pollinating 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[1]. The incorporation of such desirable traits into cultivated tomato is usually attempted by crossing with wild species. However, interspecific incongruity between many of these species limits the value of hybridization as a tool for the introduction of important traits from wild species. Genetic engineering techniques could be useful in the creation of new breeding approaches to produce new plant varieties with novel characteristics[2-4]. However, shoot or embryo regeneration is a prerequisite for these techniques. Extensive work on tissue culture has been done covering various aspects. Particularly, *in vitro* regeneration of shoots from different explants of tomato has been the main pursuit. The present study was carried out with the aim to investigate the morphogenesis in different cultivars and their hybrids.

Material and Methods

The seeds of six cultivars namely Haelani, Accession-2, TH 802 (Haelani x Accession-2), VNF 8, Punjab chuhara, TH 2312 (VNF 8 x Punjab chuhara) of *Lycopersicon esculentum* were obtained from Department of Vegetable Crops, PAU, Ludhiana (India). They were surface sterilized in 70% ethanol for 30s followed by sodium hypochlorite (4%) for 2 min and rinsed 4-5 times with sterilized distilled water. The seeds were incubated on the half strength MS (Murashige and Skoog[5]) medium for germination. Different explants were excised from 14 days old in vitro raised seedlings for shoot bud induction. For induction of shoots, different explants (cotyledon and hypocotyls) were cultured on MS medium supplemented with BAP or Kn (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 3% sucrose. The pH of medium was adjusted to 5.7 before addition of 0.8% agar (Ranbaxy India Ltd.) and autoclaves at 121°C at 15 lb/ $inch^2$ for 15 min. Molten medium (40 ml) was poured into 100 ml Erlenmeyer conical flasks. Twenty explants were used per treatment. The cultures were incubated under controlled conditions having 16 h photoperiod (3500 lux), 25±2°C temperature and 60% humidity.

The regenerated shoots were transferred to media supplemented with different concentration 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. All experiments were of complete randomized design and repeated at least twice. Data was recorded after 4 weeks of culture. Percent data was subjected to arscin transformation for proportions before analysis by ANOVA (analysis of variance) and then converted back into percentages for presentation in tables[6]. Treatment means were statistically compared by least significant difference (LSD).

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

Genotype / Explants	Percent explant response BAP (mgl^{-1}) 0.5	1.0	1.5	2.0	2.5
Haelani					
Cotyledon	25	40	60	100	100
Hypocotyl	-	-	75	75	50
Accession-2					
Cotyledon	-	-	25	80	100
Hypocotyl	-	50	66	60	80
TH 802					
Cotyledon	-	50	60	100	60
Hypocotyl	-	75	40	100	75
VFN 8					
Cotyledon	17	50	80	100	80
Hypocotyl	-	40	60	60	60
Punjab chuhara					
Cotyledon	20	40	60	100	80
Hypocotyl	-	-	40	60	70
TH 2312					
Cotyledon	-	17	67	67	50
Hypocotyl	-	-	67	75	40

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

Genotype	BAP (mgl^{-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^e	3.33^d
Shoot length	3.28	3.28	3.33	3.30	3.30
Accession-2					
No. of shoots/ explant	1.05^a	2.61^b	3.34^c	5.05^d	3.67^c
Shoot length	3.52	3.35	3.37	3.35	3.40
TH 802					
No. of shoots/ explant	1.50^a	2.89^b	3.44^c	5.17^d	3.67^c
Shoot length	3.37	3.3	3.40	3.35	3.37
VFN 8					
No. of shoots/ explant	0.22^a	1.87^b	2.83^c	5.01^e	3.72^d
Shoot length	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^e	4.11^d
Shoot length	3.43	3.34	3.37	3.30	3.22
TH 2312					
No. of shoots/ explant	0.22^a	2.72^b	3.89^c	5.11^d	4.11^c
Shoot length	3.45	3.41	3.36	3.22	3.36

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

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

Genotypes	No of roots/ plantlet	Root length (cm)
Haelani	10.44a	5.89a
Accession – 2	14.92b	7.31a
TH 802	17.55c	8.62a
VFN 8	11.32a	5.33a
Punjab chuhara	15.08b	6.58a
TH 2312	13.36a	6.44a

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

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

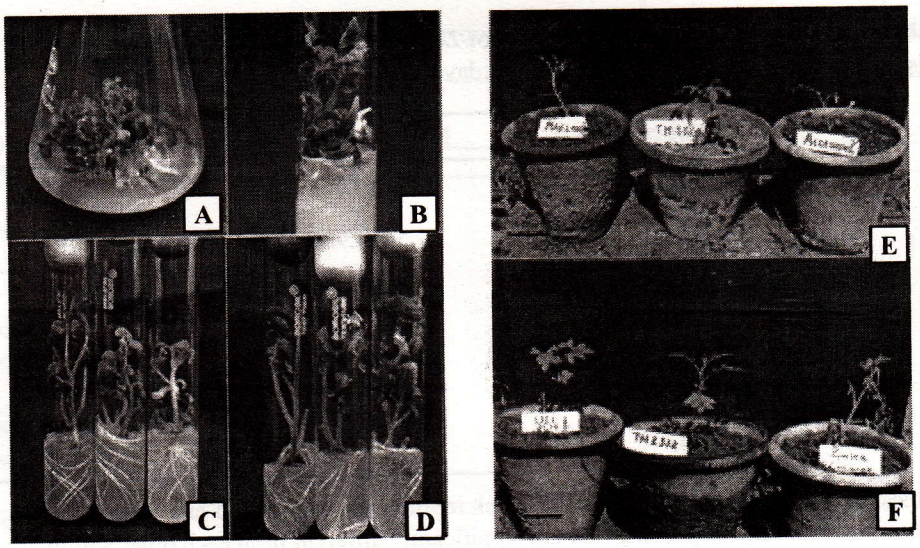
Genotype*	*In vivo* raised plants		*In vitro* raised plants	
	Plant height (cm)	No. of branches/ plant	Plant height (cm)	No. branches/ plant
Haelani	11.2±0.8	2.9±0.2	11.4±0.5	2.8±0.1
Accession – 2	13.8±0.4	3.6±0.2	14.5±0.2	3.5±0.2
TH 802	15.9±0.4	3.4±0.3	16.5±0.6	3.6±0.1
VFN 8	11.8± 0.4	2.7±02	12.2±0.5	2.8±0.5
Punjab chuhara	14.2±0.2	3.2±0.2	15.4±0.4	3.2±0.2
TH 2312	12.1±0.5	2.5±0.2	13.2±0.4	2.6±0.2

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

Results and Discussion

Shoot regeneration was significantly influenced by cytokinin type and concentration. BAP was found to be superior to Kn (Data not presented). The effectiveness of BAP can be due to the ability of plant tissue to metabolize the natural hormone more readily than artificial growth regulators or due to the ability of BAP to induce natural hormones such as Zeatin within the tissue and thus works through natural hormone[7]. Data on shoot induction in explants on different concentrations of BAP showed variation (Table 1).

Maximum percentage of explants showing shoot induction was achieved on 2.0 mgl^{-1}BAP. Cotyledons were more responsive than hypocotyl explants 67% in TH 2312, 100% in Haelani, VFN 8 and Punjab chuhara). 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). MS medium containing 2.0 mgl^{-1} BAP gave best results. This may be due to the variation in specific level of endogenous hormones as influenced by genotypes and environmental factor[8]. Maximum number of shoots per explant were observed in hybrid TH 802 (5.17) (Fig.1A) as compared to its parents: Haelani (4.22) and Accession-2 (5.05). Similarly, hybrid TH 2312 showed maximum number of shoots per explant (5.11) (Fig 1B) as compared to its parents, VFN 8 (5.01) and Punjab chuhara (4.94). Better



Figs. 1 A-F. Micropropagation of *Lycopersicon esculentum*
Fig. 1 A. Shoot regeneration from cotyledon explants of cultivar TH 802 on a medium supplemented with 2 mgl^{-1} BAP after 4 weeks of culture.; **Fig 1 B.** Shoot regeneration from cotyledon explants of cultivar TH 2312 on a medium supplemented with 2 mgl^{-1} BAP after 4 weeks of culture.; **Fig.1 C.** Rooted shoots of different cultivars on MS medium after 15 days of culture. 1) Haelani 2) TH 802 3) Accession-2; **Fig. 1 D.** Rooted shoots of different cultivars on MS medium after 15 days of culture. 1) VFN-8; 2) Th2312; 3) Punjab chuhara; **Fig. 1. E.** *In vitro* regenerated plants in pots under field conditions after 4 weeks of transplantation. 1) Haelani; 2) TH 802; 3) Accession - 2; **Fig. 1 F.** *In vitro* regenerated plants in pots under field conditions after 4 weeks of transplantation. 1) VFN - 8; 2) TH 2312; 3) Punjab chuhara.

performance of hybrids over its parents could be attributed to the heterotic type of effect in tomato[8].

The regenerated shoots (4-5 cm long) were rooted on MS basal medium. The maximum number of roots per shoot (17.55) and root length (8.62 cm) was observed in TH 802 cultivar (Table 3). Addition of auxins in the medium induced callus formation. Genotypic differences in rooting response were also observed. Hybrid TH 802 (Fig. 1C) gave best results followed by Punjab chuhara (Fig 1D).

The regenerated shoots were treated with different concentrations of glycerol (an anti-transpirant) to increase the percent survival rate and were transplanted in pots containing sand and farmyard manure (1:1:1) in the green house (Figs 1E &F). Maximum survival rate (71%) was achieved with 0.5% glycerol whereas the plantlets transplanted without glycerol treatment showed 50% survival rate (Data not presented). The hardened plants after one month were transplanted in the field. All regenerated plants exhibited normal morphological characteristics when compared with in vivo plants (Table 4). Thus, the system developed for adventitious shoot regeneration was found to be both efficient and suitable for all six cultivars studied and is, therefore, expected to pave way for molecular biology based breeding of tomato.

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