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In vitro direct plant regeneration protocol for tomato genotypes

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ABSTRACT

A high frequency direct plant regeneration method was developed for four tomato genotypes, *viz*., Castle Rock, Punjab Upma, VFN-8 and IPA-3. Cotyledons of 8-day-old *in vitro* grown seedlings of the four genotypes were excised and cultured onto Murashige an Skoog (MS) medium supplemented with different concentrations and combinations of BAP and kinetin. The frequency of plant regeneration and number of shoots per explant was influenced by the genotype and concentration of growth regulators. Among the genotypes tested, IPA-3 recoded the maximum plant regeneration (95.14%) followed by Punjab Upma (93.13%), Castle Rock (90.02%) and VFN-8 (82.09%). Among the different media compositions tried maximum average plant regeneration (90.14%) and number of shoots (6.17) were obtained using MS medium supplemented with BAP 2.0 mg l^{-1} and kinetin 1.0 mg l^{-1}. With either decrease or increase from the optimum level, decline in percent plant regeneration as well as number of shoots per explant was recorded. The shoots when attained approximately 2 cm height were transferred to the half-strength MS medium, which led to simultaneous shoot elongation as well as root formation. All the shoots recorded 100% rooting and were subsequently transferred to poly bags containing FYM: sand:soil (1:1:1) and kept in glasshouse for acclimatization.

Key words: Growth hormones, tomato, organogenesis, tissue culture.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a major vegetable crop that has achieved tremendous popularity over the last century and is grown in almost every country of the world. It is one of the most studied higher plants because of its importance as a crop species, and several advantages for genetic, molecular and physiological studies (McCormick *et al*., 8). High frequency plant regeneration base line is a pre-requisite for the development of transgenics (Bhatia *et al*., 1).

In vitro plant regeneration has been found to depend on many factors, of which most important are: genotype, explant, composition of basic medium, growth regulators, gelling agent, light intensity and quality, photoperiod, temperature, culture vessels and vessel covers (Reed, 13). *In vitro* regeneration of shoots from different explants of various *Lycopersicon* spp. has been main pursuit but in most of the cases regeneration of shoots has been obtained through callus (Bhatia *et al*., 1). It is well established that callus culture is genetically unstable, more so in tomato where polyploidation occurs even after first or second subculture. Callusing results in either epigenetic or genetic changes, *i.e*., somaclonal variations which are heritable (Peschke *et al*., 11) and may pose a serious threat to the genomic integrity of regenerated plants. The present study was therefore designed to develop a protocol direct regeneration of shoots from the cotyledons of tomato as affected by genotype and plant growth regulators.

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MATERIALS AND METHODS

The seeds of four tomato genotypes namely 'Castle Rock', 'Punjab Upma', 'VFN-8' and 'IPA-3' were surface sterilized with Ala Bleach® for 20 min. followed by four to five rinses in sterile water and germinated on half-strength (inorganic salts strength reduced to half) (Murashige and Skoog, 9) medium containing thiamine HCl (0.1 mg l^{-1}), pyridoxine HCl (0.5 mg l^{-1}), nicotinic acid (0.5 mg l^{-1}), myo-inositol (100 mg l^{-1}), sucrose (3.0 g l^{-1}) and agar (0.8%). The pH of media was adjusted to 5.8 by adding 1N NaOH/1 N HCl solution drop-wise. The *in vitro* germinated seedlings served as an explant source for direct plant regeneration studies. Cotyledons were excised from *in vitro* grown seedlings and inoculated onto MS medium supplemented with combinations and concentrations of BAP and kinetin (Table 1). The media was first dispensed (100 ml/ jar) into glass jars when still hot. These glass jars were properly capped before autoclaving at 1.05 kg cm^{-1} square pressure and 121°C for 22 min. Percent plant regeneration was calculated as the number of cotyledons showing regeneration over total number of cotyledons cultured per treatment. Experiments were replicated five times. Statistical analysis was done according to the CPCS package using factorial CRD design (Singh and Cheema, 17).

Table 1. Various concentrations and combinations of BAP and kinetin used for the direct regeneration in different tomato genotypes.

Media code	BAP (mg l^{-1})	kinetin (mg l^{-1})
R_0	0.0	0.0
R_1	0.5	0.0
R_2	1.0	0.0
R_3	1.5	0.0
R_4	2.0	0.0
R_5	1.0	0.5
R_6	2.0	1.0
R_7	3.0	2.0
R_8	4.0	3.0
R_9	5.0	4.0

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) for experimental design (Table 2) revealed the significance of genotype, hormonal concentration and their interaction for plant regeneration and number of shoots per explant. This indicated that *in vitro* response of cotyledon explants was genotype dependent and differed significantly with the kind and concentration of the growth regulators. Previous studies demonstrated that 8-10 day-old cotyledons of tomato were superior to other sources of explants for promoting shoot organogenesis (Gubis *et al.*, 6). Therefore, in this experiment 8-10 day-old seedlings were used as source of explants. Organogenic response recorded in cotyledon explants of tomato genotypes, namely, Castle Rock, Punjab Upma, VFN-8 and IPA-3, are presented in Fig. 1. It was evident from the data that no organogenesis was recorded in the basal MS medium, *i.e.*, R_0 medium. It was evident from an earlier report also that only roots were produced on the basal MS medium from the cotyledon explant (Feher, 4). Cotyledons increased in size by 3 to 4 times in length as well as width but no shoot regeneration occurred in any genotype.

Table 2. Analysis of variance for percent direct plant regeneration and number of shoots per explant in relation to genotypes and hormonal concentrations in cotyledons of tomato.

Source	df	Regeneration (%)	No. of shoots
Genotype (g)	3	151.03*	0.95*
Hormone (h)	9	10720.0*	5.79*
g x h	27	22.36*	0.11*
Error	120	0.77	0.13

* = Significant at 5% level

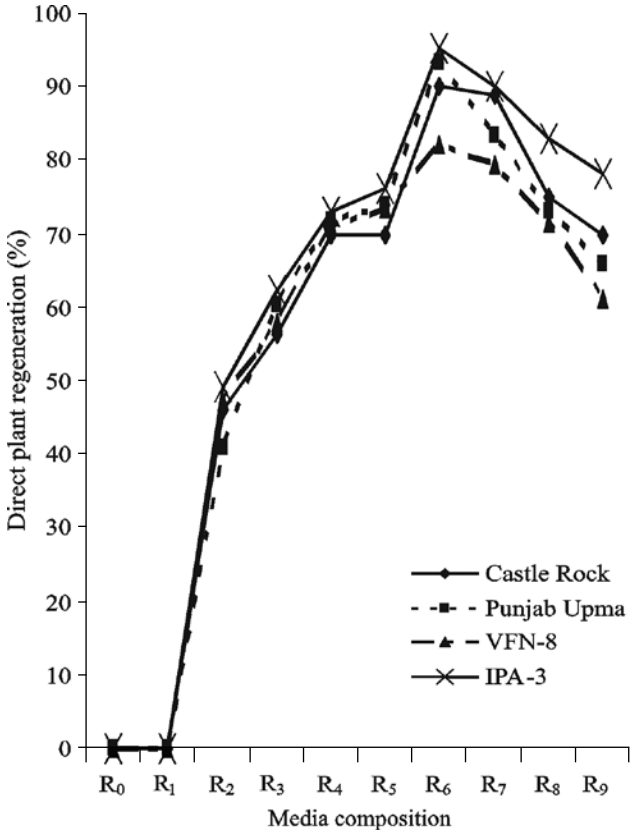

Fig. 1. Effect of media composition on percent direct plant regeneration from cotyledons of different tomato genotypes.

The addition of cytokinin to the basal MS medium led to the regeneration of explants. Although plant regeneration could be induced with BAP alone but combination of BAP and kinetin enhanced percent plant regeneration to significantly higher levels. The optimal medium for plant regeneration in all the genotypes was R_6, *i.e.*, MS medium supplemented with 2.0 mg l^{-1} BAP and 1.0 mg l^{-1} kinetin, which was significantly superior over other combinations tried. For obtaining maximum plant regeneration, cultures were needed to be sub-cultured after every 15-20 days of incubation. Although numerous rudimentary shoots aroused on the cut surface of the cotyledons, but there was not any elongation. Shoot clumps needed to be sub-cultured on regular intervals. If sub-culturing was not repeated after every 15-20 days of incubation, callus formation took place and over-powered the shoot regeneration ability of the explants. A decline was recorded in percent plant regeneration with the change in the R_6 medium composition. This indicated that hormonal concentrations and combinations had an inhibitory response on shoot regeneration ability of explants at super optimal concentrations. Among the

genotypes, maximum plant regeneration of 95.14% was recorded in genotype IPA-3 followed by Punjab Upma, Castle Rock and VFN-8, *i.e.*, 93.13, 90.02 and 82.09%, respectively. The frequency of adventitious shoot regeneration has also been reported to differ with both the type and concentration of growth regulators added to the media (Bhatia *et al.*, 1; Gubis *et al.*, 7; Shadin *et al.*, 14; Singh *et al.*, 16). The regeneration capacity was significantly influenced by cultivar and explant type (Gubis *et al.*, 5; Nogueira *et al.*, 10).

The data (Fig. 2) represent the number of shoots per explant of the four tomato genotypes. Shoots were often observed after 15-18 days of culture in all the genotypes. Shoots were obtained on all tested media compositions except in M_0, which lacked plant growth hormones and M_1 which contained only 0.5 mg l^{-1} BAP. Although shoots could be regenerated on the media which contained BAP alone but shoots appeared more prominent and healthy when BAP and kinetin were added in combination. The highest average number of shoots, *i.e.*, 8.02 per explant was recorded in genotype IPA-3 on R_6 medium in which MS medium was supplemented with BAP 2.0 mg l^{-1} and kinetin 1.0 mg l^{-1}. Genotype IPA-3 was followed by Castle Rock and Punjab Upma recorded 6.09 and 6.02 shoots, respectively. Genotype VFN-8 recorded maximum number of shoots on R_5 medium composition which contained BAP 1.0 mg l^{-1} and kinetin 0.5 mg l^{-1}. A decline was recorded in average number of shoots with either increase or decrease from these hormonal concentrations in all genotypes. The genotypic differences in the ability to regenerate shoots were also reported by various workers (Plastira and Perdikaris, 12; Bhatia *et al*., 1; Devi *et al.*, 3; Shivakumar *et al.*, 15; Bhushan and Gupta *et al.*, 2).

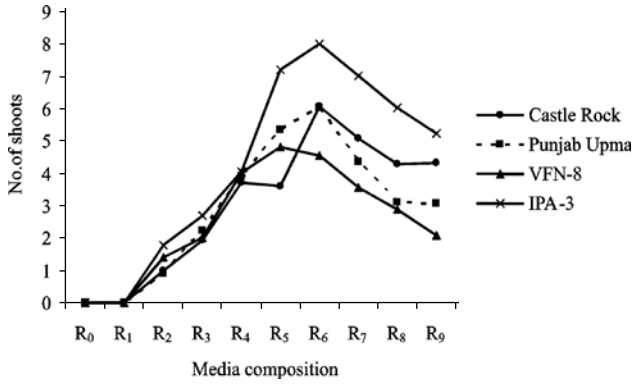

Fig. 2. Direct plant regeneration in tomato genotype IPA-3. (A) Stereo-micrographic view of direct plant regeneration, (B) showing direct plant regeneration from cotyledons on MS + BAP 2.0 mg l^{-1} and kinetin 1.0 mg l^{-1}, (C) Establishment of shoot culture on MS + BAP 2.0 mg l^{-1} and kinetin mg l^{-1}, (D) Induction of rooting on ½ MS medium.

Rate of shoot multiplication *in vitro* was studied in four tomato genotypes (Table 3). Shoot clumps derived from the cotyledon explants were cultured for five cycles of three week each on MS medium supplemented with BAP 2.0 mg l^{-1} and kinetin 1.0 mg l^{-1} starting with 22, 15, 20 and 18 culture jars of Castle Rock, Punjab Upma, VFN-8 and IPA-3, respectively. The extent of shoot multiplication ranged between 18.55 percent in Castle Rock to 22.67 percent in IPA-3.

When the regenerated shoots attained the height of 2 cm, were transferred onto half-strength MS medium. Simultaneous shoot elongation as well as rooting took place with in a week of culturing in the shoots. Roots developed fully within 15 days of transfer to the half-strength MS medium. All the stages from shoot regeneration to complete plantlet formation are presented (Fig. 3). The plantlets were washed in the running tap water to remove the adhered nutrients to the roots and kept 0.05% Bavistin® solution, in the incubation room under controlled conditions for one day. After that these plantlets were transferred to sterile normal tap water, which was changed daily to develop new roots sterlie. When plantlets leaves developed darker colour and new leaves started to come then plants were transferred on to the sterilized garden soil containing FYM: sand: soil (1:1:1) in pots under glasshouse conditions, where temperature was maintained at 25º ± 1ºC and 80% relative humidity and 2000 lux light intensity. This protocol can be efficiently used for large scale multiplication of the elite material in tomato.

REFERENCES

1. Bhatia, P., Ashwath, N., Senaratna, T. and Mid More, D. 2004. Tissue culture studies in tomato (*Lycopersicon esculentum* L.). *Pl. Cell Tiss. Org. Cult.* **78**: 1-21.

2. Bhushan, A. and Gupta R.K. 2010. Adventitious shoot regeneration in different explants of six genotypes of tomato. *Indian J. Hort.* **67**: 224-27.

3. Devi, R., Dhaliwal, M.S., Kaur, A. and Gosal, S.S. 2008. Effect of growth regulators on *in vitro*

Table 3. *In vitro* shoot multiplication of four tomato genotypes from cotyledon explants after five cycles of culture.

Genotype	No. of cultures		
	N1	N2	Multiplication rate (%)
Castle Rock	22	408	18.55
Punjab Upma	15	340	22.67
VFN-8	20	375	18.75
IPA-3	18	351	22.67

N1 = Initial number of jars cultured; N2 = Number of cultured jars after five cycles

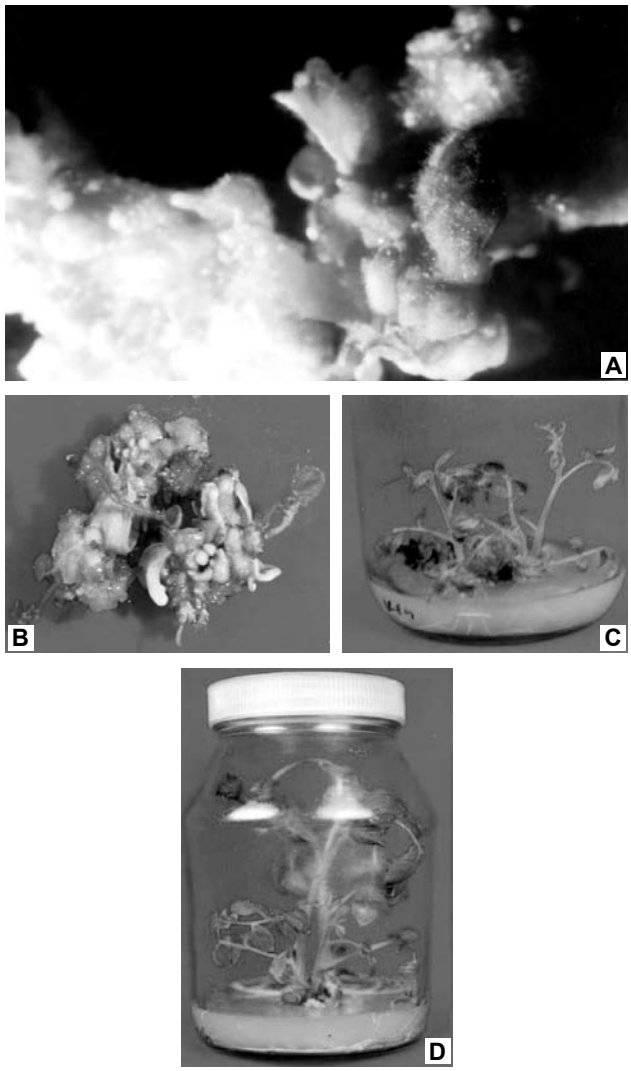

Fig. 3. Stages of regeneration in tomato (a) Ogranogenil callus, (b) Shoot bud induction, (c) Shoot elonagation, (d) Rooting of micro-shoot

morphogenetic response of tomato. *Indian J. Biotech.* **7**: 526-30.

4. Feher, T. 1979. Tomato propagation from parts of sterile seedlings. *Kerteszeti.* **11**: 59-64.

5. Gubis, J., Lajchova, Z., Farago, J. and Jurekova, Z. 2003. Effect of genotype and explant type on shoot regeneration in tomato (*Lycopersicon esculentum* Mill.) *in vitro*. *Czech J. Genet. Plant Breed.* **39**: 9-14.

6. Gubis, J., Lajchova, Z., Farago, J. and Jurekova, Z. 2004. Effect of growth regulators on shoot induction and plant regeneration in tomato (*Lycopersicon esculentum* Mill.). *Biologia Bratislava,* **50**: 405-8.

7. Gubis, J., Lajchova, Z., Klcova, L. and Jurekova, Z. 2005. Influence of growth regulators on plant regeneration in tomato. *Hort. Sci.* **32**: 118-22

8. McCormick, S., Niedermayer, J., Fry, J., Barnasm, A., Horsch, R. and Fraley, R. 1986. Leaf disc transformation of cultured tomato (*Lycopersicon esculentum*) using *Agrobacterium tumefaciens*. *Plant Cell Rep.* **5**: 81-84.

9. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant.* **15**: 473-97.

10. Nogueira, F.T.S., Costa, M.G., Figueira, M.L., Otoni, W.C. and Finger, F.L. 2001. *In vitro* regeneration of Santa Clara tomato plantlets and its natural mutant Firme. *Ciênce. e. Agrotecnologia, Lavras.* **25**: 36-71.

11. Peschke, V.M., Phillips, R.L. and Gengenbach, B.G. 1991. Genetic and molecular analysis of tissue-culture-derived Ac elements. *Theor. Appl. Genet.* **82**: 121-29.

12. Plastira, V.A. and Perdikaris, A.K. 1997. Effect of genotype and explant type on regeneration frequency of tomato *in vitro*. *Acta Hort.* **447**: 231-34.

13. Reed, B.M. 1999. Design a micropropagation system: Workshop presentation from the 1998 *SIVB Congr. on In vitro* Biology. *In Vitro Cell Dev. Biol. Plant*, **35**: 275-84.

14. Shadin, I., Magdoleen, G., Osman and Mutasim, M.K. 2009. Effects of growth regulators, explants and genotype on shoot regeneration in tomato (*Lycopersicon esculentum* cv. Omdurman). *Int. J. Sustain. Crop Prod.* **4**: 7-13.

15. Shivakumar, B., Mythilli, J.B., Anand, L. and Saiprasad, G.V.S. 2007. Influence of genotype on *Agrobacterium*-mediated transformation of tomato. *Indian J. Hort.* **64**: 251-57.

16. Singh, A., Singh, M. and Singh, B.D. 2010. Comparative *in vitro* shoot organogenesis and plantlet regeneration in tomato genotypes. *Indian J. Hort.* **67**: 224-27.

17. Singh, B. and Cheema, C.S. 1985. CPCS-computer software package, Punjab Agricultural University, Ludhiana, India.

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