Indian Journal of Biotechnology
Vol 7, October 2008, pp. 526-530

Effect of growth regulators on *in vitro* morphogenic response of tomato

Ruma Devi[1], M S Dhaliwal[1], Ajinder Kaur[2] and S S Gosal[2*]

[1]Department of Vegetable Crops and [2]Department of Plant Breeding, Genetics & Biotechnology, Punjab Agricultural University, Ludhiana 141 004, India

Received 31 August 2007; revised 26 February 2008; accepted 8 May 2008

A series of experiments were conducted to explore the *in vitro* morphogenic response of tomato genotypes viz., Castle Rock, Punjab Upma, VFN-8 and IPA-3, under different concentrations and combinations of growth regulators. The analysis of variance at 5% level of significance indicated that the differences among different genotypes, hormonal regimes as well as their interactions were statistically significant. The MS medium supplemented with BAP @ 3.0 mg L^{-1} and IAA 2.5 mg L^{-1} was optimum for callus induction, plant regeneration and number of shoots per explant. The maximum per cent callus induction and plant regeneration in the genotypes Castle Rock, Punjab Upma, VFN-8 and IPA-3. was 81.23, 76.69, 68.13 and 65.12%; and 46.91, 48.02, 57.14 and 60.23%, respectively, The respective average number of shoots per culture were 7.03, 6.92, 8.19 and 9.19. At higher and lower levels of hormones, a considerable decline was recorded in per cent callus induction, plant regeneration and number of shoots per explant. The best rooting was found to be in the ½ MS medium supplemented with 0.2 mg L^{-1} IBA. Among the four soil mixtures studied viz., vermiculite, perlite, coco-peat and mixture of three (vermiculite, perlite and cocopeat in the ratio of 1:1:1), maximum plantlet survival rate was recorded in the mixture of three for genotypes Castle Rock and Punjab Upma and in vermiculite for genotype VFN-8.

Keywords: *Lycopersicon esculentum*, callus induction, plant regeneration, acclimatization; tomato; growth regulators

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an important Solanaceous vegetable crop grown throughout the world for its versatile uses. It is one of the important 'protective foods' as it possesses appreciable quantities of vitamins and minerals[1] and sometimes rightly referred to as 'poor man's orange'. Tomato is highly amenable to physiological and cytological investigations due to its ease of culture and genetic uniformity resulting from autogamy[2]. Tissue culture has a tremendous scope for the collection, multiplication and storage of germplasm[3]. Development of high frequency plant regeneration base line is a pre-requisite for the development of transgenic plants. During the last four decades, significant advances have been made in the development of *in vitro* culture techniques, which have been extensively applied to more than 1000 different crop species[4]. The most frequently used method of regeneration in tomato is via shoot organogenesis from callus of leaf or cotyledon explants or directly from thin cell layers of the inflorescence[5]. Although advances have been made towards better understanding of metabolic processes correlated with regeneration[6], standardization of the conditions for *in vitro* plant regeneration is still largely an empirical process. Among the *Lycopersicon* species, *L. peruvianum* is considered to be highly organogenetic and regeneration of shoots from roots has already been documented[7]. Other genotypes have also been described for their ability to form shoots from calli derived from hypocotyls (*L. pimpinellifolium* cv. WV700)[8], cotyledons (*L. esculentum* cv. UC82 B)[9], suspension cells (*L esculentum* cv. VFNT)[10] and protoplasts (*L esculentum* cv. Lukullus)[11]. Although wide information is available on the morphogenesis of tomato, the techniques have not been developed to a level at which these can be utilized for large-scale multiplication of commercially important cultivars. The success of tomato regeneration has been reported to vary with the nutrient media, concentrations and combinations of growth regulators, light and temperature regimes in incubation room, genotype and explant related factors[12]. Therefore, standardization of culture conditions for desired

*Author for correspondence:
Tel: 91-161-2401960-Extn 270; Fax: 91-161-2400945
E-mail: ssgosal@rediffmail.com

genotypes deserved importance for large-scale multiplication and genetic engineering studies. In the present study, we explored the callogenic and regeneration potential of leaves on different hormone levels in four genotypes of tomato viz., Castle Rock (suitable for processing), VFN-8 (multiple disease resistance), Punjab Upma (popular variety among tomato growers) and IPA-3 (one of the parents of tomato hybrid TH1).

Materials and Methods

Seeds of four genotypes of tomato, Castle Rock, Punjab Upma, VFN-8 and IPA-3 were germinated in pots under glass house conditions. After 20-25 d of germination, third leaves from top of the seedlings were collected for use as explants. Explants were treated with 0.1% (w/v) mercuric chloride for 7-10 min in laminar air flow cabinet followed by 3-4 washings with sterile distilled water. The leaf pieces were cut into 0.5-0.8 cm^2 sizes and only those possessing central mid-rib were selected for culturing. MS[13] medium was supplemented with 100 mg L^{-1} myo-inositol and 3.0 g L^{-1} sucrose. The growth regulators (IAA and BAP) were added in varying combinations and concentrations (Table 1). The *p*H of the media was adjusted to 5.8 by adding 1N NaOH/1N HCl solution dropwise. The cultured explants were incubated under 14 h light (2500 lux) and 10 h dark periods at 25±1°C. The experiment was repeated five times. The number of explants per genotypes varied from 100-200. The observations were recorded on per cent callusing, per cent plant regeneration and number of shoots per culture. The data were analyzed following the computer software package CPCS-I using factorial CRD design.

Results and Discussion

The analysis of variance for the experimental design revealed significance of mean squares due to genotypes (g), hormone concentrations (h) and their interaction (gxh) for all the three parameters studied (Table 2). This indicated that *in vitro* response of the leaf segments was genotype dependent and differed significantly with the kind and concentration of growth regulators.

Callus Induction

The data pertaining to per cent callus induction after 4 wks of culture indicated that leaf explants of all the genotypes, Castle Rock, Punjab Upma, VFN-8 and IPA-3 did not produce callus in the absence of plant growth regulators (Fig. 1). A little callus could be induced when media were supplemented with 0.5 mg L^{-1} IAA alone that ranged between 2.34% in Castle Rock to 7.10% in Punjab Upma. The combination of IAA and BAP enhanced callus induction to significantly higher levels ranging from 7.09 to 81.23%. As the levels of IAA and BAP were increased in the media, there was a corresponding gradual increase in the frequency of callus formation,

Table 1—Different combinations and concentrations of growth hormones supplemented in MS medium

Medium	IAA (mg L^{-1})	BAP (mg L^{-1})
M_0	0.0	0.0
M_1	0.5	0.0
M_2	0.5	0.5
M_3	0.5	1.0
M_4	1.0	0.5
M_5	1.0	1.0
M_6	1.5	1.0
M_7	1.5	2.0
M_8	2.0	2.0
M_9	2.0	2.5
M_{10}	2.5	2.5
M_{11}	2.5	3.0
M_{12}	3.0	3.5
M_{13}	3.0	4.0
M_{14}	3.5	4.0
M_{15}	4.0	4.0

Table 2—Analysis of variance (mean square values) for per cent callus induction, plant regeneration and number of shoots per explant in relation to genotypes and hormonal concentrations

Source	Callus induction (%)	Plant regeneration (%)	Shoots per explant
Genotype (g)	299.71*	394.21*	1.46*
Hormone conc (h)	4,260.89*	3,714.73*	6.08*
Interaction (gxh)	31.44*	23.78*	0.29*
Error	4.09	1.14	0.47

*Significant at P=0.05

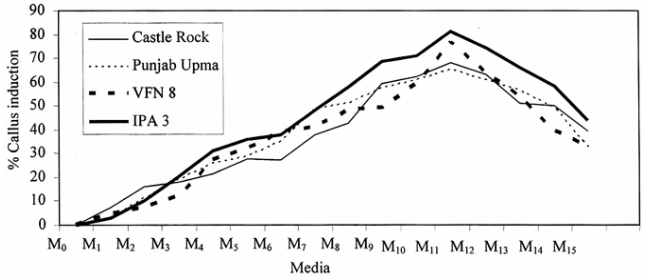

Fig. 1—Per cent callus induction in tomato as influenced by genotypes and hormonal regimes.

which reached to a maximum on M_{11} (2.5 mg L^{-1} IAA and 3.0 mg L^{-1} BAP), where IPA-3, VFN-8, Castle Rock and Punjab Upma registered 81.23, 76.69, 68.13 and 65.42% callus induction, respectively (Fig. 1). Further increase in the hormone levels led to continuous decrease in callusing, revealing toxic hormonal effects on the callus tissue.

The per cent increase or decrease in the callus induction on different media compositions varied with the genotype. Though a variable morphogenic response was recorded in different genotypes of tomato on different media compositions, yet the genotype IPA-3 remained significantly superior on most media compositions except M_1 and M_2, where Castle Rock recorded higher per cent callus induction than the other three genotypes. On M_{11} medium, genotype IPA-3 recorded significantly higher per cent callus induction than the other three genotypes. Peak callus proliferation was observed between 18-24 d after inoculation. The calli were greenish to creamish in colour, hard and compact (Fig. 2a-b). The callus turned dark brown after 30-35 d of culturing followed by rotting and death of cells with no shoot formation. Therefore, regular subculturing of callus was done after every 15-20 d on to the fresh medium. The effect of growth regulators and different tomato genotypes on per cent callus induction has already been reviewed in detail[12].

Plant Regeneration

All the obtained calli did not exhibit regeneration. Some of them remained white in colour, hard in texture and later turned black without regeneration. The data presented in Fig. 3 indicated that per cent plant regeneration varied with different hormonal concentrations in different genotypes of tomato after 4 wks of culturing. No plant regeneration was recorded on M_0 (basal MS), M_1 and M_2 media. All the four genotypes exhibited significantly superior regeneration potential on medium M_{11} i.e. MS supplemented with IAA, 2.5 mg L^{-1} and BAP, 3.0 mg L^{-1} on over all other combinations tried. Upon increase or decrease of the concentration of IAA or BAP from this level, a significant decline was recorded in per cent plant regeneration in all the four genotypes. The per cent increase or decrease varied with the genotype. In general, IPA-3 recorded significantly higher per cent plant regeneration over other genotypes in all the media compositions except M_3, M_{12} and M_{15}, where it was at par with VFN-8. Castle Rock, Punjab Upma and VFN-8 recorded also recorded at par per cent plant regeneration on M_3, M_4, M_7 and M_{10} media compositions.

Number of Shoots per Explant

The average number of shoots per culture was recorded after 8 wks of culture (Fig. 2 c-d). Corresponding to maximum per cent callus induction and plant regeneration, significantly higher number of

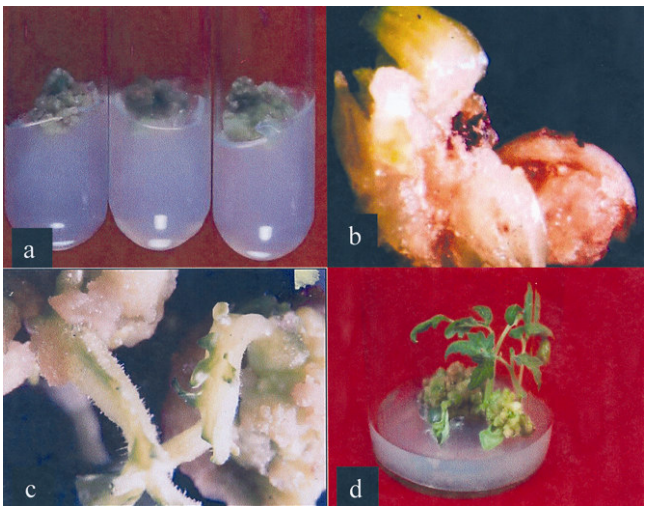

Fig. 2—Plant regeneration via callus in tomato genotype IPA-3: a-Showing callus induction on MS + BAP 3.0 mg L^{-1} + IAA 2.5 mg L^{-1} medium; b-Stereo-micrographic view of callus; c-induction of shoot regeneration from calli; & d-Elongation of shoots on MS + BAP 3.0 mg L^{-1} + IAA 2.5 mg L^{-1} medium.

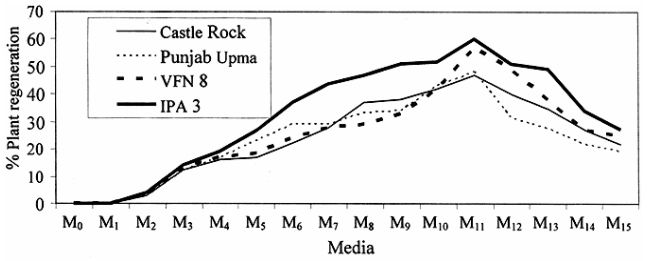

Fig. 3—Per cent plant regeneration in tomato as influenced by genotypes and hormonal regimes.

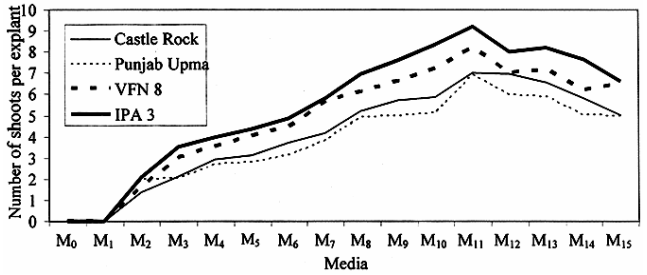

Fig. 4—Number of shoots per explant in tomato as influenced by genotypes and hormonal variations.

shoots were recorded on M_{11} i.e. MS + 2.5 mg L^{-1} IAA and 3.0 mg L^{-1} BAP medium over all other media compositions (Fig. 4). The genotypic response varied from 6.92 shoots per culture in Punjab Upma, 7.03 in Castle Rock, 8.19 in VFN-8 to 9.19 in IPA-3. No shoot formation occurred on M_0 and M_1 media. With increase in hormonal concentrations, there was a corresponding increase in the number of shoots per explant and maximum number of shoots per explant were observed on M_{11}. A gradual decline in number of shoots per explant was noticed in Castle Rock and Punjab Upma after M_{11} and in VFN-8 and IPA-3 after M_{13}. However, a non-significant difference was recorded in the number of shoots per explant on M_{12} and M_{13} nutrient media in both the genotypes, VFN-8 and IPA-3, where decline started a little later than the other two genotypes.

The genotypic means indicated that the line IPA-3 was most responsive to shoot regeneration than the other genotypes tried. The least responsive variety was found to be Punjab Upma. The four genotypes varied significantly from each other in their ability to regenerate shoots. The difference in shoot number on the similar media can be attributed to the differential response of cultivars. The leaf discs of cultivar UC 134 regenerated on an average eight shoots per disc when cultured on MS + 2.25 mg L^{-1} BAP and 0.175 mg L^{-1} IAA medium[14], whereas, cultivar UC 134-1-2 regenerated on an average 11.9 shoots per leaf segment when cultured on MS medium containing 2.5 mg L^{-1} BAP and 0.2 mg L^{-1} IAA [15].

Rooting and Acclimatization

Shoots thus obtained were isolated aseptically and placed on four different media viz., MS, ½MS (MS salts were reduced to half), MS + 0.2 mg L^{-1} IBA and ½ MS + 0.2 mg L^{-1} IBA, for the induction of rooting. Simultaneous shoot elongation and root formation took place within 7-10 d of culturing on the rooting media. Root system was fully developed within 15-20 d. Cent per cent rooting was recorded in all the genotypes in all the culture media tried. However, rooting was profuse and early in all cultures on ½ MS medium supplemented with 0.2 mg L^{-1} IBA, and hence, best for rooting in the tomato genotypes studied. Induction of rooting even on the basal MS and ½ MS media suggested that genotypes of tomato possessed sufficient levels of endogenous auxins. Therefore, exogenous application of auxins was not required for the induction of rooting. In an earlier study, profuse rooting has been reported on ½ MS + 2 mg L^{-1} IAA medium[16]. A maximum of 91.66% rhizogenesis was recorded on ½ MS + B_5 vitamin + 20 mg L^{-1} IAA medium[17].

Since micropropagated plants are raised in the most congenial environmental conditions, hardening is imperative to ensure survival of the micropropagated plants upon transfer to soil under natural conditions. Therefore, plantlets were hardened in test tubes until culture media dried and nutrients depleted completely, thus forcing the plants to gradually shift to autotrophic mode of nutrition. The plugs of test tubes were then removed and kept open for 2-3 d. Thereafter, plants were carefully washed under running tap water to remove adhered nutrients and agar. The dead and yellow portions of tissue culture plantlets were removed with the help of scalpel blade and immersed in 0.1% bavistin (w/v) for 5 min. The plantlets were transferred in simple tap water for 15 d, which was daily supplied fresh (Fig. 5a-b.). The plantlets developed new roots within 10 d of transfer to water. The plantlets were transferred to different types of soil mixtures viz., vermiculite, perlite, cocopeat and mixture of three in the ratio of 1:1:1, when the roots were well developed (Fig. 5c.). Genotypes, Castle Rock and Punjab Upma gave maximum survival of 63.6 and 58.8% in 1:1:1 mixture of vermiculite, perlite and cocopeat, whereas genotypes, VFN-8 and IPA-3 recorded maximum survival rate of 57.4 and 61.8%, respectively in vermiculite (Table 3). All genotypes recorded

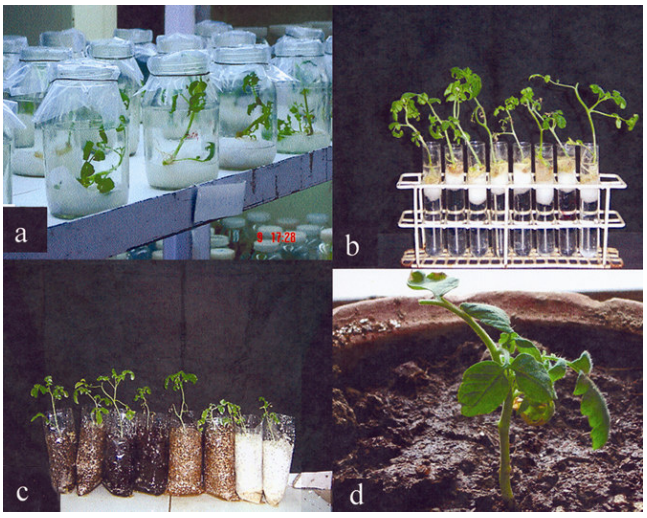

Fig. 5—Hardening and transfer of plantlets of tomato genotype IPA-3: a, b-Hardening of plantlets in simple water; c-Transfer of plantlets to various soil mixtures; & d-Establishment of plant in soil + FYM + sand (1:1:1) after 20 d of transfer.

Table 3—Per cent survival of regenerated plantlets of different tomato genotypes in different types of soil mixtures

Treatment	Genotype			
	Castle Rock	Punjab Upma	VFN-8	IPA-3
Vermiculite	60.20	58.40	57.40	61.80
Perlite	48.50	49.00	51.40	54.00
Cocopeat	38.40	39.10	40.60	42.10
1:1:1 all above	63.60	58.80	52.80	59.20

minimum per cent survival in the cocopeat. This could be attributed to its high water holding capacity, which might have clogged the fragile root system of the tissue culture regenerated plants. The plants thus obtained were then planted in the earthen pots (Fig. 5d) containing one part each of soil, well-rotten farmyard manure (FYM) and sand.

References

1 Aykroid W R, Gopalan C & Balasubramanian S C, The nutritive value of Indian foods and the planning of satisfactory diets, *Special Series ICMR*, 42 (1963) 1-255.

2 Rick C M, Tomato, in *Hybridization of crop plants*, edited by W R Fehr & H H Hadley (American Society of Agronomy, Madison) 1980, 669-680.

3 Engelman F, *In vitro* conservation of tropical plant germplasm–Review, *Euphytica*, 57 (1991) 227-243.

4 Bigot C, *In vitro* manipulation of higher plants: Some achievements, problems and perspectives, in *Cell culture techniques applied to plant production and plant breeding*, *Proc IAPTC French-British Meeting,* held on 8-9 Oct 1987, edited by J Boccon-Gibod, A Benbadis & K C E Shont, (Augers, France) 1987, 5-17.

5 Compton M E & Veilleux R E, Shoot, root and flower morphogenesis on tomato inflorescence explants, *Plant Cell Tissue Organ Cult,* 24 (1991) 223-231.

6 Cairney J, Xu N, Mackay J & Pullman J, Special symposium: *In vitro* plant recalcitrance transcript profiling: A tool to assess the development of conifer embryos. *In Vitro Cell Dev Biol-Plant,* 36 (2000) 152-162.

7 Koornneeff M, Bade J, Hanhart C, Horsman K, Schel J *et al*, Characterization and mapping of a gene controlling shoot regeneration in tomato, *Plant J*, 3 (1993) 131-141.

8 Faria R T & Illg R D, Inheritance of *in vitro* plant regeneration ability in the tomato, *Revis Brasil de Genet,* 19 (1996) 113-116.

9 Hamza S & Chupeau V, Re-evaluation of conditions for plant regeneration and *Agrobacterium*-mediated transformation from tomato (*Lycopersicon esculentum*), *J Exp Bot*, 269 (1993) 1837-1845.

10 Meredith C P, Shoot development in established callus cultures of cultivated tomato (*Lycopersicon esculentum* Mill.), *Zeit Fur Pflanz,* 95 (1979) 405-411.

11 Morgan A & Cocking E C, Plant regeneration from protoplasts of *Lycopersicon esculentum* Mill., *Zeit Fur Pflanz,* 106 (1982) 97-104.

12 Bhatia P, Ashwath N, Senaratna T & Mid More D, Tissue culture studies in tomato (*Lycopersicon esculentum* L.), *Plant Cell Tissue Organ Cult*, 78 (2004) 1-21.

13 Murashige T & Skoog F, A revised medium for rapid growth and bioassays with tobacco tissue cultures, *Physiol Plant*, 15 (1962) 473-497.

14 Frankenberger E A, Hasegawa P M & Tigchelaar E C, Influence of environment and development state on the shoot-forming capacity of tomato genotypes, *Zeit Fur Pflanz*, 102 (1981) 221-232.

15 Kurtz S M & Lineberger R D, Genotypic differences in morphogenic capacity of cultured leaf explants of tomato, *J Am Soc Hort Sci*, 108 (1983) 710-714.

16 Sukumar S & Shree Rangasamy S R, Response of *in vitro* leaf callus cultures for regeneration and evaluation of the regenerants in tomato, *Curr Sci*, 57 (1988) 890-892.

17 Selvi D T & Khader M A, *In vitro* morphogenetic capacity of tomato (*Lycopersicon esculentum* Mill.) var. PKM1, *South Indian Hort*, 41 (1993) 251-258.