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Research Paper

Effect of Plant Growth Regulators on Organogenesis in Tomato (*Lycopersicon esculentum* Mill.) cv. Dhanashri

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Abstract: *The present investigation was proposed to develop a reproducible and efficient regeneration protocol for organogenesis in tomato cv. Dhanashri. Seeds of tomato cv. Dhanashri were obtained from MPKV, Rahuri. The seedlings were raised aseptically on half-strength MS basal medium. Cotyledonary leaves and hypocotyls (1-2 cm), collected from 15days-old seedlings were excised and used as explants. Explants were cultured on MS basal medium supplemented with BAP (0.00-13.32 µM) and IAA(0.00 - 3.42 µM) alone and in various combinations. After 21–30 days when shoot buds were visible, shoots were separated from adhering callus and transferred for induction of rooting on MS basal medium supplemented with IBA (0.00-14.76 µM). The highest number of (7.8 ± 0.29) shoot per cotyledonary leaf explant was produced on MS + 6.65 µM BAP in combination with 1.14 µM IAA, which was the most optimum combination for shoot regeneration. The maximum numbers of shoots (5.6 ± 0.13) were observed on MS + 8.88 µM BAP in conjunction with 1.71 µM IAA. This combination was the optimum combination for induction of shoots from hypocotyl explants of tomato. Auxin containing medium (MS + 4.92 µM) resulted in a large proportion of rooted micro-shoots and early rooting. The results presented describe an efficient, reproducible and rapid tissue culture regeneration protocol.*

Keywords: Tomato, organogenesis.

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1. Introduction:

Tomato is considered as a prototypical plant for introduction of agronomically important genes (Wing *et al.*, 1994). Developing a reproducible *in vitro* regeneration protocol has been a subject of research because of the economic importance of the crop and its responsiveness for further improvement via genetic manipulation (Evans, 1989). It is one of the most studied higher plants because of a number of advantages for genetic, molecular and physiological studies (McCormick *et al*., 1986). The literature showed that the regeneration protocols have been made available for cultivated tomato using different explants viz., cotyledons, hypocotyl and leaf, different plant growth regulators like BAP, Kinetin, TDZ and Zeatin alone and also in combination with various concentrations of auxins (Zelcer *et al.*, 1984; Hamza and Chupeau, 1993; Ye Li and GL Zhou 1994; Plastira and Perdikaris 1997; Geetha *et al.*, 1998; Chen *et al* 1999; Gubis *et al.*, 2003; Sheeja *et al*. 2004; Rao*et al*., 2005; Ishag *et al*.2009; Mohamed *et al*., 2010; Praveen and Rama Swamy 2011; Vikram *et al*., 2011).

In vitro studies in various genotypes of tomato by means of different explants, viz. cotyledons, hypocotyls, epicotyls, meristem, leaf, stems, roots, internodes, petiole, anthers and inflorescences has been described by numerous investigators (Padmanabhan *et al.*, 1974; Behki*et.al.*, 1976; Kartha *et al.*, 1976, Ohki *et al.*, 1978; Frary and Earle, 1996; Gubis*et al.*, 2003; Raj *et al.*, 2005; Islam 2007; Chowdhury, 2008). The cotyledonary leaf segments explants reported to be the most responsive explants for tomato regeneration in various tomato varieties (McCormick, 1991; Frary and Earle, 1996; Gubis *et al.*, 2003; Islam, 2007, Chowdhury, 2008).

The success in tomato regeneration response has been genotype dependent, and also depended upon the type of explants and plant growth regulators used in the culture medium. Many workers reported that the regeneration response of tomato to plant growth regulators (PGRs) has been to be highly genotype-specific, and as such, the type and concentration appropriate for one genotype may not be most favorable for others (Frankenberger *et al*. 1982; Kurtz and Lineberger, 1983; Plastira and Perdikaris, 1997; Bhatia *et al*. 2004). However, there is no report on multiple shoots induction and regeneration from cotyledon and hypocotyl explants in tomato cv. Dhanashri.

2. Material and Methods:

Plant Material and *in Vitro* Culture:

Seeds of tomato variety Dhanashri were obtained from Department of Horticulture (Vegetable Wing), Mahatma PhuleKrushiVidhyapith, Rahuri, Ahmednagar. Seeds were thoroughly washed under running tap water, then placed in 5% Tween-20 for 5 min, followed by 3-4 rinses in sterile distilled water. They were surface sterilized with aqueous solution of 0.1% $HgCl_2$ for 4-5 min, followed by 4-5 rinses in sterile distilled water. The seeds were inoculated aseptically on half-strength MS basal medium. Cotyledonary leaves and hypocotyls (1-2 cm), collected from 15days-old seedlings were excised and used as explants. Explants were cultured on MS basal medium supplemented with BAP (0.00-13.32 µM) and IAA (0.00 - 3.42 µM) alone and in various combinations. After 21–30 days when shoot buds were visible, shoots were separated from adhering callus and transferred for induction of rooting on MS basal medium supplemented with IBA (0.00-14.76 µM).

For regeneration and rooting studies, MS media with 3% (w/v) sucrose and 0.8% (w/v) agar (Himedia, India) was used. The pH of the media was adjusted to 5.8 with 0.1N NaOH before autoclaving at 121^0C for 15 min. Each culture tube (150 X 25 mm) containing 20ml of media was inoculated with one explant and plugged with polypropylene cap. All cultures were maintained at 25 ± 2 ^{0}C under white fluorescent light (65 µE/m^2/ s) with 16 h photoperiod.

In vitro rooted plantlets were from culture vessels and washed with sterile distilled water. The plantlets were transferred to plastic cups containing garden soil mixed with vermiculite and sand (1:1:1). The plastic cups were covered with polythene bags to maintain high (70 to 80%) humidity

levels. Cups were kept in the growth room illuminated with 650-lux light intensity and 25 ± 2 ºC temperature. Pots were kept in the shade (650-lux light intensity) at temperature 25 ± 2°C with 60-55% relative humidity. These plantlets were nourished with $^{1}/_{2}$ strength MS basal medium at the interval of 4 days. These conditions were maintained for first 1 week. After 1 week polythene bags were removed and cups were kept in the shade for one week. Plantlets were exposed gradually to full sunlight, and then plantlets were taken to the field condition.

All the experiments were repeated at least three times and standard errors of the means were calculated. Data were analyzed by using analysis of variance procedure (ANOVA) in MS Excel computer program.

3. Results and Discussion

Plant growth regulators affect morphogenic response by altering various physiological processes. For tomato regeneration, a wide variety of plant growth regulators have been used at varying concentrations. The concentration of growth regulators employed was found to be dependent on the cultivar being cultured and the particular cytokinins or auxins used. Usually plantlets were regenerated either directly (Dwivedi *et al*., 1990), or from primary callus (Jawahar *et al*., 1997). A wide range of plant growth regulators (PGRs) at varying concentrations have been used along with different explants for different cultivars of tomato in various studies for callus induction and regeneration and the choice of the right explants is genotype dependent (Gubis *et al*., 2003; Park *et al*., 2003; Bhatia *et al*., 2005; Raj *et al*., 2005; Roy *et al*., 2006; Bhatia and Ashwath, 2008). Subculture of unorganized callus to a medium in which the ratio of cytokinin to auxin was increased, or in which there was only cytokinin present, lead to shoot differentiation (Gresshoff and Doy, 1972). In general, four major cytokinins viz. zeatin, 2-iP, BA, and kinetin, have been used either separately or in combination with auxins for organogenesis in tomato.

The development of viable callus and the successive regenerations into whole plants remains an experimental process. Therefore, to develop protocols for morphogenesis of commercially important cultivars is necessary for production of elite transgenic plants.

The results presented in the **Table 1 and 2, Plate I**., clearly indicates that MS + 6.65 µM BAP in combination with 1.14 µM IAA was the most optimum combination for shoot formation. At this concentration, maximum number (7.8 ± 0.29) of shoots was observed per cotyledonaryexplant.On the same line, several researchers have reported that BAP in combinations with IAA induce shoot formation in tomato (Abu-El-Heba, 2008; Sarker *et al*., 2009; Sakthivel and Manigandan, 2011; Zhang *et al*., 2012). However, the concentrations of hormones and the response varied with genotype (Gunay and Rao, 1980; Duzyaman *et al*. 1994; Plastira and Perdikaris, 1997; Costa *et al*. 2000). The regeneration response of tomato to plant growth regulators (PGRs) has been highly genotype-specific, and as such, the type and concentration appropriate for one genotype may be unfavorable for others (Frankenberger *et al*. 1982; Kurtz and Lineberger, 1983; Plastira and Perdikaris, 1997; and Bhatia *et al*. 2004).

From the results presented in the **Table 3**, **Plate I** clearly indicates that all the concentrations of IBA used in the experiments induced roots on *in vitro* grown shoot explants of tomato. IBA at 4.92 µM concentration was the best option for root induction where numbers of roots (18.4 ± 0.56) per explant was highest.

Induction of roots on *in vitro* grown shoots is essential for successful establishment of the plantlet in the soil. Auxins play an important role in the induction of roots to *in vitro* grown shoots. Rao *et al*., 2005; Chaudhry *et al*., 2010; Sakthivel and Manigandan, 2011; Vikram *et al*., 2011, 2012 have worked out the same in various genotypes of tomato.

Table 1: Effect of BAP with or without IAA on cotyledonary leaf explants of tomato cv. Dhanashri

	BAP µM	0.0	2.22	4.40	6.65	8.88	11.09	13.31
		Mean number of shoots /explant (±S.E.)						
IAA µM	0.0	------	1.8 ± 0.23	2.1 ± 0.67	3.1 ± 0.65	2.7 ± 0.86	1.6 ± 0.43	1.0 ± 0.23
	0.57	c	3.7 ± 0.98	5.1 ± 0.45	6.2 ± 0.78	4.2 ± 0.54	3.6 ± 0. 78	2.2 ± 0.83
	1.14	c, r	3.2 ± 0.20	4.2 ± 0.80	7.8 ± 0.29	4.0 ± 0.92	2.2 ± 0.54	1.6 ± 0.08
	1.71	c, r	2.8 ± 0.67	3.7 ± 0.68	4.8 ± 0.88	3.3 ± 0.38	1.4 ± 0.78	1.0 ± 0.17
	2.28	c,r	1.9 ± 0.06	2.4 ± 0.64	3.3 ± 0.87	2.8 ± 0.45	1.0 ± 0.05	c,r
	2.85	c,r	1.0 ± 0.05	1.2 ± 0.09	1.8 ± 0.44	1.0 ± 0.16	1.0 ± 0.09	c,r
	3.42	c,r	c,r	c,r	c,r	c,r	c,r	c,r

c –callus, r- rooting

Results are mean of three replicate (20 X 3) ± SE

Table 2: Effect of BAP with or without IAA on hypocotyl explants of tomato cv. Dhanashri BAP µM

	0.0		2.22	4.40	6.65	8.88	11.09	13.31
			Mean number of shoots /explant (±S.E.)					
IAA µM	0.0	------	1.2 ± 0.21	1.8 ± 0.89	2.6 ± 0.34	2.3 ± 0.86	1.2 ± 0.78	1.0 ± 0.34
	0.57	c	1.7 ± 0.87	2.8 ± 0.09	3.4 ± 0.55	3.7 ± 0.65	3.0 ± 0. 56	2.2 ± 0.83
	1.14	c, r	2.2 ± 0.76	3.2 ± 0.40	3.9 ± 0.68	5.1 ± 0.35	4.4 ± 0.87	3.6 ± 0.08
	1.71	c, r	2.8 ± 0.54	3.3 ± 0.43	4.3 ± 0.54	5.6 ± 0.13	3.9 ± 0.67	2.3 ± 0.75
	2.28	c, r	1.5 ± 0.78	2.3 ± 0.66	3.6 ± 0.76	4.1± 0.43	2.6 ± 0.71	1.2 ± 0.13
	2.85	c, r	1.0 ± 0.22	1.6 ± 0.05	2.3 ± 0.44	1.8 ± 0.39	1.5 ± 0.16	c, r
	3.42	c, r	c, r	c, r	c, r	c, r	c, r	c, r

c –callus, r- rooting

Results are mean of three replicate (20 X 3) ± SE

Table 3: Effect of IBA on rooting of *in vitro* grown shoot explants of tomato cv. Dhanashri

IBA (µM)	Mean no. of roots per explant
0.00	6.8 ± 0.59
2.46	13.8 ± 0.64
4.92	18.4 ± 0.56
7.38	15.0 ± 0.26
9.84	12.2 ± 0.52
12.3	8.4 ± 0.56

	14.76	5.6 ± 0.59

Results are mean of three replicate (20 X 3) ± SE.

Plate I

Plate I: Organogenesis in tomato cv. Dhanashri. a) 15 days old *in vitro* grown seedlings of tomato. b) Shoot regeneration from cotyledonary leaf explants on MS basal + 6.65 µM BAP + 1.14 µM IAA. c) Shoot regeneration from hypocotyl explants on MS basal + 8.88 µM BAP + 1.71 µM IAA. d) Rooting of *in vitro* grown shoots on MS basal + 4.92 µM IBA. e) Hardened plantlet in plastic cup.

4. Conclusions:

This study has shown that cotyledonary leaf explants was better responsive in terms of number of shoots per explant than hypocotyl explants. Cotyledonary leaf explants showed maximum shoots in MS basal medium supplemented with 6.65 µM BAP in conjunction with 1.14 µM IAA was better for shoots induction from cotyledonary leaf explants tomato cultivar Dhanashri. The best rooting was observed in MS basal medium supplemented with 4.92 µM IBA. This study is a baseline to carry further research on this tomato cultivar for improvement by using gene transfer technology.

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