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Effect of explants and media on plant regeneration in different tomato genotypes

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ABSTRACT

The explants were cultured on Murashige and Skoog (1962) medium supplemented with different concentrations of BAP and kinetin in combination with IAA and IBA 0.2 mg/l. Two media, i.e., MS basal medium fortified with BAP 2.5 mg/l + IAA 0.2 mg/l and kinetin 2.5 mg/l + IAA 0.2 mg/l showed the highest number of shoot formation and number of shoots per explant among 24 different media combinations tested. The cotyledon explant was found to be more efficient in shoot formation. The MS basal medium containing BAP 2.5 mg/l + IAA 0.2 mg/l for hypocotyl and MS basal medium supplemented with kinetin 2.5 mg/l + IAA 0.2 mg/l for cotyledon were found the best for days to shoot formation, percent shoot formation and number of shoots per explant. The regenerated shoots produced roots (100%) in both the cultivars Hisar Lalima and Hisar Anmol on MS medium containing 0.2 mg/l IAA and were successfully hardened and transferred to the screen-house.

Key words: Tomato, *Solanum lycopersicum*, explants, regeneration, genotype.

INTRODUCTION

Plant tissue culture techniques are recognized useful tools in crop improvement. Genetic transformation with regeneration *in vitro* has been successfully used for genetic improvement (Lindsey, 7). Resistance to biotic and abiotic stresses, tolerance to herbicide and improvement of quality are the most important goals of genetic plant modification. Biotechnology has great promises for the improvement and micro-propagation and offers an opportunity for rapid clonal multiplication of important plant species. Shoot formation from explants of apical meristems, cotyledons, stems, petioles, leaves, anthers and inflorescences has been reported in tomato (Rzepka-Plevnes, 13; Bhushan and Gupta, 2; Madhulatha *et al*., 8). These reports reveal that there is a differential response of various explants to different concentrations of auxins and cytokinins, which suggests that the hormonal requirements are highly specific for the different genotypes, differentiation and developmental stage of the explants, etc.

The most successful procedure up to date is regeneration through adventitious organogenesis (Osman *et al*., 11). *In vitro* plant regeneration has been found to depend on many factors, of which most important are composition of the basic medium, growth regulators, gelling agent, light intensity and quality photoperiod, temperature, cultivation vessels and vessel covers. The present studies were, therefore, undertaken to determine the effect of different explants and media combinations on plant regeneration from tomato genotypes.

MATERIALS AND METHODS

The experiment was conducted on cultivated tomato cultivars Hisar Lalima and Hisar Anmol in the laboratory of the Department of Biotechnology and Molecular Biology, CCS Haryana Agricultural University, Hisar during 2006-07. The seeds obtained from the Department of Vegetable Science were sterilized for 3 min. in 0.06% mercuric chloride ($HgCl_2$) and rinsed 7 to 8 times with sterile distilled water. The cultured seeds were germinated in glass containers with MS (Murashige and Skoog, 10) medium containing 3% sucrose and 0.6% agar. The cultures were maintained under 16 h light and 8 h dark conditions. Hypocotyls and cotyledons were used as the source of explants, which were excised in a laminar air-flow from 25-to 30-day-old seedlings grown in aseptic conditions at 25 ± 1ºC temperature. Hypocotyls were cut into small pieces about 2 to 3 mm, and then, placed on shoot proliferation medium (MS basal medium, sucrose 3.0% and agar 0.8%). Each cotyledon was transversally cut

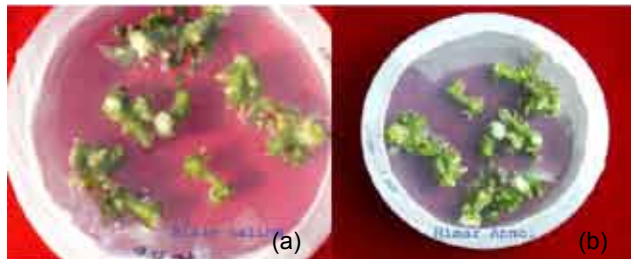

Fig. 1. Shoot initiation from hypocotyl explant in tomato cvs. (a) Hisar Lalima and (b) Hisar Anmol.

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into a proximal and a distil half-segment and placed horizontally with the adaxial surface in contact with the medium. The media were adjusted to pH 5.8 prior to autoclaving at 121ºC with 15 psi for 15 min. The regeneration ability of explants was assessed 6 to 7 weeks later. The parameters evaluated were percent shoot formation, the number of shoots per explant and percent root formation. The data related to different characters were analyzed statistically by applying the analysis of variance technique as suggested by Cochran and Cox (4).

RESULTS AND DISCUSSION

The response of each genotype used in regeneration study was different due to genetic variability. The regeneration frequency was found to be dependent on the concentration of the different cytokinins and combinations of cytokinins with auxins used in the culture media. Overall, 24 different media were used for the *in vitro* regeneration. Different responses were obtained from different explant sources, i.e., hypocotyls and cotyledons, however, the cotyledon was found to be the best source for callus and percent shoot formation. The maximum percent shoot formation was obtained from Hisar Lalima (81.50%) followed Hisar Anmol (65.43%) under MS medium supplemented with kinetin 2.5 mg/l + IAA 0.2 mg/l (Table 1). These results are in compliance with the results of Raziuddin *et al*. (12) who found 94.48% callus formation in the cv. Roma

Table 1. Percent shoot formation from cotyledon explants of two tomato genotypes.

MS medium +	Hisar Lalima	Hisar Anmol	Mean
BAP 0.5 mg/l	10.83 (17.36)	03.70 (09.33)	(13.35)
BAP 1.0 mg/l	37.95 (38.30)	33.61 (35.71)	(37.00)
BAP 2.5 mg/l	51.50 (46.12)	31.73 (34.56)	(40.34)
BAP 5.0 mg/l	21.90 (33.36)	08.33 (12.80)	(23.08)
Kin 0.5 mg/l	00.00 (04.05)	00.00 (04.05)	(04.05)
Kin 1.0 mg/l	26.66 (31.38)	39.16 (38.95)	(35.16)
Kin 2.5 mg/l	56.60 (49.06)	23.43 (29.16)	(39.11)
Kin 5.0 mg/l	25.56 (30.68)	12.10 (15.14)	(22.91)
BAP 0.5 + IAA 0.2 mg/l	00.00 (04.05)	00.00 (04.05)	(04.05)
BAP 1.0 + IAA 0.2 mg/l	38.00 (38.37)	50.33 (45.46)	(41.88)
BAP 2.5 + IAA 0.2 mg/l	51.83 (46.32)	60.24 (51.19)	(48.75)
BAP 5.0 + IAA 0.2 mg/l	59.53 (50.82)	45.16 (42.48)	(46.65)
Kin 0.5 + IAA 0.2 mg/l	00.00 (04.05)	00.00 (04.05)	(04.05)
Kin 1.0 + IAA 0.2 mg/l	41.63 (40.42)	44.14 (42.06)	(41.24)
Kin 2.5 + IAA 0.2 mg/l	81.50 (60.09)	65.43 (54.33)	(49.71)
Kin 5.0 + IAA 0.2 mg/l	43.33 (41.42)	37.47 (38.01)	(39.71)
BAP 0.5 + IBA 0.2 mg/l	00.00 (04.05)	00.00 (04.05)	(04.05)
BAP 1.0 + IBA 0.2 mg/l	20.10 (26.96)	24.90 (30.23)	(28.59)
BAP 2.5 + IBA 0.2 mg/l	30.43 (33.76)	13.50 (21.80)	(27.78)
BAP 5.0 + IBA 0.2 mg/l	40.31 (39.69)	18.70 (25.97)	(32.83)
Kin 0.5 + IBA 0.2 mg/l	00.00 (04.05)	00.00 (04.05)	(04.05)
Kin 1.0 + IBA 0.2 mg/l	00.00 (04.05)	13.33 (21.57)	(12.81)
Kin 2.5 + IBA 0.2 mg/l	45.90 (42.91)	25.66 (30.75)	(36.83)
Kin 5.0 + IBA 0.2 mg/l	00.00 (04.05)	00.00 (04.05)	(04.05)
Mean	28.81 (29.18)	22.96 (25.16)	
CD (p = 0.05)			
Genotype	1.583		
Medium	5.484		
Genotype x medium	7.756		

*Figures in parenthesis are angular transformed values.

Table 2. Percent shoot formation from hypocotyl explant in two tomato genotypes.

MS medium +	Hisar Lalima	Hisar Anmol	Mean
BAP 0.5 mg/l	00.00 (04.05)	26.75 (31.45)	(17.75)
BAP 1.0 mg/l	35.11 (36.61)	67.86 (55.99)	(46.30)
BAP 2.5 mg/l	61.46 (52.91)	62.70 (52.69)	(52.85)
BAP 5.0 mg/l	27.42 (31.87)	41.16 (40.15)	(36.01)
Kin 0.5 mg/l	00.00 (04.05)	00.00 (04.05)	(04.05)
Kin 1.0 mg/l	12.70 (21.13)	28.96 (32.82)	(26.98)
Kin 2.5 mg/l	23.33 (29.18)	40.56 (39.80)	(34.49)
Kin 5.0 mg/l	00.00 (04.05)	11.66 (17.75)	(10. 90)
BAP 0.5 + IAA 0.2 mg/l	00.00 (04.05)	00.00 (04.05)	(04.05)
BAP 1.0 + IAA 0.2 mg/l	50.35 (45.47)	47.66 (43.92)	(44.70)
BAP 2.5 + IAA 0.2 mg/l	69.44 (56.97)	68.73 (56.32)	(56.65)
BAP 5.0 + IAA 0.2 mg/l	46.65 (43.34)	44.33 (41.99)	(42.66)
Kin 0.5 + IAA 0.2 mg/l	00.00 (04.05)	00.00 (04.05)	(04.05)
Kin 1.0 + IAA 0.2 mg/l	56.11 (48.79)	42.95 (41.21)	(45.00)
Kin 2.5 + IAA 0.2 mg/l	47.19 (43.65)	62.06 (52.26)	(47.95)
Kin 5.0 + IAA 0.2 mg/l	48.50 (44.40)	38.36 (38.54)	(41.47)
BAP 0.5 + IBA 0.2 mg/l	00.00 (04.05)	00.00 (04.05)	(04.05)
BAP 1.0 + IBA 0.2 mg/l	00.00 (04.05)	00.00 (04.05)	(04.05)
BAP 2.5 + IBA 0.2 mg/l	28.52 (32.54)	30.43 (33.76)	(33.15)
BAP 5.0 + IBA 0.2 mg/l	31.94 (34.68)	33.76 (35.79)	(35.23)
Kin 0.5 + IBA 0.2 mg/l	00.00 (04.05)	10.00 (13.87)	(08.90)
Kin 1.0 + IBA 0.2 mg/l	40.86 (40.01)	09.52 (13.57)	(26.70)
Kin 2.5 + IBA 0.2 mg/l	31.61 (34.50)	23.70 (28.87)	(31.68)
Kin 5.0 + IBA 0.2 mg/l	00.00 (04.05)	00.00 (04.05)	(04.05)
Mean	26.46 (26.35)	28.77 (28.96)	
CD (p = 0.05)			
Genotype	2.126		
Medium	7.367		
Genotype x medium	10.42		

*Figures in parenthesis are angular transformed values.

and 92.15% in the cv. Rio when cotyledon explants were cultured on the medium containing kinetin 2.0 mg/l + IAA 0.2 mg/l, and Camillo *et al*. (3) also used cotyledon explants for *in vitro* studies due to its high calli production ability.

The hypocotyls of cv. Hisar Lalima showed the highest shoot regeneration frequency (69.45%) than that of cv. Hisar Anmol (68.75%) on MS medium fortified with BAP 2.5 mg/l + IAA 0.2 mg/l (Table 2). The results observed in the present study are in accordance with the results of Sukumar and Rangaswamy (15). In present study, the BAP was found to be the effective plant growth regulator for hypocotyls indicating cytokinin specificity for shoot bud regeneration and multiple shoot induction in these tissues. These results are also in close conformity with the earlier results of Singh *et al*. (14) who also found the BAP to be more suitable as compared to kinetin for maximum shoot bud differentiation as well as multiple shoot induction. Similarly, Lengliz *et al*. (6) achieved shoot organogenesis on media containing zeatin/IAA combination.

The MS basal medium containing BAP 2.5 mg/l + IAA 0.2 mg/l with sucrose 30 g/l resulted in maximum number of shoots in cvs. Hisar Lalima and Hisar Anmol (8.07 and 7.53 shoots per hypocotyl explant, respectively), however, the number of shoots regenerated from cotyledon explants showed the maximum shoots per explant (8.39) in cv. Hisar Lalima followed by 8.06 shoots in Hisar Anmol on the same

Table 3. Number of shoots regenerated on hypocotyl explant in two tomato genotypes.

MS medium +	Hisar Lalima	Hisar Anmol	Mean
BAP 0.5 mg/l	0.00 (1.22)	1.66 (1.63)	(1.42)
BAP 1.0 mg/l	2.30 (1.94)	2.91 (2.09)	(2.01)
BAP 2.5 mg/l	2.03 (1.87)	6.08 (2.75)	(2.31)
BAP 5.0 mg/l	1.66 (1.77)	1.85 (1.83)	(1.41)
Kin 0.5 mg/l	0.00 (1.22)	0.00 (1.22)	(1.22)
Kin 1.0 mg/l	0.00 (1.22)	1.91 (1.83)	(1.52)
Kin 2.5 mg/l	2.00 (1.86)	3.58 (2.24)	(2.05)
Kin 5.0 mg/l	1.58 (1.74)	0.94 (1.54)	(1.64)
BAP 0.5 + IAA 0.2 mg/l	0.00 (1.22)	0.00 (1.22)	(1.22)
BAP 1.0 + IAA 0.2 mg/l	4.46 (2.44)	4.58 (2.46)	(2.45)
BAP 2.5 + IAA 0.2 mg/l	8.07 (3.09)	7.53 (3.00)	(3.04)
BAP 5.0 + IAA 0.2 mg/l	2.73 (2.05)	3.13 (2.15)	(2.10)
Kin 0.5 + IAA 0.2 mg/l	0.00 (1.22)	0.00 (1.22)	(1.22)
Kin 1.0 + IAA 0.2 mg/l	2.98 (2.11)	2.83 (2.07)	(2.09)
Kin 2.5 + IAA 0.2 mg/l	6.30 (2.79)	4.41 (2.43)	(2.61)
Kin 5.0 + IAA 0.2 mg/l	4.71 (2.49)	3.33 (2.19)	(2.34)
BAP 0.5 + IBA 0.2 mg/l	0.00 (1.22)	0.00 (1.22)	(1.22)
BAP 1.0 + IBA 0.2 mg/l	0.00 (1.22)	0.00 (1.22)	(1.22)
BAP 2.5 + IBA 0.2 mg/l	4.65 (2.48)	2.48 (1.99)	(2.23)
BAP 5.0 + IBA 0.2 mg/l	1.83 (1.82)	1.50 (1.73)	(1.77)
Kin 0.5 + IBA 0.2 mg/l	0.00 (1.22)	0.00 (1.22)	(1.22)
Kin 1.0 + IBA 0.2 mg/l	1.41 (1.70)	2.25 (1.93)	(1.81)
Kin 2.5 + IBA 0.2 mg/l	2.01 (1.87)	2.58 (2.02)	(1.94)
Kin 5.0 + IBA 0.2 mg/l	0.00 (1.22)	0.00 (1.22)	(1.22)
Mean	2.03 (1.79)	2.21 (1.85)	
CD (p = 0.05)			
Genotype	0.031		
Medium	0.109		
Genotype x medium	0.155		

*Figures in parenthesis are square root transformed values.

MS medium supplemented with kinetin 2.5 mg/l + IAA 0.2 mg/l (Tables 3 & 4). These results are in agreement with the earlier results of Moghaieb *et al*. (9) who obtained about 7.2 shoots per explant using 1 mg/l zeatin in the medium.

Cent percent rooting was recorded in both the genotypes on MS medium + IAA 0.2 mg/l, which is in compliance with the results of Devi *et al*. (5) who reported the maximum rooting (91.66%) on half-strength MS medium + B_5 vitamin + IAA 2.0 mg/l. In the cultivars Hisar Anmol and Hisar Lalima, the maximum rooting (86.66 and 76.66%, respectively) took place on simple MS basal medium. The cvs. present findings are in line with those of Bhatia *et al*. (1) who observed that the morphogenesis response seems to be highly dependent PGRs used in the media, which is again cultivar and genotypic specific, however, the maximum root formation was observed on containing IAA rooting medium (Table 5).

The regenerated plants from hypocotyl and cotyledon explants of both the cultivars were established successfully, *i.e.*, 100% in the pots in the screen-house.

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Table 4. Number of shoots regenerated per cotyledon explants of two tomato genotypes.

MS medium +	Hisar Lalima	Hisar Anmol	Mean
BAP 0.5 mg/l	1.33 (1.67)	0.33 (1.34)	(1.50)
BAP 1.0 mg/l	2.16 (1.91)	1.80 (1.81)	(1.86)
BAP 2.5 mg/l	3.10 (2.14)	2.61 (2.02)	(2.08)
BAP 5.0 mg/l	1.38 (1.69)	1.53 (1.70)	(1.69)
Kin 0.5 mg/l	0.00 (1.22)	0.00 (1.22)	(1.22)
Kin 1.0 mg/l	2.55 (2.01)	1.65 (1.77)	(1.89)
Kin 2.5 mg/l	1.60 (1.76)	1.69 (1.78)	(1.77)
Kin 5.0 mg/l	1.11 (1.61)	0.50 (1.39)	(1.50)
BAP 0.5 + IAA 0.2 mg/l	0.00 (1.22)	0.00 (1.22)	(1.22)
BAP 1.0 + IAA 0.2 mg/l	3.63 (2.26)	4.06 (2.35)	(2.30)
BAP 2.5 + IAA 0.2 mg/l	3.56 (2.25)	4.75 (2.49)	(2.37)
BAP 5.0 + IAA 0.2 mg/l	5.84 (2.70)	5.75 (2.69)	(2.69)
Kin 0.5 + IAA 0.2 mg/l	0.00 (1.22)	0.00 (1.22)	(1.22)
Kin 1.0 + IAA 0.2 mg/l	2.67 (2.04)	3.20 (2.16)	(2.10)
Kin 2.5 + IAA 0.2 mg/l	8.39 (3.14)	8.06 (3.09)	(3.11)
Kin 5.0 + IAA 0.2 mg/l	3.60 (2.23)	3.66 (2.27)	(2.25)
BAP 0.5 + IBA 0.2 mg/l	0.00 (1.22)	0.00 (1.22)	(1.22)
BAP 1.0 + IBA 0.2 mg/l	1.66 (1.77)	2.15 (1.91)	(1.88)
BAP 2.5 + IBA 0.2 mg/l	4.09 (2.36)	1.48 (1.72)	(2.04)
BAP 5.0 + IBA 0.2 mg/l	2.65 (2.03)	1.31 (1.67)	(1.85)
Kin 0.5 + IBA 0.2 mg/l	0.00 (1.22)	0.00 (1.22)	(1.22)
Kin 1.0 + IBA 0.2 mg/l	0.00 (1.22)	2.16 (1.91)	(1.56)
Kin 2.5 + IBA 0.2 mg/l	5.11 (2.57)	2.71 (2.05)	(2.31)
Kin 5.0 + IBA 0.2 mg/l	0.00 (1.22)	0.00 (1.22)	(1.22)
Mean	2.25 (1.86)	2.07 (1.81)	
CD (p = 0.05)			
Genotype	0.034		
Medium	0.119		
Genotype x medium	0.170		

*Figures in parenthesis are square root transformed values.

Table 5. Percent root formation on regenerated micro-shoots in tomato.

Medium	Hisar Lalima	Hisar Anmol
MS basal	76.66	86.66
MS basal + IAA 0.2 mg/l	100.0	100.0
CD (p = 0.05)	9.50	9.50

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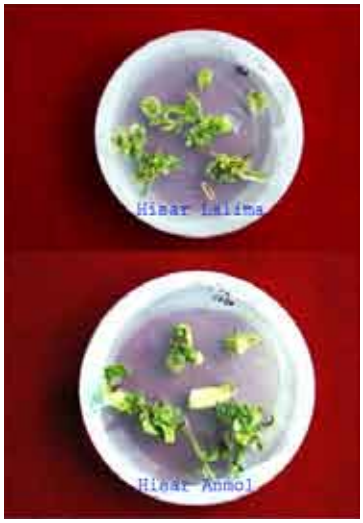

Fig. 2. Shoot initiation from cotyledon explants.

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