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Effect of explant orientation on shoot regeneration in tomato (*Lycopersicon esculentum*)

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ABSTRACT

Effects of explant orientation on percent shoot regeneration in tomato (*Lycopersicon esculentum* M.) were studied using two commercially important cultivars, viz Hisar Arun and Hisar Lalit. The explant orientation affected shoot regeneration in both genotypes. Both cotyledon and leaf explants placed in abaxial (lower surface facing down) orientation produced higher percent regeneration and higher number of shoots per explant as compared to those placed in adaxial (upper surface facing down) orientation. In Hisar Arun, when cotyledon explants cultured in abaxial orientation on medium containing zeatin 1.5 mg/l, maximum per cent shoot formation was 80.63% while in adaxial orientation it was decreased to 42.50%. Similar pattern of decrease in percent regeneration from 75.73% to 27.31%, on changing the orientation of cotyledon explants, was observed in cv. Hisar Lalit on medium containing BAP 1.5 mg/l. In leaf explants of cv. Hisar Arun, on medium supplemented with zeatin 1.5 mg/l, percent shoot regeneration reduced from 46.93% to 32.78% on changing the orientation from abaxial to adaxial. In cv. Hisar Lalit, per cent shoot regeneration was reduced to less than half (75.00% to 36.11%), on medium containing BAP 1.5 mg/l when the leaf explant were placed adaxially. The present study revealed that orientation of explant had significant effect on per cent regeneration.

Key words: Abaxial, Adaxial, BAP, NAA, Orientation, Zeatin

Tomato (*Lycopersicon esculentum* M.) is the second most popular vegetable crop next to potato in the world. Tomato is mostly grown from hybrid seeds, which are expensive to produce due to involvement of manual labor for emasculation and pollination. An efficient tissue culture system may produce hybrid plantlets at low cost. As tomato is grown world-wide, including in marginal and submarginal lands, a good regeneration system may aid in genetic engineering techniques to develop cultivars resistant to various biotic and abiotic stresses. The majority of researchers who have worked on regeneration of tomato have tested one or a few cultivars of tomato for their ability to produce callus and shoots (Costa *et al*. 2000a, Costa *et al*. 2000b, Venkatachalam *et al*. 2000). Since the genotypes differ markedly in their response (Stommel and Sinden 1991, El-Farash *et al*. 1993), it is important to test a wide range of cultivars to develop a universally applicable protocol for shoot regeneration in tomato. The regeneration response of tomato to plant growth regulators (PGRs) has been observed to be highly genotype-specific, and as such, the type and concentration suitable for one genotype may not be optimal for others (Frankenberger *et al*. 1981, Kurtz and Lineberger 1983, Plastira and Perdikaris 1997, Bhatia *et al*. 2004, Mamidala and Nanna 2011).

The success of regeneration depends not only on the type of the explant chosen, but also the way explants are placed on the culture media (Duzyaman *et al*. 1994). Explants can be inoculated on the culture media in polar (straight up, with the physiological base in the medium) or apolar (upside down, physiological base out of the medium) position for the hypocotyls and shoots, and abaxial (lower surface facing down) or adaxial (upper surface facing down) orientation for the cotyledons and leaves (George 1993). The current study was undertaken to evaluate the effects of explant orientation on regeneration capacity of cotyledon and leaf explant of two cultivars of tomato.

MATERIALS AND METHODS

The seeds of *Lycopersicon esculentum* cultivars Hisar Arun and Hisar Lalit were obtained from Department of Vegetable Sciences, College of Agriculture, CCS Haryana Agricultural University, Hisar, India. The seeds were washed several times under running tap water containing two drops

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of teepol. After this, the seeds were sterilized with freshly prepared 0.1% mercuric chloride ($HgCl_2$) solution for one minute in laminar air flow followed by several washings with sterilized distilled water and then aseptically inoculated on the germination medium. The tomato seeds were germinated in the culture room at 25 ± 1°C and 16 hr light/ 8 hr dark photoperiod. Explants were excised from 15-20 days old seedlings in a laminar airflow hood to procure the desired explants. Cotyledons and leaves were used as explants for shoot regeneration. Cotyledons were excised only when there were no or minimal true leaves present on the seedlings. These explants were placed on the culture medium in both abaxial and adaxial orientation.

MS basal medium (Murashige and Skoog 1962) was used for *in vitro* seed germination and the plant regeneration media. The pH of the medium was adjusted to 5.8 using 1 N NaOH or 1 N HCl prior to adding gelling agent. The MS medium fortified with different concentrations of auxins and cytokinins was used for regeneration experiments (Table 1).

The culture plates were placed in a culture room at 25 ± 1°C and 16 hr light/8 hr dark photoperiod. The experiments were repeated thrice and the data was transformed using angular transformation and analyzed following factorial CRD.

RESULTS AND DISCUSSION

The results of the current experiment demonstrated shoot

Table 1 Regeneration response in two cultivars of tomato on MS medium supplemented with different concentrations and combinations of growth regulators (BAP:NAA – AB1 to ABN4; zeatin:NAA – AZ1 to AZN4; in mg/l)

Media code	Hisar Arun				Hisar Lalit			
	Cotyledon Abaxial	Cotyledon Adaxial	Leaf Abaxial	Leaf Adaxial	Cotyledon Abaxial	Cotyledon Adaxial	Leaf Abaxial	Leaf Adaxial
AB1 (1.5:0)	65.12 (53.77±0.8)	32.32 (34.56±0.6)	23.91 (29.25±0.7)	22.74 (28.43±1.1)	75.73 (60.48±1.0)	27.31 (31.49±0.5)	75.00 (59.99±0.8)	36.11 (36.91±0.8)
AB2 (2.0:0)	57.09 (49.05±0.4)	28.81 (32.45±0.6)	13.32 (21.39±0.5)	16.96 (24.29±0.8)	70.42 (57.03±0.6)	24.07 (29.35±0.6)	46.67 (43.07±0.9)	14.26 (22.10±1.3)
AB3 (3.0:0)	43.88 (41.47±0.3)	13.39 (21.45±0.1)	12.09 (20.24±0.9)	14.56 (22.36±1.3)	42.19 (40.49±0.5)	19.64 (26.29±0.6)	30.20 (33.30±1.0)	10.37 (18.61±1.9)
AB4 (4.5:0)	16.67 (24.03±1.2)	11.13 (19.47±0.3)	11.66 (19.87±1.4)	10.94 (19.27±0.8)	21.12 (27.32±0.8)	14.30 (22.18±0.8)	24.44 (29.45±2.8)	8.88 (17.31±0.5)
ABN1 (1.5:0.1)	13.33 (21.32±1.4)	13.33 (21.30±1.6)	17.50 (24.69±0.9)	18.33 (26.44±2.0)	28.76 (32.41±0.5)	19.43 (26.10±1.1)	25.00 (29.96±1.0)	12.42 (20.58±1.0)
ABN2 (2.0:0.1)	9.50 (17.87±0.7)	10.17 (18.59±0.1)	12.93 (21.02±1.1)	11.67 (19.87±1.4)	20.52 (26.90±0.7)	13.26 (21.34±0.3)	14.03 (21.98±0.5)	5.83 (13.90±0.9)
ABN3 (3.0:0.1)	7.50 (15.95±0.5)	6.29 (14.47±0.9)	12.50 (20.63±1.2)	10.00 (18.42±0.0)	18.21 (25.24±0.4)	12.48 (20.52±1.8)	12.80 (20.85±1.6)	4.30 (13.71±2.6)
ABN4 (4.5:0.1)	2.97 (9.90±0.3)	2.94 (9.86±0.0)	10.83 (19.18±0.7)	9.46 (17.86±1.4)	16.27 (23.75±0.8)	11.12 (19.39±1.4)	9.73 (17.90±2.3)	3.13 (10.16±0.5)
AZ1 (1.5:0)	80.63 (63.89±1.0)	42.50 (40.67±0.4)	46.93 (43.21±1.3)	32.78 (34.90±0.9)	74.47 (59.63±0.7)	32.37 (34.65±1.0)	59.15 (50.25±0.8)	29.26 (32.70±1.2)
AZ2 (2.0:0)	68.42 (55.78±0.7)	29.28 (32.73±0.6)	45.56 (42.43±0.3)	26.09 (30.69±0.8)	69.38 (56.40±1.1)	24.08 (29.34±1.2)	52.71 (46.53±0.7)	21.35 (27.47±1.2)
AZ3 (3.0:0)	67.67 (55.33±0.8)	15.83 (23.40±1.0)	39.04 (38.65±0.5)	23.89 (29.20±1.3)	59.88 (50.68±0.4)	20.67 (26.99±1.2)	33.33 (35.23±1.0)	16.67 (24.03±1.2)
AZ4 (4.5:0)	15.00 (22.45±1.1)	9.17 (17.34±2.3)	18.33 (22.29±1.2)	8.89 (17.27±1.1)	23.81 (29.16±1.0)	16.62 (24.02±0.9)	25.00 (29.98±0.0)	11.34 (19.64±0.9)
AZN1 (1.5:0.1)	23.33 (28.65±3.0)	21.66 (27.69±1.1)	12.96 (21.01±1.3)	13.17 (21.18±1.6)	33.25 (35.17±1.3)	22.57 (28.34±0.6)	34.07 (35.67±1.5)	14.17 (21.89±2.4)
AZN2 (2.0:0.1)	18.89 (25.73±0.8)	12.22 (20.41±0.9)	11.11 (19.42±0.9)	6.47 (14.72±0.2)	22.33 (28.13±1.5)	19.19 (25.92±1.3)	22.59 (28.30±1.7)	6.87 (15.12±1.1)
AZN3 (3.0:0.1)	8.33 (16.59±1.8)	5.83 (13.90±0.9)	6.69 (14.87±1.2)	5.55 (13.47±1.4)	17.17 (24.47±0.3)	12.27 (20.43±1.2)	14.44 (22.29±0.8)	5.60 (13.67±0.4)
AZN4 (4.5:0.1)	5.00 (12.91±0.0)	0.00	3.98 (11.39±1.1)	0.00	14.80 (22.55±1.3)	11.76 (19.95±1.5)	8.59 (16.97±1.0)	4.17 (11.64±1.2)

*Values in parenthesis are transformed values with corresponding standard error.

regeneration in two tomato cultivars of high commercial importance. Cultured explants showed signs of regeneration within three weeks. Shoot clusters emerged mostly from the proximal end of the cotyledons. Occasionally, shoots were also initiated from the remaining parts of the cotyledons. Morphogenic response varied with respect to cultivar, explant type, orientation and hormone combination. Genotypes exhibited significant variation in regeneration response ranging from 3.98% to 80.63% in abaxial orientation and from zero to 42.50% in adaxial orientation. Better shoot regeneration response in abaxial orientation was evident for both the genotypes.

In cv. Hisar Arun, the cotyledon explants when cultured abaxial surface touching to the medium (Table 1) containing BAP (1.5 mg/l to 4.5 mg/l) and zeatin (1.5 mg/l to 4.5 mg/l), the maximum shoot regeneration observed was 65.12% (on medium AB1) and 80.63% (AZ1), respectively and on changing the cotyledon orientation to adaxial, maximum shoot regeneration on these media was reduced to 32.22% and 42.50%, respectively. In case of media supplemented with zeatin, the per cent shoot regeneration was higher as compared to medium containing BAP. The per cent shoot regeneration decreased with increase in BAP and zeatin concentration. Average number of shoots per explant was higher on media with BAP and zeatin alone than on media with NAA. It was also observed that number of shoots per explant was lower in cotyledon explants cultured in adaxial orientation as compared to the explants cultured in abaxial orientation.

In leaf explants (abaxial orientation) of cv. Hisar Arun, maximum shoot regeneration was 46.93% with 1.96 average number of shoots per explant (Table 1 and 2). When the orientation of leaf explants changed from abaxial to adaxial, maximum shoot regeneration reduced to 32.78%. Not only per cent shoot regeneration but number of shoots per explant was also less in leaf explants cultured in adaxial orientation as compared to the explants cultured in abaxial orientation.

Similar results were obtained in cv. Hisar Lalit. In cv. Hisar Lalit, the cotyledon explants when cultured abaxial surface touching to the medium, maximum shoot regeneration was 75.73% that reduced to less than half (27.31%) on changing the orientation from abaxial to adaxial on the same medium. In leaf explants cultured abaxially the maximum shoot regeneration (75.00%, Table 1) was observed on medium AB1 with average number of shoots per explant 1.4. Medium AB1 also showed maximum regeneration response on changing the orientation of leaf explants but it was only 36.11%. The per cent shoot regeneration in leaf (abaxial) explant was higher in cv. Hisar Lalit than in cv. Hisar Arun except on medium ABN4. Shoot regeneration was less on media containing zeatin than on media containing BAP that was not in case of cotyledon explants. Shoot regeneration decreased with increase in zeatin concentration and also on addition of NAA as was observed with cotyledon explants.

In our study, the per cent regeneration response decreased when the orientation of cotyledon and leaf explants changed from abaxial to adaxial even on the same medium. Bhatia *et al.* (2005) studied the effect of orientation on shoot

Table 2 Mean shoots per explant in two cultivars of tomato on MS medium supplemented with different concentrations and combinations of growth regulators (BAP:NAA – AB1 to ABN4; zeatin:NAA – AZ1 to AZN4; in mg/l)

Media code	Hisar Arun				Hisar Lalit			
	Cotyledon Abaxial	Cotyledon Adaxial	Leaf Abaxial	Leaf Adaxial	Cotyledon Abaxial	Cotyledon Adaxial	Leaf Abaxial	Leaf Adaxial
AB1 (1.5:0)	1.97±0.1	2.50±0.3	1.80±0.3	2.13±0.3	1.86±0.0	1.53±0.1	1.46±0.0	1.10±0.1
AB2 (2.0:0)	1.93±0.2	1.63±0.3	1.36±0.2	1.80±0.1	1.87±0.1	1.30±0.1	1.57±0.1	1.63±0.2
AB3 (3.0:0)	1.97±0.1	1.60±0.3	1.80±0.1	1.30±0.1	1.33±0.0	1.40±0.0	1.33±0.1	1.00±0.0
AB4 (4.5:0)	2.33±1.0	2.83±0.6	1.00±0.0	1.13±0.1	1.33±0.1	1.33±0.0	1.17±0.1	1.00±0.0
ABN1 (1.5:0.1)	1.27±0.1	1.20±0.2	1.80±0.1	1.20±0.1	1.20±0.1	1.27±0.1	1.93±0.1	1.00±0.0
ABN2 (2.0:0.1)	1.17±0.1	1.33±0.3	1.27±0.0	1.10±0.1	1.43±0.1	1.40±0.2	1.36±0.2	1.17±0.1
ABN3 (3.0:0.1)	1.10±0.1	2.10±0.7	1.20±0.1	2.00±0.0	1.23±0.1	1.50±0.1	1.80±0.1	2.10±0.7
ABN4 (4.5:0.1)	1.00±0.0	1.00±0.0	1.67±0.2	1.53±0.2	1.63±0.1	1.00±0.0	1.33±0.1	1.00±0.0
AZ1 (1.5:0)	2.37±0.1	2.36±0.3	1.96±0.0	1.37±0.1	2.00±0.1	1.23±0.1	1.27±0.2	1.20±0.1
AZ2 (2.0:0)	2.07±0.2	2.76±0.1	2.50±0.1	2.50±0.1	2.40±0.1	1.43±0.1	1.70±0.1	1.83±0.1
AZ3 (3.0:0)	1.67±0.1	1.33±0.1	2.00±0.2	2.10±0.2	1.57±0.0	1.16±0.1	1.53±0.0	1.20±0.1
AZ4 (4.5:0)	1.83±0.3	1.56±0.2	1.80±0.1	1.83±0.2	1.60±0.0	1.33±0.1	1.67±0.2	1.70±0.2
AZN1 (1.5:0.1)	1.40±0.2	1.00±0.0	1.60±0.3	1.56±0.2	1.27±0.0	1.33±0.1	1.16±0.1	1.00±0.0
AZN2 (2.0:0.1)	1.33±0.0	1.00±0.0	1.70±0.2	1.67±0.3	1.23±0.1	1.17±0.1	1.27±0.0	1.10±0.1
AZN3 (3.0:0.1)	1.16±0.1	1.17±0.1	1.10±0.1	1.00±0.0	1.23±0.1	1.19±0.1	1.10±0.1	1.00±0.0
AZN4 (4.5:0.1)	1.33±0.3	0.00	1.33±0.3	0.00	1.00±0.0	1.10±0.1	1.00±0.0	1.33±0.3

regeneration from cotyledon explants in ten cultivars of tomato and found that per cent shoot regeneration decreased when the cotyledon explants were cultured in adaxial orientation. In contrary to this Costa *et al*. (2000a) reported that position of cotyledon segments did not affect the regeneration frequency.

Results of this experiment indicate that the abaxial orientation induces better shoot regeneration than its adaxial counterpart. However, the extent of the response varied with the cultivars being used, as demonstrated by the significant genotype × explant orientation interaction. The current observations are contrary to those reported by Costa *et al.* (2000b), who found that the orientation of the cotyledonary segments had no effect on the average regeneration frequency and shoot number. In agreement with the results of this experiment, a significant effect of explant orientation was reported by Duzayman *et al.* (1994). However, in contrast to current results, Duzayman *et al.* (1994) reported better regeneration from adaxial than abaxial orientation. The total surface area of the explants that comes into contact with the medium in two orientations is different. For example, the cotyledons at the adaxial orientation curl at both ends, resulting in reduction in the area of contact. The shoot regeneration in tomato occurred only at the proximal end (which was attached to the cotyledonary node) and mostly from the adaxial surface. In the adaxial orientation (upper surface facing down), shoots originated from the adaxial surface, protruded inside the medium and then turned upwards, trying to emerge from the medium. This resulted in a reduced number of shoots being produced per explant and also reduced shoot height (Bhatia *et al.* 2005).

In summary, the present study has demonstrated that the abaxial orientation of both the explants (cotyledon and leaf) in each cultivar induced better shoot regeneration. Till date very less reports have been published on effect of orientation of explants on per cent shoot regeneration. Provision of a good regeneration system for commercial cultivars and breeding lines will significantly aid in the development of a transformation system for these genotypes. However, the protocol may require adjustment to obtain the best possible results. Successful coupling of a regeneration system and gene transfer procedures will assist in addressing both basic and applied research issues.

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