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In vitro Plant Regeneration from Seedlings-derived Explants of Tomato (*Lycopersicon esculentum* Mill.)

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Abstract

Tomato is one of the most important vegetable crops in India. In vitro tissue culture of tomato could serve as an important means for its improvement through genetic transformation. To improve the regeneration capacity of tomato, the effect on plant regeneration of donor plant type, basal medium, and plant growth regulators were evaluated using explants derived from the cotyledon, hypocotyl, leaf and petioles. Cotyledon and hypocotyl excised from 10-15 days old and leaf and petiole excised from 25-30 days were optimal explants. Explants were cultured on MS (Murashige and Skoog) basal medium supplemented with different concentrations and combinations of BA-IAA, BA-NAA, Kn-IAA and Kn-NAA. The highest regeneration efficiency was obtained on MS basal medium containing combinations of BA-IAA and Kn-IAA. The best response in terms of the percentage of shoot regeneration (77%) was obtained from petiole explant cultured on MS basal medium supplemented with 1.0 mg/l Kn + 1.0 mg/l IAA. Successful rooting was achieved by placing the shoots onto MS basal medium supplemented with 0.20 mg/l NAA. The combination of sand: soil (1:1) was the best for plant acclimatization as 90% of the plants survived and became established.

Highlights

We reported plant regeneration in tomato cv. Solan vajr through different seedling explants. The maximum regeneration efficiency (77%) was obtained on MS basal medium supplemented with 1.0 mg/l Kn + 1.0 mg/l IAA through petiole explants. Successful rooting was achieved by placing the shoots onto MS basal medium supplemented with 0.20 mg/l NAA.

Keywords: Lycopersicon esculentum, seedling explants, cotyledon, hypocotyl, leaf and petiole, shoot regeneration, acclimatization

The availability of an efficient and reproducible protocol for regeneration of plants through adventitious shoot formation from explants is essential for the application of micropropagation and genetic engineering for the improvement of plants. Plant regeneration in tomato is known to be genotype dependent (Zelcer *et al.,* 1983). Although plant regeneration from tomato is no longer limited to a small number of genotypes, a rapid development/ regeneration of shoots has only been obtained when specific procedures were applied in combination with culture medium and plant growth regulators suitable for the genotype of donar tissues. Tomato is one of the most important vegetable crops of solanaceae family grown all over the world for its special nutritive value. Tomato is the rich source of vitamins A, B and C contains 93.10 g moisture, 1.90 g protein, 0.10 g fat, 0.60 g minerals and 3.60 g carbohydrates (Rao and Aggarwal, 2000). Being an economically important vegetable crop around the world, much effort

has been made towards its improvement through tissue culture and plant genetic engineering. The techniques for plant regeneration depend on the use of plant growth regulators in complex and nearly empirical combination adapted to each particular situation. The potential of plant regeneration in tomato has been reported by several workers (El-Bakry, 2002; Gubis *et al.,* 2003; Plana *et al.,* 2005; Madhulatha *et al.,* 2006; Afroz *et al.,* 2009; Sharma and Srivastava, 2013; Devi *et al.,* 2013; Mohamed *et al.,* 2010; Srakar *et al.,* 2009; Chandra *et al.,* 2013; Harish *et al.,* 2010; Aishwarya and Philip Robinson, 2013) where different concentration of cytokinins and auxins in combination or alone, various type of explant or cultivar were used for shoot regeneration. To date, adventitious shoot regeneration has been demonstrated from a variety of in vitro cultured explants of cultivated tomato (Lycopersicon esculentum Mill.) through direct and indirect organogenesis and somatic embryogenesis. These include cotyledon explants (EI-Farash *et al.,* 1993; Ichimura and Oda, 1995; Lu *et al.,* 1997, Muthuvel *et al.,* 2005; Ouyang *et al.,* 2003; Madulatha *et al.,* 2006; Sarkar *et al.,* 2009; Harish *et al.,* 2010; Devi *et al.,* 2013; Chandra *et al.,* 2013), hypocotyl (Newman *et al.,* 1996; Davis *et al.,* 1994; El-Enany, 1995; Jawahar *et al.,* 1997; Venkatchalam *et al.,* 2000; Afroz *et al.,* 2009; Gubis *et al.,* 2003; Plana *et al.,* 2005; Rao and Suvartha, 2007; Ghada *et al.,* 2008; Harish *et al.,* 2010; Chandra *et al.,* 2013), leaf (Dwivedi *et al.,* 1990; Selvi and Khader, 1993; Ali and Li, 1994; Plastira *et al.,* 1997; Mamidala and Nanna, 2009; Villers *et al.,* 1993; Chandra *et al.,* 1995; Lech *et al.,* 1996; Khan *et al.,* 2006; Chandel and Katiyar, 2000; Gubis *et al.,* 2003; Harish *et al.,* 2010; Cruz-Mendivil *et al.,* 2011; Sharma and Srivastava, 2013), petiole (Gubis *et al.,* 2003; Sharma and Srivastava, 2013), stem (Pongtongkam *et al.,* 1993; Selvi and Khader, 1993), anther culture (Brasileiro *et al.,* 1999; Park *et al.,* 2001; Jose and Femando, 2006; Aishwarya and Philip Robinson, 2013), embryos (Young *et al.,* 1987; Guimaraes *et al.,* 1998) and protoplast (Latif *et al.,* 2006; Chen and Adachi, 1998). The present investigation was carried out to develop a reliable and reproducible protocol for adventitious shoot regeneration and development of complete plantlets from various explants i.e. cotyledon, hypocotyl, leaf and petiole and also to compare the organogenetic potential of different explants type.

Materials and Methods

Plant material

The certified seeds of tomato (Lycopersicon esculentum Mill. cv. Solan vajr) were procured from the Department of Vegetable crops, Dr. Y.S. Parmar University, Solan (H.P.). The cotyledon and hypocotyl explants were excised from 10-15 days old and leaf and petiole were excised from 25-30 days old glass house grown seedlings. The explants were cut into small pieces (0.5-1.0 cm size) and washed with water containing 1-2 drops of tween-20 for 2 min and rinsed two times with sterile water. Explants were surface sterilized with 0.1% w/v mercuric chloride ($HgCl_2$) solution for 20 sec and four times with sterile water. The adhering water were removed by placing the explants on sterile filter paper, approximately (0.5-0.8 cm) size explants were prepared using a sterile scalpel and forceps from surface sterilized cotyledon, hypocotyl, leaf and petiole.

Media Preparation, culture conditions and shoot regeneration

All the experiments were conducted in 100 ml of conical flasks containing 25 ml of MS basal medium supplemented with various concentrations and combinations of growth regulators. Shoot regeneration medium (SRM) composed of Murashige and Skoog (1962) basal medium fortified with 3% w/v sucrose, 0.8% agar and different combinations and concentration of plant growth regulators. The pH of the medium was adjusted to 5.8 with 1N NaOH before autoclaving at 1210C for 20 min. Five explants were cultured in each flask and incubated in an environmentally controlled growth chamber at 16/8 h day/night photoperiod with photosynthetic photon flux density of 60µmol m-2s-1 provided by cool white fluorescent lights (Philips Kolkata, India), 25±20C and 70% relative humidity. The explants were sub-cultured to fresh medium every one month. Shoot regeneration was performed with 45 explants per set in three replicates for each growth regulator combination. After culturing for 4 weeks adventitious regenerated shoots were transferred to same shoot regeneration medium for shoot elongation.

Root regeneration and acclimatization

When shoots reached 2-3 cm in height, they were excised and transferred onto MS basal medium containing different concentration of auxins. Hence, the same method was followed for inducing roots from the shoots obtained from other explants. Rooted shoots were washed with distilled water to remove traces of agar and planted in 10 cm diameter plastic pots containing autoclaved sand: soil (1:1). All the plantlets were covered with polythene bags for one week and watered once a day during the acclimatization phase.

Data analysis

Shoot regeneration experiments were conducted in three replicates of 45 explants, while rooting experiments had replicates of 10 plantlets per treatments. All the experiments were repeated at least three times. Data of plant (the percentage of shoot formation and the average number of shoots per explant) were collected after in vitro growth for 8 weeks and data of rooting (the percentage of shoots rooted per shoot) were collected after in vitro cultivation for 4 weeks. Data had shown represent the mean of three replicates and statistically analyzed by using completely randomized block design. Mean comparisons were made by least significant difference at the 5% probability to proportional data before ANOVA (Gomez and Gomez, 1984).

Results and Discussion

Initial experiments compared the cytokinin BA and Kn in combination with different auxins IAA and NAA. The results showed that only callus was induced from all (cotyledon, hypocotyl, leaf and petiole) the explants cultured on NAA containing medium supplemented BA and Kn (data not shown).

(I) Shoot regeneration from cotyledon explants

(a) Effect of BA and NAA on shoot regeneration

Nine different combinations and concentrations of BA-NAA were used for shoot regeneration. During the initial days of culture, the color of explants changed from green to whitish green and there was no change in the colour of the media. The callus initiation was observed after 10-12 days and only callus was formed in all the combinations of BA-NAA. No shoot bud differentiation was observed in any of the combination BA-NAA.

Table 1. Effect of different concentrations and combinations of BA and IAA (in MS basal medium) on shoot regeneration from cotyledon explants of tomato (Lycopersicon esculentum cv. solan vajr)

Sr. No.	Medium composition	Average number of shoot formed per explant	Percent shoot regeneration
1.	MS basal medium + 1.0 mg/l BA + 0.1 mg /l IAA	No shoot regeneration	
2.	MS basal medium + 2.0 mg/l BA + 0.1 mg/l IAA	0.5	22.22 (28.12)
3.	MS basal medium + 2.5 mg/l BA + 0.1 mg/l IAA	0.9	31.11 (33.90)
4.	MS basal medium + 1.0 mg/l BA + 0.25 mg/l IAA	1.2	37.78 (37.93)
5.	MS basal medium + 2.0 mg/l BA + 0.25 mg/l IAA	1.9	44.44 (41.81)
6.	MS basal medium + 2.5 mg/l BA + 0.25 mg/l IAA	1.8	46.67 (43.09)
7.	MS basal medium + 1.0 mg/l BA + 0.5 mg/l IAA	3.2	66.67 (54.74)
8.	MS basal medium + 2.0 mg/l BA + 0.5 mg/l IAA	3.0	60.00 (50.77)
9.	MS basal medium + 2.5 mg/l BA + 0.5 mg/l IAA	2.4	53.33 (46.91)
CD0.05		0.10	0.34
SE+		0.07	0.15

The data in parentheses are arc sine transformed values.

(b) Effect of BA and IAA on shoot regeneration

Nine different concentrations and combinations of BA-IAA were used for shoot regeneration. The callus initiation was observed after 10-12 days and shoot initiation started after 20-22 days at cut ends of the explants through callus formation in combination of BA-IAA (Fig.). Maximum per cent shoot regeneration (66.67%) and average number of shoots per explant (3.2) were observed on MS basal medium containing 1.0 mg/l BA + 0.5 mg/l IAA. Minimum percentage of shoot regeneration (22.22) with 0.5 average numbers of shoots were observed on MS basal medium containing 2.0 mg/l BA + 0.1 mg/l IAA (Table 1 & Fig 1.).

(II) Shoot regeneration from hypocotyl explants in tomato

Hypocotyl explants were excised from 10-15 days old in vivo grown seedlings and cultured on MS basal medium supplemented with different concentrations and combinations of BA-NAA and BA-IAA.

(a) Effect of BA and NAA on shoot regeneration

Different combinations of BA-NAA were tried for shoot regeneration. During early days, the explant increased in size and colour of hypocotyls explants changed from dark green to light green. Slight callusing was observed around cut surface of explants after 10-15 days. The only callus was formed in all the combinations of BA-NAA.

(b) Effect of BA and IAA on shoot regeneration

Nine different concentrations and combinations of BA-IAA were tried for shoot regeneration and, which showed different percentage of shoot regeneration and average number of shoots per explants. Adventitious shoot bud formation/shoot regeneration from hypocotyl explant started after 20-25 days of culturing in the combination of BA-IAA. Maximum per cent shoot regeneration (44.44%) and average number of shoots per explant (1.7) were obtained from the hypocotyl explants on the MS basal medium containing 2.0 mg/l BA + 0.5 mg/l IAA (Table 2 & Fig 1.).

Table 2. Effect of different concentrations and combinations of BA and IAA (in MS basal medium) on shoot regeneration from hypocotyl explants of tomato (Lycopersicon esculentum cv. solan vajr)

Sr. No.	Medium composition	Average number of shoots per explant	Percent shoot regeneration
1.	MS basal medium + 1.0 mg/l BA + 0.1 mg/l IAA	No shoot regeneration	
2.	MS basal medium + 2.0 mg/l BA + 0.1 mg/l IAA	No shoot regeneration	
3.	MS basal medium + 2.5 mg/l BA + 0.1 mg/l IAA	No shoot regeneration	
4.	MS basal medium + 1.0 mg/l BA + 0.25 mg/l IAA	No shoot regeneration	
5.	MS basal medium + 2.0 mg/l BA + 0.25 mg/l IAA	No shoot regeneration	
6.	MS basal medium + 2.5 mg/l BA + 0.25 mg/l IAA	No shoot regeneration	
7.	MS basal medium + 1.0 mg/l BA + 0.5 mg/l IAA	No shoot regeneration	
8.	MS basal medium + 2.0 mg/l BA + 0.5 mg/l IAA	1.7	44.44 (41.81)
9.	MS basal medium + 2.5 mg/l BA + 0.5 mg/l IAA	1.2	33.33 (35.26)
CD0.05		0.08	0.003
SE+		0.03	0.001

The data in parentheses are arc sine transformed values.

(III) Shoot regeneration from leaf explants

Leaf explants were excised from 25-30 days old glass house grown seedlings and cultured on shoot regeneration medium supplemented with different concentration and combinations of BA-NAA, BA-IAA, Kn-NAA and Kn-IAA.

(a) Effect of BA-NAA on shoot regeneration

All the nine different combinations and concentrations of BA-NAA were tried in MS basal medium resulted in callus initiation after 8-10 days of culturing. No shoot/shoot bud differentiation was observed in any of the combination and only callus was formed in all the combinations of BA-NAA. The callus was greenish and nodular.

(b) Effect of BA-IAA on shoot regeneration

Nine different combinations and concentrations of BA-IAA were tried which showed different percentage of shoot regeneration and average number of shoots per explant. Shoot initiation started after 15-20 days at cut ends of explant and through callus formation. Maximum per cent shoot regeneration (71.11%) and average number of shoots per explant (3.3) were obtained from the leaf explants on MS basal medium supplemented with 2.5 mg/l BA and 1.0 mg/l IAA (Table 3 & Fig 1.).

(c) Effect of Kn-NAA on shoot regeneration

Various combinations and concentrations of Kn-NAA tried and only callus was formed in all the combinations. No shoot/bud differentiation was observed in any of the combination.

(d) Effect of Kn-IAA on shoot regeneration

Nine different concentrations and combinations of Kn-IAA were tried for shoot regeneration. Shoot initiation started after 15-20 days through callus formation. Maximum per cent shoot regeneration (68.88%) and average number of shoots per explant (3.0) were obtained from the leaf explants on MS basal medium containing 1.0 mg/l Kn and 1.0 mg/l IAA (Table 4).

(IV) Shoot regeneration from petiole explants

Petiole were excised from 25-30 days old *in vivo* grown seedlings and cultured on shoot regeneration medium supplemented with combinations of BA-NAA, BA-IAA, Kn-NAA and Kn-IAA.

Table 3. Effect of various combinations and concentrations of BA and IAA (in MS basal medium) on shoot regeneration from leaf explants of tomato (Lycopersicon esculentum cv. solan vajr)

Sr. No.	Medium composition	Avearge number of shoots formed per explant	Percent shoot regeneration
1.	MS basal medium + 1.0 mg/l BA + 0.2 mg/l IAA	0.5	26.67 (31.09)
2.	MS basal medium + 2.0 mg/l BA + 0.2 mg/l IAA	1.1	33.33 (35.26)
3.	MS basal medium + 2.5 mg/l BA + 0.2 mg/l IAA	2.1	44.44 (41.81)
4.	MS basal medium + 1.0 mg/l BA + 0.5 mg/l IAA	2.7	57.77 (49.47)
5.	MS basal medium + 2.0 mg/l BA + 0.5 mg/l IAA	3.0	60.00 (50.77)
6.	MS basal medium + 2.5 mg/l BA + 0.5 mg/l IAA	3.2	66.66 (54.73)
7.	MS basal medium + 1.0 mg/l BA + 1.0 mg/l IAA	2.5	55.55 (48.19)
8.	MS basal medium + 2.0 mg/l BA + 1.0 mg/l IAA	3.0	62.22 (52.07)
9.	MS basal medium + 2.5 mg/l BA + 1.0 mg/l IAA	3.3	71.11 (57.49)
CD0.05		0.17	0.33
SE+		0.08	0.15

The data in parentheses are arc sine transformed value

Table 4. Effect of various combinations and concentrations of kinetin and IAA (in MS basal medium) on shoot regeneration from leaf explants of tomato (Lycopersicon esculentum cv. solan vajr)

Sr. No.	Medium composition	Average number of shoot formed per explant	Percent shoot regeneration
1.	MS basal medium + 1.0 mg/l Kn + 0.2 mg/l IAA	Only callus formation and no shoot regeneration	
2.	MS basal medium + 2.0 mg/l Kn + 0.2 mg/l IAA	Only callus formation and no shoot regeneration	
3.	MS basal medium + 2.5 mg/l Kn + 0.2 mg/l IAA	2.8	64.44 (53.39)
4.	MS basal medium + 1.0 mg/l Kn + 0.5 mg/l IAA	1.9	48.88 (44.36)
5.	MS basal medium + 2.0 mg/l Kn + 0.5 mg/l IAA	2.5	55.55 (48.19)
6.	MS basal medium + 2.5 mg/l Kn + 0.5 mg/l IAA	2.4	62.22 (52.07)
7.	MS basal medium + 1.0 mg/l Kn + 1.0 mg/l IAA	3.0	68.88 (56.09)
8.	MS basal medium + 2.0 mg/l Kn + 1.0 mg/l IAA	2.8	66.66 (54.39)
9.	MS basal medium + 2.5 mg/l Kn + 1.0 mg/l IAA	Callus formation and no shoot regeneration	
CD0.05		0.14	0.008
SE+		0.06	0.003

The data in parentheses are arc sine transformed values.

Table 5. Effect of various combinations and concentrations of BA and IAA (in MS basal medium) on shoot regeneration from petiole explants of tomato (Lycopersicon esculentum cv. solan vajr)

Sr. No.	Medium Composition	Average number of shoots per explant	Percent shoot regeneration
1.	MS basal medium + 1.0 mg/l BA + 0.2 mg/l IAA	1.9	44.44 (41.81)
2.	MS basal medium + 2.0 mg/l BA + 0.2 mg/l IAA	2.0	57.77 (49.47)
3.	MS basal medium + 2.5 mg/l BA + 0.2 mg/l IAA	2.6	62.22 (52.07)
4.	MS basal medium + 1.0 mg/l BA + 0.5 mg/l IAA	1.9	42.22 (40.53)
5.	MS basal medium + 2.0 mg/l BA + 0.5 mg/l IAA	2.0	51.11 (45.64)
6.	MS basal medium + 2.5 mg/l BA + 0.5 mg/l IAA	2.6	55.55 (48.19)
7.	MS basal medium + 1.0 mg/l BA+ 1.0 mg/l IAA	2.0	64.44 (53.09)
8.	MS basal medium + 2.0mg/l BA + 1.0 mg/l IAA	3.5	68.88 (56.09)
9.	MS basal medium + 2.5 mg/l BA + 1.0mg/l IAA	3.0	66.66 (54.73)
CD0.05		0.17	0.01
SE+		0.08	0.004

The data in parentheses are arc sine transformed values.

(a) Effect of BA-NAA on shoot regeneration

Nine different combinations and concentrations of BA-NAA were tried for shoot regeneration and only callus was formed in all the combinations of BA-NAA. No shoot/bud differentiation was observed in any of the combination.

(b) Effect of BA-IAA on shoot regeneration

Different combinations of BA-IAA were tried for shoot regeneration, which showed different percentage of shoot regeneration and average number of shoots per explant. Shoot initiation started after 7-10 days at cut ends of explant. After 18-20 days of culturing, shoots attained 2-3 cm height on the same medium. Maximum percentage of shoot regeneration (68.88%) and average number of shoots per explant (3.5) were obtained on MS medium with 2.0 mg/l BA + 1.0 mg/l IAA (Table 5.).

(c) Effect of Kn-NAA on shoot regeneration

Various combinations and concentrations of Kn-NAA were tried for shoot regeneration, which showed callus initiation after 8-10 days of culturing and only callus was formed in all the combinations of Kn-NAA. The growth of callus continued upto 30 days. The callus was light greenish and nodular.

(d) Effect of Kn-IAA on shoot regeneration

Nine different combinations of Kn-IAA were tried for shoot regeneration, which showed different percentage of shoot regeneration and average number of shoots per explant. Shoot initiation started after 7-10 days at cut ends of explant and after 18-20 days of culturing, shoots attained 2-3 cm height on the same medium. Maximum per cent shoot regeneration (77.77%) and average number of shoots per explant (3.6) were obtained from the petiole explants on MS basal medium + 1.0 mg/l Kn + 1.0 mg/l IAA (Table 6 and Fig 1.).

Table 6. Effect of various concentrations and combinations of kinetin and IAA (in MS basal medium) on shoot regeneration from petiole explants of tomato (Lycopersicon esculentum cv. solan vajr)

Sr. No.	Medium composition	Average number of shoot formed per explant	Percent shoot regeneration
1.	MS basal medium + 1.0 mg/l Kn + 0.2 mg/l IAA	2.5	57.77 (49.47)
2.	MS basal medium + 2.0 mg/l Kn + 0.2 mg/l IAA	3.1	62.22 (52.07)
3.	MS basal medium + 2.5 mg/l Kn + 0.2 mg/l IAA	3.9	71.11 (57.49)
4.	MS basal medium + 1.0 mg/l Kn + 0.5 mg/l IAA	3.0	66.66 (54.73)
5	MS basal medium + 2.0 mg/l Kn + 0.5 mg/l IAA	3.4	68.88 (56.09)
6.	MS basal medium + 2.5 mg/l Kn + 0.5 mg/l IAA	3.2	62.22 (52.07)
7.	MS basal medium + 1.0 mg/l Kn + 1.0 mg/l IAA	3.6	77.77 (61.87)
8.	MS basal medium + 2.0 mg/l Kn + 1.0 mg/l IAA	2.7	64.44 (53.39)
9.	MS basal medium + 2.5 mg/l Kn + 1.0 mg/l IAA	2.0	60.00 (50.77)
CD0.05		0.17	0.33
SE+		0.08	0.15

The data in parentheses are arc sine transformed values.

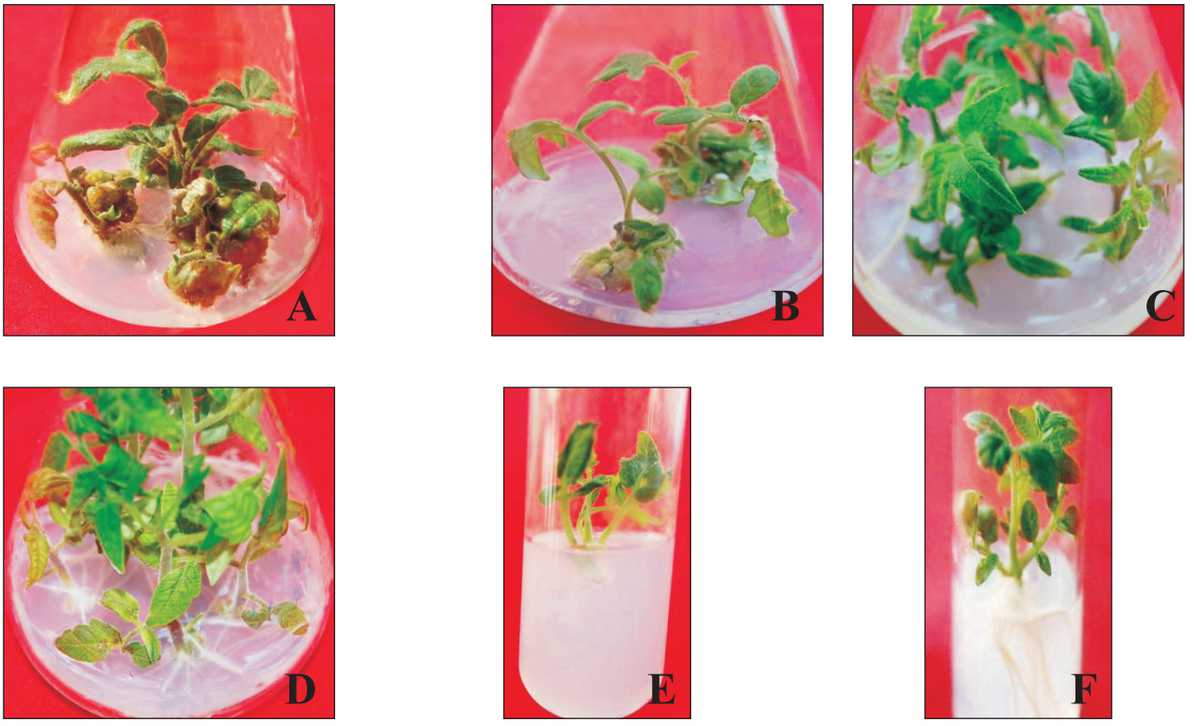

Fig 1. In vitro regeneration in tomato (Lycopersicon esculentum Mill. cv. Solan vajr) using seedling explants
(a) Shoot regeneration from cotyledon explants on MS (basal medium) + 1.0 mg/l BA + 0.5 mg/l IAA
(b) Shoot regeneration from hypocotyl explants on MS (basal medium) + 2.0 mg/l BA + 0.5 mg/l IAA
(c) Shoot regeneration from leaf explants on MS (basal medium) + 2.0 mg/l BA + 0.5 mg/l IAA
(d) Regeneration of shoots from petiole explants on MS (basal medium) + 1.0 mg/l Kn + 1.0 mg/l IAA
(e) Regenerated shoots transferred to root regeneration medium containing MS (basal medium) + 0.20 mg/l NAA
(f) Regeneration of roots on root regeneration medium {MS (basal medium) + 0.20 mg/l NAA} after 4 weeks

Table 7. Effect of different concentrations of various auxins on per cent root regeneration from in vitro developed shoots of tomato (Lycopersicon esculentum cv. solan vajr)

Sr. No.	Auxins (in MS medium)	Concentrations (mg/l)		
		0.05	0.10	0.20
1.	IAA	60 (50.77)	65 (53.73)	70 (56.79)
2.	NAA	70 (56.79)	75 (60.00)	80 (63.44)
3.	IBA	30 (33.21)	38 (38.06)	45.33 (42.32)

	SE+	CD0.05
Treatment (T)	0.294	0.618
Concentrations (C)	0.294	0.618
T x C	0.509	1.070

The data in parentheses are arc sine transformed values.

Fig 2. Hardening of in vitro regenerated plantlets of tomato (Lycopersicon esculentum Mill. cv. Solan vajr)
(a) *In vitro* regenerated plantlets of tomato
(b) *In vitro* regenerated plantlets of tomato kept for hardening in pots containing mixture of sand : soil (1:1) planting substrate at 0 day covered with polythene bags
(c) *In vitro* regenerated plantlets successfully acclimatized to sand and soil (1:1) planting substrate after 21 days
(d) Acclimatized young healthy plantlets transferred to soil in plastic pots after one months of hardening

Similar studies In vitro plant regeneration in Punica granatum cv. Nana from leaf explant. 2003, 6(1)
(a) in vitro plant regeneration studies in three indica rice varieties. 2012, 5(4)
(b) in vitro shoot regeneration of stevia rebaudiana through callus and nodal segments, 2012. 5(2).

(V) Multiplication and elongation of shoots

The regenerated shoots were multiplied on the best shoot regeneration medium. Elongation of shoots was observed on the same medium.

(VI) Root regeneration in vitro developed shoots and acclimatization of plantlets

The same rooting method was followed in from seedling explants in this study. Roots developed on the shoots after 10-15 days in most of the cultures. The elongated shoots of all the explants (cotyledon, hypocotyl, leaf and petiole) were transferred to MS basal medium containing different concentration of auxins NAA, IBA & IAA. Although the roots could be induced by all the concentrations of auxins IBA, IAA & 2, 4-D. In comparison, root induction was maximum (80%) by NAA (0.20 mg/l) compared with other growth regulators (Table 7 & Fig 2). Rooted plantlets were transferred to plastic pots containing sand: soil (1:1) and 90% of the plantlets survived in the greenhouse and acclimatized

If tomato is to be a good candidate for genetic transformation it is essential that we be able to regenerate whole plantlets from the explants. Plant regeneration was achieved from cotyledon, hypocotyl, leaf and petiole explants of tomato. In the present studies, there was however a significant difference in the efficiency of shoot regeneration among the explants studied with petiole exhibiting the highest in vitro response.

All the explants (cotyledon, hypocotyl, leaf and petiole) cultured on MS basal medium containing different concentrations and combinations of auxins and cytokinins. The percentage of callus formation and the percentage of explants regenerating shoots varied significantly depending on the concentration of plant growth regulators (PGR) present in the medium. There was also a significant interaction between the factors ($P<0.05$), indicating that the effects of PGR concentration on in vitro adventitious shoot regeneration are dependent on explant types. There was significant difference among the different types of explants in terms of the percentage of shoot regeneration. Duzyaman *et al.,* (1994) compared the growth of hypocotyl, cotyledon and leaf explants of

tomato cultivars. The degree of shoot regeneration was in the order of leaves, cotyledon and hypocotyl and all cultivars responded similarity. Plastira and Predikaris (1997) reported differential regeneration frequency of various explants in the order of hypocotyl>cotyledon>leaf. Gubis *et al.,* (2003) studied regeneration capacity of six types of explants (segments from hypocotyl, cotyledon, epicotyl, leaf, internodes and petiole) in 13 cultivars of tomato and reported that hypocotyl and epicotyls showed up to 100% regeneration on MS + 1.0 mg/l zeatin + 0.1 mg/l IAA. Madhulatha *et al.,* (2006) reported that among three explants (cotyledon, hypocotyl and leaves), cotyledon was found most effective in shoot regeneration ability. Khoudi *et al.,* (2009) reported that primary leaves are three times more efficient then cotyledons in terms of shoot regeneration frequency. In the present study, petiole explants were found more efficient then others in terms of shoot regeneration. Rashid and Bal (2010) reported that direct regeneration was significantly influenced by the genotype, hormone and concentration. They obtained maximum shoot regeneration and number of shoots per explant from hypocotyl on MS medium supplemented with kinetin (0.5 mg/l) and BAP (0.5 mg/l).

The maximum shoot regeneration was obtained on combination of Kn-IAA from the petiole explants. The concentrations of different cytokinins and auxins in combination or alone, required for shoot regeneration, varies with the type of explant or cultivar used (Duzyman *et al.,* 1994; El-Farash *et al.,* 1993; Davis *et al.,* 1994; Lech *et al.,* 1996; Lu *et al.,* 1997; Plastira *et al.,* 1997; El-Bakry, 2002; Gubis *et al.,* 2003; Madhulatha *et al.,* 2006; Harish *et al.,* 2010; Sharma and Srivastava, 2013; Devi *et al.,* 2013; Nahar Liza *et al.,* 2013; Nasher Mohamed *et al.,* 2010). Duzyaman *et al.,* (1994) compared the growth of hypocotyl, cotyledon and leaf explants of tomato cultivars. The use of MS basal medium supplemented with 0.20 mg/l IAA + 2.30 mg/l BA resulted in greater shoot regeneration than one with 1.0 mg/l BA and 1-2 mg/l Kinetin. El-Bakry (2002) observed maximum number of shoots on MS basal medium supplemented with 2.0mg/l BA and 0.20 mg/l IAA in five cultivars from cotyledon explants. Madhulatha *et al.,* (2006) reported that out of the various combinations of cytokinins and auxins tried, MS medium containing 2.5 mg/l BAP + 0.5 mg/l IAA was the best combination for multiple shoot induction. Devi *et al.,* (2013) studied effect of different media compositions on plant regeneration in four genotypes of tomato and obtained high percentage of regeneration of four genotypes (IPA-3, Punjab Upma, Castle Rock, VFN-8) on MS medium supplemented with BAP (2.0 mg/l) and kinetin (1.0 mg/l). Sharma and Srivastava (2013) obtained maximum regeneration percentage from leaf (68%) and petiole (80%) explants on MS medium supplemented with 1 mg/l BAP + 0.5 mg/l IAA and 1 mg/l Kn + 1 mg/l IAA respectively.

No significant differences were observed in the root regeneration responses of shoots harvested from seedling explants. Maximum 80% root regeneration was observed on the 0.20 mg/l NAA containing medium. Root regeneration media containing different concentrations of IAA, NAA and IBA were also used by several workers for root regeneration of in vitro developed shoots of tomato (Jawahar *et al.,* 1997; Plastira *et al.,* 1997; Venkatachalam *et al.,* 2000; Park *et al.,* 2001; Madhulatha *et al.,* 2006; Khan *et al.,* 2006; Kant and Srivastava, 2003; Ouyang *et al.,* 2003; Plana *et al.,* 2005). Results indicated that petiole explants were found best for regeneration and showed shoot regeneration on combination of Kn-IAA. In conclusion this efficient shoot regeneration system for tomato will contribute to the production of transgenic technologies for this important plant species and present a protocol that for tomato improvement.

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