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Standardization of high efficient and rapid regeneration protocol for *Agrobacterium* mediated transformation of tomato *(Solanum lycopersicum* L.) *cv.* Pusa Ruby, Vaibhav and Arka Meghali.

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Key words: BAP-Benzyl Amino Purine, AS-Adenine Sulphate, TDZ-Thidiazuron, Zn-Zeatin, RM-Regeneration Media, IAA- Indole-3-Acetic Acid and MS- Murashige and Skoog.

Abstract

In the present investigation, three tomato cultivars Pusa Ruby, Vaibhav and Arkamegali (*Solanum lycopersicum* L.) were selected for multiple shoot induction. The explants cotyledon, hypocotyl and epicotyl of all the cultivars of tomato were cultured on MS medium fortified with different concentrations of BAP (1.0 to 4.0 mg/l), TDZ (1.0 to 4.0 mg/l), Zeatin (0.5 to 1.0 mg/l), AS (5.0 to 20 mg/l) and 0.1 mg/l IAA . Adventitious shoot buds were induced at the cut ends of the explants after three weeks of culture. The shoots were induced from cotyledon, hypocotyl and epicotyl explants on different concentrations of growth regulators (RM 1 to RM 10). Among ten media combinations tested, RM 9 was the best combination for induction of shoots from cotyledon, hypocotyl and epicotyl explants compared to RM 2. The highest number of shoots was induced on 0.5 mg/l Zn + 10 mg/l AS + 0.1 mg/l IAA (RM 9) followed by 2.0 mg/l BAP + 10 mg/l AS + 0.1mg/l IAA (RM 2). Among the three genotypes tested Pusa ruby showed highest number of shoots per explant followed by Vaibhav and Arkamegali. Highest number of adventitious shoots was induced from cotyledonory compared to hypocotyl and epicotyl explants in Pusa ruby and Arka meghali, where as Vaibhav induced highest shoots from epicotyl explants. Rooting was achieved on MS medium supplemented with 0.1 mg/l IAA in all the genotypes. Hence the plant regeneration was found to be influenced by the genotype, different explants with hormonal combinations in the media.

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Introduction

Tomato is one of the most important vegetable crops with highest production and consumption worldwide. It's a perennial plant belonging to *Solanaceae* family grown in tropical, sub-tropical and temperate regions (Atherton & Rudich, 1986). It ranks third among vegetable crops (next to potato and sweet potato) with an annual production of 1617 million metric tonnes (FAO statistical database, 2012).

The production and yield of tomato is adversely affected by various abiotic factors such as salinity, drought, floods, heat and cold stress and biotic factors like pests and diseases (Bhatnagar-Mathur *et al.,* 2008). To tackle these problems conventional breeding methods are the best approaches, but time consuming. Hence, breeding associated with biotechnological tools for transfer of desired genes to elite varieties without modifying the genetic makeup will lead to development of high yielding varieties. However, transfer of genes depends on rapid and efficient *in-vitro* regeneration protocols.

Mass multiplication of tomato has been attempted using shoot tip culture (Izadpanah and Khosh Khui, 1992), somatic embryogenesis (Kaprakis and Alderson, 2002), direct organogenesis from intact explants (Ichimura and Oda, 1998). Chaudhry *et al*., 2007 reported the effect of genotype, explants and plant growth hormones on callus proliferation and regeneration of three tomato cultivars. Shoot apex, nodal segments and root segments were successfully used for callus induction and regeneration (Jatoi *et al*., 2001). Similarly, combination of IAA + BAP for shoot regeneration from different explants of tomato was found to be more effective (Gunay and Rao, 1980; Kurtz and Lineberger, 1983; Selvi and Khader, 1993; Villiers *et al*., 1993; Duzyman *et al*., 1994; Chen *et al*., 1999., Chandel and Katiyar, 2000;). None of the protocols are efficient and reliable, hence an efficient regeneration protocol is required for a given plant species before transformation (Potrykus *et al*., 1995). In this regard, an attempt was made to reveal the factors influencing high efficient and rapid regeneration protocol for tomato cultivars using various explants by using different growth hormones.

Material and methods

Plant material and culture conditions

Seeds of high yielding tomato cultivars from three different institutes, 'Pusa Ruby' were obtained from the Indian Agricultural Research Institute, New Delhi, and 'Vaibhav' from University of Agricultural Sciences Bangalore and 'Arka Meghali' from Indian Institute of Horticulture Research respectively. Seeds were sterilized with two different sterilants; sodium hypochlorite (1- 4 *per cent*) and mercuric chloride (0.1 - 0.4 *per cent*) were tried. Seeds were treated with 5 *per cent* teepol detergent for 15 minutes, soaked them under running tap water for 30-45 minutes and washed with sterile water twice. Then surface sterilized for 3 minutes in 70 *per cent* ethanol and washed with sterile water followed by sterilizing in 4 *per cent* sodium hypochlorite solution along with 4-5 drops of Tween-20 for 10 minutes, followed by three rinses with sterile water. The surface sterilized seeds were blot dried and 25 to 30 sterilized seeds were inoculated into culture bottles containing half strength MS medium with 1.5 *per cent* sucrose, gelled with 0.7 *per cent* agar having pH 5.8. Culture bottles were incubated initially for two days in dark at 25° C and later exposed to light intensity of 2000 lux with photoperiod of 16/8 hours of light and dark cycles.

Culture media, hormones

MS-medium (Murashige and Skoog, 1962) supplemented with various hormonal combinations. Benzylaminopurine (BAP-1.0 mg/l to 4.0 mg/l), Thidiazuron (TDZ-1.0 mg/l to 4.0 mg/l), Zeatin (Zn-0.5 mg/l to 2.0 mg/l), Adenine sulphate (5 to 20 mg/l), and Indole-3-acetic acid (lAA-0.1 mg/l) labeled from RM 1 to RM 10 were used for shoot and root regeneration (Table 1).

Explant selection

Three types of explants, Cotyledons, hypocotyls and epicotyls from ten days old *in-vitro* grown seedlings were used for preparation of explants. Cotyledons excised at the proximal petiole edge and 1 millimeter width transverse strips of tissue prepared from the

proximal region of the cotyledons were incubated with the adaxial side on shoot induction medium. Transverse hypocotyl discs of 0.5–1.0 millimeter thick were sliced from a 10 millimeter hypocotyl segment cut immediately below the cotyledonary node and placed with their basal cut. Similarly epicotyls were cut into two pieces and cultured on the regeneration medium. Each bottle was inoculated with 10 explants of cotyledons, hypocotyls and epicotyls separately and incubated in growth chamber with 16/8 hour photoperiod with light intensity of 2000 lux. The observations were recorded for parameters like regeneration percentage, shoot primordia per explants, number of shoots per explant and shoot length. The regenerating explants were observed and revived onto fresh medium at every 15 days.

Result and discussion

The plant tissues cultured under *in-vitro* condition are greatly influenced by different components of culture media composition and concentrations of growth hormones in the morphogenetic differentiation of the explant. Hence evaluation of different hormones in combinations is necessary towards better regeneration of tomato.

Table 1. Combination of various growth hormones used in association with MS basal medium for standardization of regeneration in tomato cultivars.

Media code	Nutrient medium	BAP (mg/L)	TDZ (mg/L)	Adenine sulphate (mg/L)	ZR (mg/L)	IAA (mg/L)
RM 1	MS	1.0	-	5	-	0.1
RM 2	MS	2.0	-	10	-	0.1
RM 3	MS	3.0	-	15	-	0.1
RM 4	MS	4.0	-	20		0.1
RM 5	MS	-	1.0	5	-	0.1
RM 6	MS	-	2.0	10	-	0.1
RM 7	MS	-	3.0	15	-	0.1
RM 8	MS	-	4.0	20	-	0.1
RM 9	MS	-	-	10	0.5	0.1
RM 10	MS	-	-	15	1.0	0.1

Table 2. Percentage of regeneration in three cultivars of tomato in MS medium supplemented with growth regulators.

Media	Arka Meghali				Vaibhav				Pusa Ruby				Mean
	Hypocotyl	Epicotyl	Cotyledon	Mean	Hypocotyl	Epicotyl	Cotyledon	Mean	Hypocotyl	Epicotyl	Cotyledon	Mean	
RM 1	2.88±0.03	2.62±0.03	41.55±0.05	15.68^{d}	5.07±0.03	32.85±0.05	7.14±0.04	15.02^{d}	64.56±0.03	53.95±0.03	76.49±0.04	65.00^{d}	31.90
RM 2	90.24±0.04	88.42±0.03	94.89±0.04	91.18^{b}	70.08±0.03	90.63±0.08	90.32±0.03	83.68^{b}	91.59±0.04	90.72±0.02	95.85±0.04	93.55^{b}	89.47
RM 3	80.17±3.33	68.87±5.33	80.72±3.06	76.59^{e}	51.52±4.47	67.92±4.47	52.24±3.33	57.23^{f}	83.56±3.27	80.17±3.33	89.54±2.67	84.42^{e}	72.74^{e}
RM 4	40.36±4.66	27.29±3.27	52.24±3.27	39.96^{g}	30.41±4.42	50.12±3.33	37.76±5.16	39.43^{g}	69.82±4.47	59.98±4.22	73.79±5.20	67.86^{g}	49.09^{g}
RM 5	4.57±3.33	3.37±3.27	16.98±5.33	8.31^{i}	1.84±2.66	5.97±6.29	3.61±4.47	3.80^{i}	50.93±4.42	30.41±4.42	56.75±5.54	46.03^{i}	19.38^{i}
RM 6	81.85±4.47	71.78±5.33	84.72±2.67	79.45^{d}	66.07±4.27	74.30±4.27	58.34±5.96	66.24^{d}	86.50±3.05	84.72±2.67	86.50±3.05	85.91^{d}	77.20^{d}
RM 7	77.98±4.67	68.87±3.27	74.30±4.27	73.72^{f}	61.66±4.67	70.79±3.33	54.45±3.06	62.30^{e}	74.82±3.06	79.62±5.16	80.17±4.22	78.20^{f}	71.41^{f}
RM 8	3.37±3.51	3.37±3.51	7.08±4.71	4.61^{j}	1.84±2.94	3.37±3.51	2.49±3.33	2.57^{j}	8.39±3.33	4.57±3.51	7.57±5.77	6.84^{j}	4.67^{j}
RM 9	92.55±0.05	90.24±0.03	95.91±0.03	93.23^{a}	80.12±0.03	92.73±0.03	90.34±0.02	87.73^{a}	92.70±0.05	92.85±0.05	96.95±0.03	96.95^{a}	92.49
RM 10	90.34±0.04	89.03±0.08	92.79±0.04	90.72^{c}	77.55±0.05	85.30±0.02	83.55±0.05	82.13^{c}	88.79±0.04	85.14±0.04	94.96±0.02	89.63^{c}	87.49
Mean	56.33^{b}	51.16^{c}	63.84^{a}	57.1^{b}	44.40^{c}	56.93^{a}	47.87^{b}	49.73^{c}	71.32^{b}	65.88^{c}	75.96^{a}	71.05^{a}	
SEm	0.01	0.01	0.02		0.01	0.02	0.02		0.02	0.01	0.02		
CD (1%)	0.07	0.05	0.09		0.06	0.09	0.08		0.10	0.07	0.11		
CV	0.04	0.04	0.05		0.03	0.05	0.04		0.05	0.04	0.07		

Data - Mean± SE of 10 replications. The data were taken 3 weeks after culture. The values followed by same letters are not significantly different @ P= 0.05.

The results indicated that the seeds treated with mercuric chloride had low seed germination rate, long germinating time and un-uniform germination, where as the seeds treated with sodium hypochlorite had higher rate of seed germination, short germination time and uniform germination, Similar results were observed by Gubis *et al.,* 2003. In the present study the additional step in the explant surface sterilization with 70 per cent alcohol helped in removal of microorganisms. The addition of tween-20 into 4 *per cent* sodium hypochlorite has increased the germination rate to 95-100%, devoid of bacterial and fungal contaminations. Similar results were obtained by Gubis (2004) with explant immersion time of 15 min in 4 per cent sodium hypochlorite in tomato cultivars "Hana" and "Premium". Similarly, Javier (2001) treated the explants with one *per cent* sodium hypochlorite for 20 min and obtained 100% regeneration in tomato cultivars "Ailsa craig", "UC82B" and "Rutgers".

Table 3. Shoot primordia per regenerating explants in three cultivars of tomato in MS medium supplemented with growth regulators.

Media	Arka Meghali				Vaibhav				Pusa Ruby				Mean
	Hypocotyl	Epicotyl	Cotyledon	Mean	Hypocotyl	Epicotyl	Cotyledon	Mean	Hypocotyl	Epicotyl	Cotyledon	Mean	
RM1	2.44±0.04	1.86±0.03	5.68±0.03	3.33^{d}	2.04±0.03	5.04±0.03	2.39±0.04	3.16^{d}	7.77±0.03	7.59±0.03	9.23±0.03	8.20^{d}	4.89
RM 2	11.42±0.03	8.78±0.03	12.71±0.05	10.97^{b}	8.59±0.04	11.84±0.04	9.84±0.03	10.09^{b}	17.88±0.03	14.28±0.03	20.42±0.03	17.53^{b}	12.86
RM 3	7.16±0.24	6.64±0.30	11.12±0.44	8.31^{e}	5.54±0.26	7.56±0.26	6.86±0.23	6.65^{e}	15.67±0.33	13.32±0.49	19.77±0.32	16.25^{c}	10.40^{e}
RM 4	3.82±0.93	3.60±0.84	9.27±0.26	5.56^{g}	3.28±0.55	6.54±0.30	3.26±0.57	4.36^{g}	13.67±0.30	10.06±0.31	17.48±0.26	13.74^{e}	7.88^{f}
RM 5	2.40±0.81	1.78±0.53	5.19±0.94	3.123^{h}	1.35±0.47	2.38±1.27	2.40±0.81	2.04^{i}	8.07±0.23	4.78±0.86	10.87±0.27	7.91^{i}	4.36^{i}
RM 6	7.76±0.24	7.17±0.20	10.98±0.21	8.64^{d}	6.86±0.23	11.69±0.53	10.36±0.30	9.64^{c}	12.36±0.33	10.35±0.33	17.37±0.33	13.36^{f}	10.55^{d}
RM 7	5.03±1.44	5.80±0.34	7.93±0.33	6.26^{f}	5.69±0.70	5.93±1.39	4.69±1.26	5.44^{f}	11.08±0.23	7.67±0.21	15.35±0.42	11.37^{g}	7.69^{g}
RM 8	1.64±0.42	1.60±0.37	1.91±0.35	1.72^{j}	1.20±0.21	1.64±0.42	1.34±0.26	1.39^{j}	4.80±1.30	2.72±1.07	4.55±1.88	4.022^{j}	2.38^{j}
RM 9	11.85±0.05	10.08±0.03	17.22±0.03	13.05^{a}	8.79±0.04	15.92±0.03	11.16±0.02	11.96^{a}	20.48±0.03	16.09±0.04	24.89±0.04	20.49^{a}	15.16
RM 10	10.00±0.05	8.04±0.04	12.95±0.01	10.33^{c}	5.82±0.03	11.76±0.04	7.04±0.02	8.21^{c}	11.90±0.05	11.85±0.03	19.28±0.03	14.34^{c}	10.96
Mean	6.23^{b}	5.46^{c}	9.34^{a}	7.01^{b}	4.82^{c}	7.93^{a}	5.85^{b}	6.20^{c}	12.31^{b}	9.82^{c}	15.76^{a}	12.6^{a}	
SEm	0.01	0.01	0.02		0.02	0.01	0.01		0.01	0.01	0.01		
CD (1%)	0.05	0.05	0.10		0.08	0.06	0.07		0.06	0.07	0.05		
CV	0.28	0.36	0.37		0.18	0.17	0.23		0.23	0.36	0.32		

Data - Mean± SE of 10 replications. The data were taken 3 weeks after culture. The values followed by same letters are not significantly different @ P= 0.05.

Effects of different hormonal combinations in tomato shoot differentiation

Murashige and Skoog medium supplemented with various cytokines and auxins in balanced proportion play an important role in the development of shoot primordia, shoot elongation, number of shoots and shoot length.

The regeneration frequency was studied using different explants, combinations of hormones in all the three varieties. The cotyledonory differentiation rate was highest in Pusa Ruby (96.95%) followed by Arka Meghali (95.91%) in RM 9 (MS + Zn 0.5mg/l+ Adenine sulphate (AS) 10 mg/l+ IAA 0.1mg/l) followed by RM 2 (MS + BAP 2mg/l+ Adenine sulphate (AS) 10 mg/l+ IAA 0.1 mg/l) with 95.85 % and 94.89 % respectively , where as the variety Vaibhav showed maximum differentiation rate in epicotyl explant on RM 9 (92.72%) followed by RM 2 (90.63%). The induced primary shoot buds were strong, less blank, less deformed. Average budding number of 5.5 per explant was observed. The combination of BAP + AS and IAA also induced primary shoot buds, but the number of induced shoots per explant was less than that of the combination of Zn + AS and IAA. It is interesting that the combination of BAP + AS and IAA showed best callus growth compared to other combinations with respect to size and structure of the calli (Table-2). The size of the calli was twofold larger than others, the

calli were green, swollen and loose with out bud formation. The results are in confirmation with Ling *et al* 1998, Gubis *et al.*, 2003 as they used 8 to 10 days old cotyledons, which were superior to other explants.

The MS medium supplemented with hormone Zeatin was the most efficient medium for tomato regeneration (NoGueira *et al.,* 2001).

Table 4. Number of shoots per regenerating explants in three cultivars of tomato in MS medium supplemented with growth regulators.

Media	Arka Meghali				Vaibhav				Pusa Ruby				Mean
	Hypocotyl	Epicotyl	Cotyledon	Mean	Hypocotyl	Epicotyl	Cotyledon	Mean	Hypocotyl	Epicotyl	Cotyledon	Mean	
RM 1	3.12±0.01	3.05±0.03	4.29±0.10	3.49^{d}	2.07±0.01	4.77±0.08	2.07±0.02	2.97^{d}	5.01±0.06	3.87±0.06	6.84±0.01	5.24^{d}	3.90
RM 2	5.63±0.05	4.39±0.01	6.16±0.03	5.75^{a}	4.63±0.04	5.84±0.02	5.06±0.05	5.11^{c}	9.66±0.07	7.39±0.02	11.73±0.47	9.59^{b}	6.81
RM 3	4.14±0.24	3.37±0.16	5.14±0.24	4.22^{f}	3.37±0.16	4.55±0.22	3.73±0.24	3.88^{d}	8.68±0.21	7.69±0.15	10.88±0.23	9.08^{c}	5.73^{c}
RM 4	2.52±0.22	2.63±0.21	3.73±0.24	2.96^{g}	3.46±0.16	3.70±0.29	2.90±0.25	3.35^{f}	7.06±0.23	5.46±0.22	8.97±0.25	7.16^{e}	4.49^{g}
RM 5	3.05±0.32	1.58±0.21	4.00±0.31	2.87^{h}	2.05±0.24	3.34±0.34	2.63±0.21	2.67^{h}	4.72±0.29	3.54±0.22	6.15±0.24	4.81^{g}	3.45^{h}
RM 6	5.05±0.23	3.68±0.29	6.45±0.26	5.06^{b}	3.02±0.23	5.65±0.26	4.33±0.26	4.33^{c}	6.86±0.23	5.81±0.34	8.85±0.31	7.18^{e}	5.52^{d}
RM 7	4.23±0.26	3.17±0.30	5.95±0.25	4.45^{c}	2.81±0.23	4.74±0.24	3.84±0.23	3.80^{e}	6.14±0.29	4.55±0.22	8.46±0.26	6.39^{f}	4.88^{f}
RM 8	1.32±0.16	1.32±0.16	1.58±0.21	1.41^{j}	1.23±0.15	1.58±0.21	1.28±0.22	1.37^{i}	4.35±0.22	3.10±0.24	5.32±0.30	4.26^{i}	2.34^{j}
RM 9	4.47±0.04	3.33±0.01	7.22±0.01	4.47^{c}	5.92±0.06	7.75±0.08	5.86±0.04	6.51^{a}	10.99±0.12	8.66±0.04	12.82±0.06	10.82^{a}	7.26
RM 10	3.98±0.02	4.38±0.06	5.60±0.03	4.84^{b}	5.39±0.02	5.63±0.08	4.16±0.02	5.13^{b}	7.62±0.03	6.68±0.02	10.96±0.14	8.42^{c}	6.13
Mean	3.54^{b}	2.77^{c}	4.80^{a}	3.70^{c}	3.17^{c}	4.72^{a}	3.52^{b}	3.80^{b}	6.87^{b}	5.54^{c}	8.97^{a}	7.12^{a}	
SEm	0.13	0.04	0.02		0.02	0.02	0.04		0.03	0.02	0.02		
CD (1%)	0.66	0.19	0.09		0.08	0.10	0.19		0.13	0.09	0.09		
CV	2.70	0.99	0.55		0.83	0.94	1.36		1.00	0.87	1.06		

Mean number of shoots per regenerating explants, presented as mean± SE of 10 replications. Data was recorded at 5 weeks of culture. The values followed by same letters are not significantly different @ P= 0.05.

Table 5. Shoot length per regenerating explants in three cultivars of tomato in MS medium supplemented with growth regulators.

Media	Arka Meghali				Vaibhav				Pusa Ruby				Mean
	Hypocotyl	Epicotyl	Cotyledon	Mean (cm)	Hypocotyl	Epicotyl	Cotyledon	Mean (cm)	Hypocotyl	Epicotyl	Cotyledon	Mean (cm)	
RM 1	5.06±0.01	5.25±0.01	3.57±0.01	4.63^{b}	5.95±0.01	7.80±0.00	7.85±0.01	7.87^{c}	4.83±0.03	5.71±0.00	3.97±0.03	4.84^{b}	5.78
RM 2	4.51±0.04	5.51±0.02	6.35±0.07	4.30^{d}	4.82±0.00	9.81±0.02	5.77±0.00	6.13^{d}	3.26±0.04	4.86±0.02	5.72±0.01	3.40^{d}	4.61
RM 3	5.54±0.10	2.88±0.03	2.60±0.12	5.16^{b}	3.64±0.10	7.31±0.08	5.27±0.08	5.41^{g}	3.36±0.10	4.75±0.07	3.25±0.07	3.79^{e}	4.79^{d}
RM 4	2.45±0.11	3.35±0.10	0.63±0.03	2.14^{f}	2.23±0.11	4.76±0.09	3.15±0.09	3.38^{i}	1.61±0.07	3.23±0.07	1.15±0.07	1.99^{h}	2.51^{h}
RM 5	2.92±0.10	3.42± 0.13	2.70±0.12	3.01^{e}	6.39±0.19	8.80±0.10	7.86±0.07	8.02^{b}	2.88±0.11	4.12±0.13	2.73±0.07	3.91^{d}	4.98^{c}
RM 6	2.06±0.12	2.67 ±0.09	1.74±0.12	2.16^{f}	4.50±0.08	8.20±0.11	5.98±0.08	6.23^{d}	2.81±0.12	4.35±0.10	2.51±0.12	3.22^{f}	3.87^{f}
RM 7	1.27±0.10	1.88±0.11	0.75±0.06	1.30^{g}	4.51±0.09	6.82±0.17	5.69±0.10	5.67^{f}	2.54±0.11	3.24±0.09	1.64±0.09	2.47^{g}	3.15^{g}
RM 8	0.69±0.03	1.06 ±0.06	0.55±0.03	0.77^{h}	3.76±0.07	5.63±0.10	4.28±0.04	4.56^{h}	1.59±0.07	2.45±0.11	1.19±0.07	1.74^{i}	2.36^{i}
RM 9	5.57±0.02	4.55±0.03	7.71±0.03	5.61^{a}	7.53±0.01	9.86±0.01	8.79±0.02	8.46^{a}	5.52±0.03	5.50±0.01	6.70±0.03	5.91^{a}	6.66
RM 10	4.51±0.01	5.88±0.04	3.04±0.03	4.48^{c}	6.80±0.02	9.43±0.05	8.66±0.01	8.30^{b}	2.87±0.03	5.09±0.01	5.32±0.03	4.64^{c}	5.80
Mean	3.30^{b}	4.31^{a}	2.23^{c}	3.28^{c}	4.97^{c}	7.84^{a}	6.30^{b}	6.37a	3.32^{b}	4.67^{a}	2.56^{c}	3.52^{b}	
SEm	0.01	0.01	0.01		0.01	0.02	0.01		0.02	0.01	0.01		
CD (1%)	0.03	0.07	0.03		0.05	0.10	0.04		0.08	0.07	0.07		
CV	0.16	0.32	0.22		0.65	0.89	0.32		0.93	0.61	0.52		

Mean number of shoot length per regenerating explants, presented as mean± SE of 10 replications. Data was recorded at 5 weeks of culture. The values followed by same letters are not significantly different @ P= 0.05.

Shoot primordia per regenerating explant

Shoot primordia plays very important role in development of shoots. The shoot primordia per explant was highest in Pusa Ruby on RM 9 (24.89) followed by RM 2 (20.42), similarly the response of Arka Meghali was highest in RM 9 (17.22) followed by RM 2 (12.71) but Vaibhav responded quite differently by producing highest shoot primordia on RM 9

(15.92) followed by RM 2 (11.84) on epicotyls (Table-3). The shoot bud formation started at 12 days and the shoot regeneration response to obtain a shoot length of 8-10 mm was around 22 days in cotyledons.

Fig. 1. Different stages of regeneration in tomato cultivar Pusa Ruby.

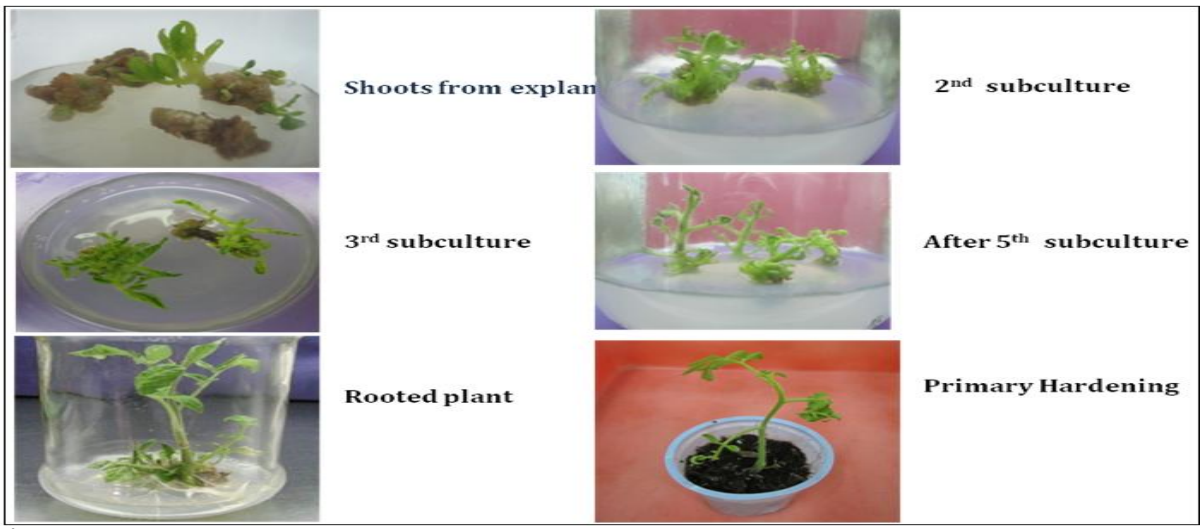

Fig. 2. Different stages of regeneration in tomato cultivar Vaibhav.

Number of shoots per explant and shoot length

Multiple shoots arising from a single explant reduces the time and resources for mass multiplication. Variability in shoot production was recorded for different explant types and cultivars. The highest number of shoots per explant was produced by cotyledonory explants of Pusa ruby and Arka Meghali in RM 9 (12.82) and (7.22) respectively, followed by Vaibhav (7.75) with epicotyl explant and least number of shoots was recorded in hypocotyl explant (Table-4). Shoot length per explant was recorded after 15 to 20 days of culture, shoot length was contradictory to cotyledonory explants as the highest shoot length was recorded in epicotyl explant of Vaibhav in RM 9 (9.86) followed by cotyledonory explants of Arka Meghali (7.71) and cotyledonory explants of Pusa ruby (6.70) (Table-5). Gubbis *et al.*, 2003 reported highest number of shoots produced from epicotyl and hypocotyl explants but the cotyledonory explants produced less number of shoots in tomato cultivars of Hana, premium and Robura. These results are in confirmation with the present results of tomato cultivar Vaibhav. Moghaieb *et al.*, 1999 also obtained highest shoots per explant. In contradictory these results obtained highest shoots (5.8-6.0) in wild tomato using leaf and cotyledonory explants on Zeatin supplemented medium (Arriliga *et al.,* 2001). The elongated shoots were excised individually and

transferred onto fresh half strength MS supplemented with 0.1 mg/l IAA. Subsequently, rooted plantlets were acclimatized on sterilized soil Rite (Tissue culture grade) under *in-vitro* conditions for one week followed by pots containing soil composed of coco peat: sand: soil (2:1:1, v:v:v), then covered with plastic bags to increase the humidity and grown under controlled greenhouse with a photoperiod of 16/8 hours conditions. Plants were hardened by removing the plastic bags gradually after 7 - 10 days. The similar results were reported by Lee *et al*., 1999, Abu-El-Heba *et al.,* 2008, that use of half strength along with 0.1 mg/l IAA induced roots.

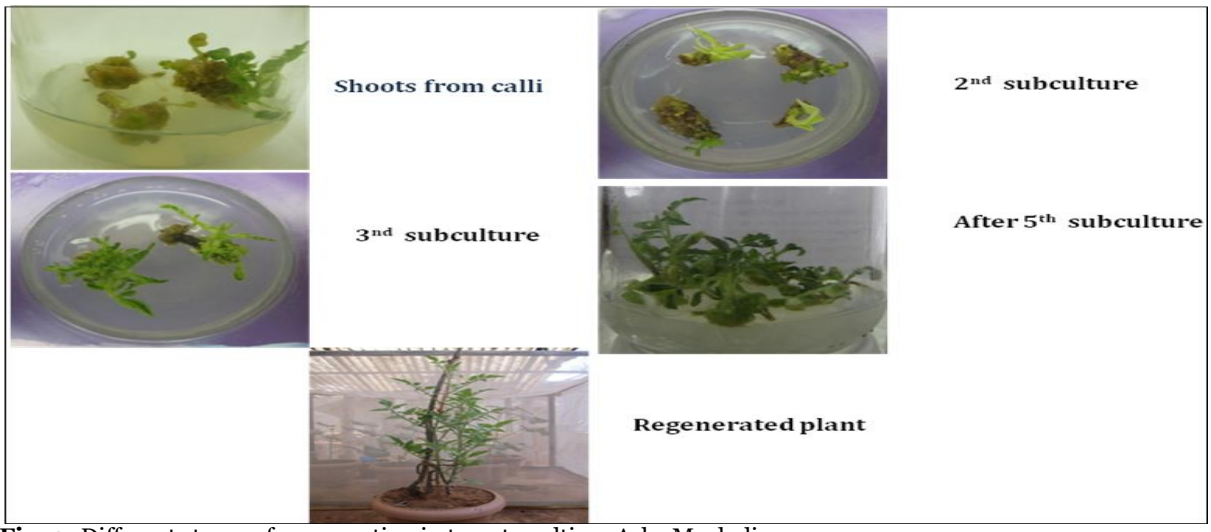

Fig. 3. Different stages of regeneration in tomato cultivar Arka Meghali.

Conclusion

We are the first to report the effect of cytokinin Adenine sulphate in shooting response of tomato cultivars Pusa ruby, Arka Meghali and Vaibhav. The addition of Adenine sulphate increased the induction of multiple shoots in cotyledonory, epicotyl and hypocotyl explants. MS medium supplemented with Zeatin (0.5mg/l) + Adenine sulphate (10mg/l) + IAA (0.1mg/l) was the best hormonal combination for all the explants. The above media also reduced the number of days required for complete plant regeneration from 120 days to 75-80 days. Pusa Ruby was the best cultivar followed by Vaibhav and Arka Meghali. Hence this rapid, highly efficient and reproducible regeneration protocol can be utilized for genetic transformation with desired genes to develop high yielding, biotic and abiotic resistant varieties.

References

Abu-El-Heba GN, Hussein GM, Abdalla NA. 2008. A rapid and efficient tomato regeneration and transformation system, Agriculture and Forestry Research **58,** 103-110.

Agarwal S, Rao AV. 2000. Role of antioxidant lycopene in cancer and heart disease. Journal of American College Nutrition **19(5),** 563-569.

Atherton JG, **Rudich.** 1986. Journal In: Tomato crop Chapman and Hall, London, New York **661,**

Arriliga I, Gis bert C, Sales E, Rogi L, Moreno V. 2001. *in-vitro* plant regeneration and gene transfer in the wild tomato *Lycopersicon cheesmanii*. Journal of Horticultural Science. Biotechnology **76,** 413–418.

Bhatia P, Ashwath N, Senaratna T, David M. 2004. Tissue culture studies of tomato (*Lycopersicon esculentum*) Plant Cell Tissue and Organ Culture **78,** 1–21.

Bhatnagar-Mathur P, Vadez _K V, Sharma K. 2008. Transgenic approaches for abiotic stress tolerance in plants:retrospect and prospects., Plant Cell Reports **27,** 411–424.

Chandel G, Katiyar SK. 2000. Organogenesis and

somatic embryogenesis in tomato (*Lycopersicon esculantum* Mill.). Advance in Plant Science **13,** 11-17.

Chen H, Zhang J, Zhuang T, Zhou G. 1999. Studies on optimum hormone levels for tomato plant regeneration from hypocotyls explants cultured *in-vitro*. Acta Agriculture Scandinavica-a **15,** 26-29.

Chaudhary Z, Afroz A, Rashid H. 2007. Effect of variety and plant growth regulators on callus proliferation and regeneration response of three tomato cultivars (*Lycopersicon esculentum*). Pakistan Journal of Botany **39(3),** 857-869.

Chaudhry Z, Feroz I, Ahmed W, Rashid H, Mizra B, Qureshi A. 2001. Varietal response of *Lycopersicon esculentum* to callogenesis and regeneration. *Online*, Journal of Biological Science **1,** 1138-1140.

Compton ME, Veilleux RE. 1991. Shoot, root and flower morphogenesis on tomato influores- cence explants. Plant Cell Tissue and Organ Culture **24,** 223-231.

Duzyman E, Tanrisever A, Gunver G. 1994. Comparative studies on regeneration of different tissues of tomato *in-vitro*, Acta Horticulture **34,** 235-242.

Gubis J, lajchova Z, farago J, jurekova Z. 2004. Effect of growth regulators on shoot induction and plant regeneration in tomato (*Lycopersicon esculentum* Mill.). Biological, Bratislava **59,** 405-408.

Gunay AL, Rao PS. 1980. *in-vitro* propagation of hybrid tomato plants (*Lycopersicon esculentum* L.) using hypocotyl and cotyledon explants. Annals of Botany **45,** 205-207.

Jatoi SK, Sajid GM, Sappal H, Baloch MS, Qureshi A, Anwar R. 2001. Differential *in- vitro* response of tomato hybrids against a multitude of hormonal regimes. Online Journal of Biological Science **1,** 1141-1143.

Javier Pozueta-Romero, Guy Houln´e, Luis Cañas, Rodolphe Schantz, Jes´us Chamarro. 2001. Enhanced regeneration of tomato and pepper seedling explants for *Agrobacterium*-mediated transformation. Plant Cell Tissue and Organ Culture. **67,** 173–180.

Koblitz H. 1982. Versuche zur Gewebekultur in der *Gattung lycopersicon* Mill. Ubersicht. Kulturpflanze, Berlin **27-43,**

Kurtz SM, Lineberger RD. 1983. Genotypic differences in morphogenic capacity of cultured leaf explants of tomato. Journal of American Society for Horticultural Science **108,** 710-714.

Lee KS, Schottler F, Collins JL, Lanzino G, Couture D, Rao A, Hiramatsu KI, Goto Y, Hong SC, Caner H, Yamamoto H, Chen Z-F, Bertram E, Berr S, Omary R, Scrable H, Jackson T, Goble J, Eisenman L. 1997. A genetic animal model of human neocortical heterotopia associated with seizures. Journal of Neuroscience Research **17,** 6236-6242.

Ling HQ, Kriseleit D, Ganal MW, 1998. Effect of ticarcillin/potassium clavulanate on callus growth and shoot regeneration in *Agrobacterium* mediated transformation of tomato (*Lycopersicon esculentum Mill.*). Plant Cell Reports **17,** 843-847.

Moghaieb REA, Saneoka H, Fujita K. 1999. Plant regeneration from hypocotyl and cotyledon explant of tomato (*Lycopersicon esculentum* Mill.). Soil Science and Plant Nutrition **45,** 639–646.

Nogueira FTS, Costa MG, Figueira ML, Otoni WC, Finger FL. 2001. *in-vitro* regeneration of Santa Clara" tomato plantlets and its natural mutant/Firme". Science in Agrotechnology. Lavras **25,** 36-71.

Potrykus I, Spangenberg G. 1995. Gene transfer

to plants. Berlin: Springer **361,**

Selvi DT, Khader MA. 1993. *in-vitro* morphogenetic capacity of tomato (*Lycopersicon esculentum* Mill.) var. PKM.1. South Indian Horticulture **41,** 251-258.

Villiers RPD, Vuuren RJV, Ferreira DI, Staden JV. 1993. Regeneration of adventitious buds from leaf discs of *Lycopersicon esculentum* cv. Rodade: optimization of culture medium and growth conditions. Journal of South African Society of Horticultural Sciences **3,** 24-27.