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Efficient Plantlet Regeneration in Tomato (*Lycopersicon esculentum* Mill.)

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Abstract

In vitro culture response was assessed in three varieties of tomato (*Lycopersicon esculentum* Mill.) for optimum callus induction and plantlet regeneration. Callus induction was achieved within seven - ten days and plantlet regeneration was observed in 20 days across varieties. Presence of 2.0 mg/l BAP and 0.5 mg/l Kn produced maximum shootlets. N6 medium displayed the best regeneration percentage, plantlet regeneration, maximum number and length of shootlets, while MS produced tall whole plantlets. Appropriate levels of gelling agents, phytohormones and carbon sources and adjuvents like agar and agarose (2 and 0.8%), Kn (2.0 mg/l) and IAA (0.5 mg/l), glucose and sucrose (1.5% each), folic acid (0.25 mg/l), biotin (0.5 mg/l), coconut water (5%) and use of young hypocotyl explants were found to enhance plantlet regeneration and length of plantlets. For whole plantlet regeneration proximal end of cotyledons were most compatible. Plain half strength of MS was found to be the best rooting medium, however addition of IAA (1.0 mg/l) was found essential to induce longer roots.

Introduction

Establishment of an efficient tissue culture protocol is an essential prerequisite in harnessing the advantage of cell and tissue culture for genetic improvement. Efficient plantlet regeneration in tomato was reported from meristems (Mirghis et al. 1995), leaf (Behki et al. 1976, Kartha et al. 1976, Padmanabhan et al. 1974), stems, anthers (Zamir et al. 1970) and hypocotyls (Ohki et al. 1978). Organogenesis in callus cultures of tomato was reported to be less owing to the number of factors acting individually or synergistically. The present study

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encompasses the effects of seven important biological and physio-chemical factors in modulating *in vitro* culture response in tomato. Comprehensive understanding of the factors deems to be instrumental in developing an efficient culture protocol for maximum callus induction and increased plantlet regeneration. This would help to achieve prolific *in vitro* propagation in tomato. Promising HYVs with high *in vitro* culture response were selected based on recurrent evaluation in our laboratory.

Materials and Methods

Three popular high yielding, *in vitro* culture responsive tomato (*Lycopersicon esculentum* Mill. varieties, which showed good *per se* were used for these experiments. Seeds were surface sterilized (Chandra et al. 1995) and stem and leaf explants from four-week-old *in vitro* grown seedlings were employed. About 2 cm length explants were excised 1 cm below the apex of the primary and axillary shoots of three - four weeks old seedlings. Cross sections of 1 cm^2 dimension were cut from three - four weeks old seedlings using a sharp scalpel and placed onto medium keeping abaxial side up. In case of cotyledons and hypocotyls, each cotyledon from 15 days old seedlings were cut into three pieces proximal near to hypocotyls, distal (away from hypocotyls) and the middle portion. In case of hypocotyls two pieces of about 0.5 cm length (viz. tip with meristem, shoot bud and adjacent portion) were used.

The explants were cultured (Chandra et at. 1995) to a height of about 2.5 - 3.0 cm. Roots were carefully washed after removing agar and planted in plastic cups of perlite drenched with 1/4th Hoagland solution. After 20 days they were transferred to open range for further growth and development.

Factors evaluated: Different explants *viz.* root, leaf, stem, hypocotyl and cotyledon were evaluated. The explants were initially cultured on callus induction medium (CIM) supplemented with MS salts and 2.0 mg/l BAP for three weeks and transferred to regeneration medium (RM) consisting of MS with 2.0 mg/l BAP and 0.5 mg/l Kn. Diverse media like MS, N6, LS and B5 were evaluated in an experiment (Table 1). Different phytohormones like GA_3 (0.5 - 2.0 mg/l), BAP and Kn (1.0 - 3.0 mg/l) were evaluated in 20 different combinations (Table 2). Organic adjuvants evaluated were casein hydrolysate (100 mg/l), coconut water (10%), tryptophan (50 mg/l), arginine (50 mg/l), D-biotin and folic acid (0.25, 0.5 and 1.0 mg/l). The various stressing agents used were abscisic acid (ABA; 1.0 and 2.0 mg/I), agar (0.8 and 2%), mannitol (0.4, 0.5, 0.6 M) and heat and cold shock (37 and 14°C for 6 h) (Table 3). Different carbon

Table 1. Influence of diverse media in governing plantlet regeneration response in tomato (after 60 days of explant inoculation).

		Genotypes								
		Pant 11			Pant 5			Le 79		
*Medium	Explant	[e]Av. No. of shootlets/ explant	[e]Av. No. of whole/ explant	Plantlet regenera-tion (%)	[e]Av. No. of shootlets/ explant	[e]Av. No. of whole/ explant	Plantlet regenera-tion (%)	[e]Av. No. of shootlets/ explant	[e]Av. No. of whole/ explant	Plantlet regenera-tion (%)
N6	Stem	8.75 (0.9)	3.5 (1.5)	58	7 (0.7)	2 (1.2)	68	7 (0.7)	2 (1.2)	100
	Leaf	4.2 (1)	2.5 (2)	78	16 (1.1)	0	100	16 (1.1)	0	100
LS	Stem	4.7 (0.92)	0	78	6.14 (0.3)	0	58.4	12.86 (2.18)	2 (1.2)	100
	Leaf	5 (0.2)	0	50	7 (0.48)	0	86	5 (0.8)	0 (1)	100
B5	Stem	2.5 (0.7)	2 (1)	15	6 (1)	1 (1.75)	25	5.75 (0.95)	0	58
	Leaf	3 (1.2)	0	17	6.25 (0.6)	0	57	5.75 (0.7)	2.4 (2.3)	72
MS	Stem	3.9 (0.8)	2.4 (1.2)	90	8.1 (0.6)	3.1 (1.8)	100	7.4 (0.8)	3.1 (1.7)	83
	Leaf	12.4 (0.9)	3.4 (1.0)	80	3.1 (1.0)	0.3 (1.1)	73	5.2 (0.6)	3.0 (1.5)	88
CD (0.05)										
V		0.52 (0.19)	0.39 (0.12)	5.02						
M		0.60 (0.22)	0.45 (0.14)	5.80						
E		0.42 (0.15)	0.31 (0.10)	4.10						
VM		1.04 (0.38)	0.78 (0.25)	10.05						
ME		0.85 (0.31)	0.63 (0.20)	8.20						
VE		0.74 (0.27)	0.55 (0.17)	7.10						
VME		1.48 (0.54)	1.10 (0.35)	14.21						

*Medium contains 2.0 mg/l BAP and 0.5 mg/l Kn. [e]Figures in parentheses indicate length of shootlets and plantlets in cm; Scale 1 = excellent; 2 - 3 = very good; 4 - 5 = good; 6 - 7 = moderate; 8 = poor; 9 = very poor.

Table 2. Effects of diverse auxins and cytokinins on callus culture and plantlet regeneration.

	Pant 5						Pant 11						Le 79					
	Leaf			Stem			Leaf			Stem			Leaf			Stem		
Treatment	Calli (%)	Regeneration (%)	No. of shoots (wp)	Calli (%)	Regeneration (%)	No. of shoots (wp)	Calli (%)	Regeneration (%)	No. of shoots (wp)	Calli (%)	Regeneration (%)	No. of shoots (wp)	Calli (%)	Regeneration (%)	No. of shoots (wp)	Calli (%)	Regeneration (%)	No. of shoots (wp)
1	100	20	2 (0)	0	0	0	100	-	-	100	-	-	91	-	.	100	20	1(0)
2	100	60	1.33(0)	100	80	2(0)	100	-	-	100	20	2(1)	100	60	3ef)	100	10	1(0)
3	100	80	8.75(0)	100	40	1.5(0)	100	-	-	100	20	6(0)	100	40	2(2)	100	50	3.5 (0)
4	100	60	3(0)	100	-	.	100	10	1(0)	55	40	2.5(1)	100	60	3(1)	100	0	0
5	100	40	4.5(0)	100	10	1(0)	100	100	2.4(0)	100	10	3(0)	100	.	.	100	-	-
6	100	90	7.25(0)	100	10	2(0)	100	60	5(0)	100	.		100	95	4.2(1)	100	-	.
7	100	60	9.3(0)	100	10	1(0)	100	60	6(0)	90	-	.	100	90	10.25 (0)	100	.	-
8	100	90	6(0)	100	5	1(0)	100	40	4(0)	100	60	5.7(0)	100	60	3.4(0)	100	-	-
9	100	20	5(0)	100	80	4.25(0)	100	35	2(0)	100	50	2.3(0)	100	40	4.5hY	100	80	15.25(0)
10	73	16	1.5(0)	100	83.3	3.8(0)	50	5	0.5(0)	100	10	1(0)	88	50	3.0(0)	83	16.6	2.5 (0)
11	73	40	5.5(1)	77	60	3.0 (0.8)	100	40	7.5(1)	100	.	.	100	98	25(2)	100	100	4(1)
12	100	60	4(3)	100	75	6.75 (3)	100	80	3.5(0)	100	80	4(1)	100	60	7.7(0)	100	90	11.75(0)
13	100	10	1 (0)	100	-	3.8(2)	100	-	.	100	-	.	100	60	4.6(0)	100	65	6.7 (0)
14	100	95	9.9(3)	100	90	9(1)	100	40	6.5 (0.5)	100	20	3(0)	100	100	8.9(5)	100	70	6.9 (0)
15	100	100	18(2)	100	70	7.7(2)	100	50	3.5 (0.5)	100	80	3.5(2)	100	100	10.69 (2)	100	60	6.2 (2)
16	100	100	8(3)	84	34	5(2)	100	95	3.4 (0.5)	100	100	8(3)	100	100	11.5 (4)	100	50	2.5 (0.5)
17	100	100	6.25(3)	100	60	4.4 (0.5)	100	80	5(0.5)	100	100	6.7(0.3)	100	70	11.3 (3)	82	40	4(1)
18	100	100	22.2(3)	100	60	3.5 (0.3)	100	80	10(0.5)	91	60	4.2(1.3)	100	100	7.2(2)	100	83.3	3(1)
19	71.4	60	4(0.3)	100	86	3.8 (0.5)	100	67	11.25 (1)	100	50	2.7(0.1)	100	80	4(0.8)	100	100	1(1)
20	50	20	1(0.2)	51	40	6.5 (0.5)	100	60	2(0.3)	100	40	8(1)	100	86	10 (0.5)	100	25	1 (0.3)

1. 1.0 mg/l BAP+ 0.2 mg/l IAA; 2. 1.0 mg/l B + 0.5 mg/l IAA; 3. 1.0 mg/l BAP + 1.0 mg/l IAA; 4. 2.0 mg/l BAP + 0.2 mg/l IAA; 5. 2.0 mg/l BAP + 0.5 mg/l IAA; 6. 2.0 mg/l BAP + 1.0 mg/l IAA; 7. 3.0 mg/l BAP + 0.2 mg/l IAA; 8. 3.0 mg/l BAP+ 1.0 mg/l IAA; 10. Control; 11. 1.0 mg/l Kn + 0.2 mg/l IAA; 12. 1.0 mg/l Kn + 0.5 mg/l IAA; 13. 1.0 mg/l Kn + 1.0 mg/l IAA; 14. 2.0 mg/l Kn + 0.2 mg/l IAA; 15. 2.0 mg/l Kn + 0.5 mg/l IAA; 16. 2.0 mg/l Kn + 1.0 mg/l IAA; 17. 3.0 mg/l BAP + 0.2 mg/ l IAA; 18. 3.0 mg/l Kn + 1.0 mg/l IAA.

sources like glucose, fructose, maltose (1.5, 3.0 and 2.0 % singly or in combination) and gelling agents like agar, agarose and phytagel were used singly. Rooting medium was standardized using combinations of NAA, BAP, Kn, IAA (0.5 - 1.0 mg/l) and half strength MS. Ten replications were kept in each treatment. Uniform pieces from the same type of explant were used in combinatorial treatments except in case of experiments where different explant

Table 3. Effect of folic acid and biotin on whole plantlet and shootlet regeneration in tomato.

		Pant 11		Pant 5		Le 79	
Treatment (mg/l)	Explant	*Av. No. of shootlets/ explant	* Av. No. of whole plantlets/ explant	*Av. No. of shootlets/ explant	*Av. No. of whole plantlets/ explant	*Av. No. of shootlets/ explants	Av. No. of whole plantlets/ explant
Biotin							
0.25	Stem	0	1 (0.3)	6.5 (0.75)	2 (1.25)	6.66 (0.4)	2.75 (3.5)
	Leaf	5.6 (0.6)	2.25 (1.8)	3 (0.45)	0	2.5 (0.75)	4.3 (4.4)
0.50	Stem	4.15 (0.65)	2 (1.5)	9.2 (0.4)	0	6 (0.72)	1 (2.2)
	Leaf	4 (0.8)	1 (1)	2.13 (0.85)	1.88 (2.8)	7 (0.75)	3.5 (1.85)
1.0	Stem	4.16 (0.63)	.2.5 (1.8)	6.23 (0.42)	3.3 (1.9)	4.6 (1.1)	2.3 (3.8)
	Leaf	2.76 (0.86)	1.5 (1.1)	6.8 (0.6)	3.5 (1.6)	0	5.5 (2.5)
Folic acid							
0.25	Stem	9.66 (0.86)	3 91.85)	6.6 0.6)	1.6 (2.6)	2.3 (2.2)	3.3 (2.13)
	Leaf	1.75 (1.1)	2 (2.6)	6.3 (0.76)	1.7 (3.0)	5.8 (0.92)	2.3 (2.2)
0.50	Stem	0	0	7.8 (0.6)	1.7 (1.5)	3 (3.3)	3.66 (2.6)
	Leaf	5 (0.8)	1.88 (3.1)	10.8 (0.7)	2 (2.8)	7 (0.63)	3 (3.3)
1.0	Stem	7.8 (1.82)	6 (2)	9 (0.8)	1.5 (2)	4.5 (0.75)	2 (1.2)
	Leaf	3.16 (0.7)	1 (4.25)	8.1 (0.5)	1.8 (1.8)	2.5 (1.5)	2.5 (1.5)
Control	Stem	4 (0.5)	1 (1)	3.5 (0.8)	0.5 (1)	0.5 (1)	0.3 (1)
	Leaf	2 (0.3)	0	1 (0.5)	0.2 (1)	1 (0.5)	0.5 (1)
CD (0.05)	T	0.42 (0.04)	2.5 (0.17)				
	V	0.28 (0.02)	1.68 (0.11)				
	TV	0.74 (0.07)	4.45 (0.31)				
	E	0.22 (0.02)	1.37 (0.09)				
	VE	0.39 (0.03)	2.37 (0.16)				
	TE	0.60 (0.06)	3.63 (0.25)				
	TVE	1.04 (0.10)	6.29 (0.43)				

*Figures in parentheses indicate length of shootlets and whole plantlets (cm).

types were used for comparison. Observations across treatment variety wise were averaged. Each experiment was repeated thrice. Observations were recorded for callus colour, callus health, % callus induction, plantlet regeneration, average number of plantlets and shootlets developed. Mean data were statistically analyzed and interpreted.

Results and Discussion

Cultured explants showed signs of callus induction within seven - ten days and regeneration within 20 - 30 days. Morphogenic response varied with respect to variety, media, hormone and organic adjuvants used. N6 was found to show maximum plantlet regeneration (%), shoot length and whole plantlets, number and length of shootlets, while MS was found to be most appropriate for development of maximum healthy tall whole plantlets and LS for faster regeneration (Fig. 1). Earlier studies prospect extensive use of MS in tomato tissue culture (Mirghis et al. 1995, Behki et al. 1976, Zamir et al. 1970, Kartha et al. 1976, Ohki et al. 1978). In contradiction the present study recommends the utility of N6 medium in tomato tissue culture. The probable reason might be due to the increased dose of $(NH_4)_2SO_4$ (5 mM), which perhaps helped in enhancing plantlet regeneration as reported by Khanna and Raina (1997) and Poddar et al. (1997) in rice.

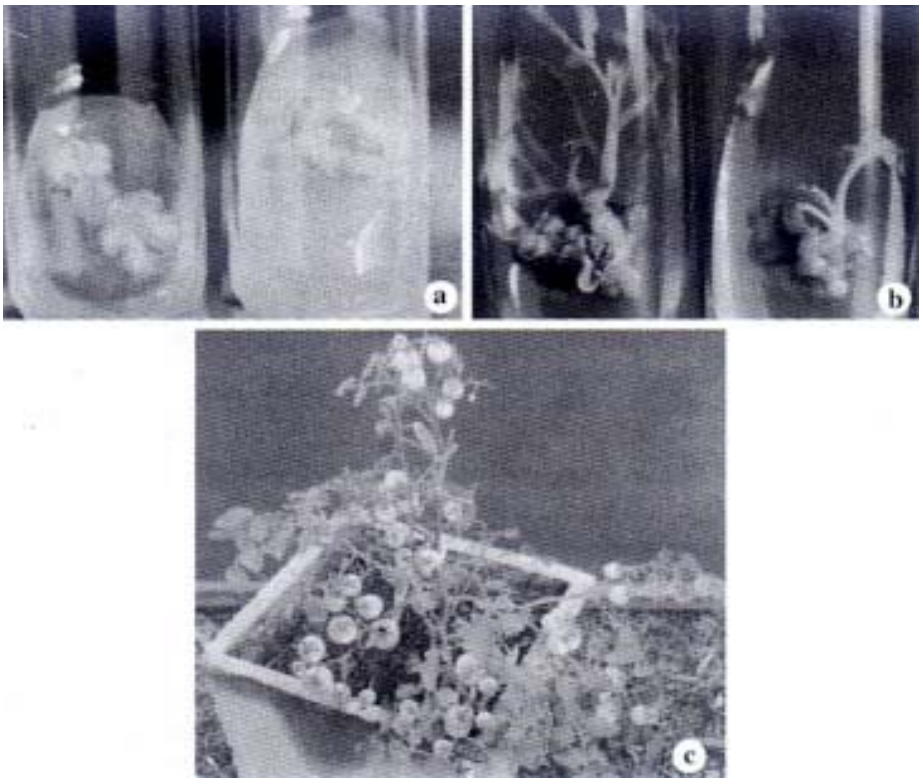

Fig. 1. Somatic tissue culture in tomato. (a) Leaf and stem explants showing prolific callus induction on MS with 2.0 mg/l BAP, (b) redifferentiation of callus into shootlets following organogenesis, (c) selected somaclones at SC2 in varieties Pant 5.

Both leaf and stem explants displayed best performance on 0.8% agar and 0.8 % agarose, respectively with maximum shoot length and tall whole plantlets. It is mentionable that use of gelling agents other than agar in tomato *in vitro* culture are extremely limited, except in cases where it was employed in optimizing efficiency of *Agrobacterium*-mediated genetic transformation (Frary et al. 1996). Earlier it was reported that the same gelling agent at various concentrations have profound influence in retention of water and regulation of moisture regime of the medium, which influences plantlet regeneration response immensely (Suprasanna et al. 2000). High concentration of gelling agent was found to improve plantlet regeneration in rice (Maiti 2001). The present study shows only an increase of length of whole plantlets at 2% agar. Among the various explants hypocotyls performed the best in terms of average number of shootlets and tall whole plantlets, while for whole plantlet development proximal end of cotyledons and hypocotyls showed an on par response. This could probably be attributed to the age effects in response to juvenile vs adult explant sources (Durzan et al. 1984), where younger explants showed better callus induction and organogenetic response (George and Sherrington 1984). The superiority of hypocotyls explant derived callus in terms of plantlet regeneration corroborates with the findings of Locky (1983).

Use of low dose of IAA (0.5 mg/l) with moderate dose of Kn was found to be optimum (Locky 1983) for enhanced plantlet regeneration. This contradicts the observations of Kartha et al. 1977 and Chandra et al. 1995 where high levels of IAA were found to be promising probably due to genotypic and explant specificity (Kurtz et al. 1983). Maximum whole plantlets were produced at 2.0 mg/l Kn and 1.0 mg/l lAA as per the observations of Kartha et al. 1976. Even medium devoid of auxins irrespective of cytokinin concentration produced adventitious roots on explants due to high endogenous auxins reported in tomato (Delanghe 1974, Shyluk and Constabel 1976).

A combination of glucose with sucrose (1.5 % each) performed excellently. Sucrose was found to be beneficial in rice (Maiti 2001) and tomato (Sabapathi et al. 1985, Chandra et al. 1995). Organic adjuvants like folic acid and biotin at 0.25 and 0.5 mg/l, respectively was found to enhance plantlet regeneration and length of shootlets. In tomato similar reports were made by Chandra et al. (1995). However, presence of these adjuvents found to be inhibitory to root development. Coconut water @ 10% level enhanced plantlet regeneration as well as length of whole plantlets. Casein hydrolysate was found to hault callus induction and enhance plantlet regeneration in this study (Table 2). A cold shock of 14_C for 6 h increased shootlet regeneration involving stem explants. The best rooting medium turned out to be half strength MS devoid of

any synthetic hormones with maximum survival per cent. Reduction in sucrose levels to 2% and increase in agar content to 0.9% was found to enhance rooting corroborates the earlier findings of Chandra et al. 1995. Maximum root length was observed in presence of 2.0 mg/l BAP and 1.0 mg/l lAA.

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