J. Bangladesh Agril. Univ. 5(2): 213–216, 2007 ISSN 1810-3030

In vitro regeneration of tomato plant from leaf and internode segments

M.A. Karim and M.A. Kayum[1]
Department of Crop Botany, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[1] Agricultural Botany, Patuakhali Science & Technology University, Dumki, Patuakhali, Bangladesh

Abstract

With a view to develop and establish a reproducible protocol for rapid regeneration in tomato, leaf and internodes explants of tamato were used. Leaf and internodes explants were cultured on MS medium containing 30 gl^{-1} sucrose, 8 gl^{-1} agar supplemented with α-Naphthalen acetic acid (NAA), Indole acetic acid (IAA) and 6-Benzyl amino purine (BAP). Explants were sterilized and cultured on MS medium supplemented with BAP at 2 to 3 mgl^{-1} in combination with NAA at 0.1, 0.25 and 0.5 mgl^{-1} for callus induction. The combination of BAP at 3mgl^{-1} and NAA at 0.25 mgl^{-1} was the most suitable treatment for callus formation. Internode explants produced higher amount of callus than the other explants. Leaf explants produced highest number of shoot at 1.0 mgl^{-1} BAP. Regenerated shoots were excised and then transferred to medium containing various levels of IAA for rooting. IAA at 0.25 mgl^{-1} produced large number of roots. Internode explants were more suitable for root production.

Keywords: *In vitro*, Regeneration, Tomato plant, Leaf, Internode

Introduction

Tomato is one of the most important nutritious and popular vegetables in the world. It belongs to the family Solanaceae. It is grown widely in many countries of the world including Bangladesh for its good source of vit. C and high nutritional quality and for its adaptability to wide range of soil and climate. Ripe tomato is used mostly in salad and other forms of food products, such as jam, jelly, ketchup, pickle, sauce etc. Every year, 30 thousand children's are affected by night blindness due to deficiency of vitamin-A in Bangladesh (FAO 2002). Tomato can play a vital role to reduce such kind of deficiency. Tomato is grown usually during winter in Bangladesh. The demand of tomato is increasing day by day in the agro and food industries of the country. To meet the increasing demand of tomato in Bangladesh, it is necessary to develop good varieties with quality, higher yield potential and wide adaptability. Conventional techniques of crop improvement are lengthy process. The techniques of plant tissue culture have been developed as a new and powerful tool for crop improvement.

Regeneration of plant by tissue culture technique is an important and essential component of biotechnological research as well as genetic manipulation of plants. Cultures are conducted in aseptic environment with the assurance of the production of diseases free plants and without risk of reinfection. High frequencies regeneration of plants from *in vitro* cultured tissues is a prime objective for successful application of tissue culture techniques for crop improvement. Tomato seed is not considered as explants because responses of seed in invitro cultured is associated with various problems such as time consuming, high rate of contamination and very poor callus initiation but leaf and internode segment as explants are very suitable for tissue culture techniques because very high callus initiation and proliferation and low rate of contamination. The objective of the present study was to determine the most suitable explants as well as optimum formulation of media compositions for rapid and reproducible protocol for regeneration of tomato plant.

Materials and Methods

Internodes and leaf of tomato (BINA Tomato -3) were used in this experiment as explants at the tissue culture laboratory of the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh during January, 2003 to April, 2004. Murashige and Skoog (MS)

medium was used for micro propagation. Proper sterilization of MS medium, test tube, pipette, petriplates, beakers, conical flasks, transfer hood, forceps, scalpels and growth chamber made were followed. Incubation chamber was properly maintained.The leaf and internode segments of size 0.5 to 1.0 cm used as explants and then the explants were taken in beaker and were thoroughly washed in running tap water for 8-10 minutes and then washed with sterile water. They were surface sterilized with 70% alcohol for 30 seconds then treated with 0.1% mercuric chloride ($HgCl_2$) solution with 2-3 drops of twin-20/100ml for 10-15 minutes. The surface sterilized explants were then washed 4-5 times with sterile distilled water to remove all traces of $HgCl_2$.

Explants were cultured on MS medium supplemented with 2 to 3mgl^{-1} BAP with 0.10, 0.25 and 0.50 mgl^{-1} NAA for callus initiation and proliferation. Calli from leaf and internodes were sub cultured on the same medium. The explants were cultured on MS medium supplemented with 0.5 mgl^{-1}, 1.0 mgl^{-1} and 2.0 mgl^{-1} BAP for shoot proliferation. Media used for rooting composed of full strength of MS salts plus vitamins, 30 gl^{-1} sucrose and 8 gl^{-1} agar supplemented with 0.15, 0.25, 0.50 and 1.0 mgl^{-1} IAA. Cultured flasks were incubated at 22 ± 2^0 c under 16 hours photoperiod with light intensity of 3000 lux. It was tried to keep the incubation room free from all kinds of microorganism.

The initiation and proliferation of calli and shoots were recorded at 15, 30 and 45 days after culture. Number of roots initiated were counted and length of roots produced in each media were measured after 15, 30 and 45 days of culture. To study the effects of different kinds of treatments, data were collected on callus initiation, fresh weight of callus (g), fresh weight of shoots with callus(g),length of shoots(cm),number of root initiation etc. All experiments were carried out followed by a Randomized Complete Block Design (RCBD) with at least eight replications. The mean separation was done by LSD test.

Results and Discussion

Internode and leaf explants when cultured on MS medium containing a cytokinin BAP plus auxin NAA callus initiated and started to proliferate new callus after 5 days of culture. The greatest amount of callus was obtained from both explants on the medium containing 3.00 mgl^{-1} BAP plus 0.25 mgl^{-1} NAA (Singh Bezai, 2002). The other combinations of BAP and NAA was less effective. Table 1 shows 3.00 mgl^{-1} BAP and 0.5 mgl^{-1} NAA had highest amount of callus (2.080g) at 45 days of culture. Similar results were obtained by others (Locy, 1983, Geetha *et al.,* 1998).In the present studies 3.00 mgl^{-1} BAP plus 0.25 mgl^{-1} NAA might be more balanced than other treatments. This may be an important factor for enhancing the rate of cell division resulting more fresh weight of callus. This findings supports the reports of Capote *et al.(*2000), Selvi and Khader (1993) while working with leaf tissue and stem segments of different cultivars with BAP+NAA combination of PGRs and with leaf disc, stem or shoot tip of tomato cv.PKM.1 with2.50mgl^{-1} BAP+ 0.20 mgl^{-1} NAA+ 3% sucrose respectively. Usually compact callus is considered as best for its high regeneration capacity. More compact callus was also produced from the internode explants. Initially the callus was yellow green, but it turned dark-brown within 5-6 weeks (Fig. 1A, 1B).

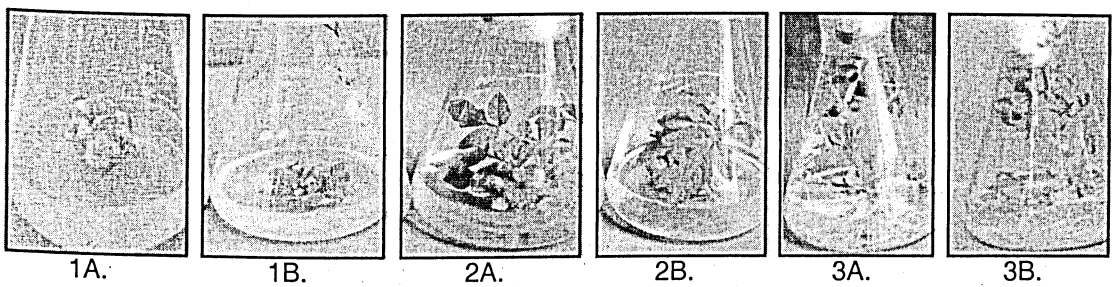
Cultured explants (internode & leaf) produced calli on MS medium containing BAP began to increase in size and started to proliferate new shoots after 3 weeks of culture. The highest number of shoots were obtained from both explants when cultured on medium supplemented with 1.00 mgl^{-1} BAP (Table-2). The culture medium containing 1.00 mgl^{-1} BAP showed the highest shoot proliferation at 45 days of culture. Soniya *et al.* (2001) repoted similar results from the leaf explants of tomato. Leaf explants produced more shoots than internode explants (Table-2).Venkatachalam *et al.* (2000) found the same result on MS medium supplemented with the 1.00 mgl^{-1} BAP. Following 45 days of culturethe calli produced about 3 to 4 shoots in each vessel on the medium containing 1.00 mgl^{-1} of BAP (Fig-2A, 2B).

Table 1. Effects of plant growth regulators on the fresh weight of calli developed from internode and leaf explant

Explants	Concentration and combination of PGR(mgl^{-1})		Fresh weight(g) of callus at different days of culture		
	BAP	NAA	15 days	30 days	45 days
Internode	2.00	0.10	0.515	1.168	1.752
	2.00	0.25	0.519	1.244	1.849
	2.00	0.50	0.483	1.102	1.740
	3.00	0.10	0.585	1.174	1.740
	3.00	0.25	0.641	1.320	1.715
	3.00	0.50	0.509	1.122	2.080
	Control		0.370	0.669	0.957
Leaf	2.00	0.10	0.479	1.095	1.621
	2.00	0.25	0.487	1.225	1.840
	2.00	0.50	0.462	1.098	1.600
	3.00	0.10	0.518	1.136	1.762
	3.00	0.25	**0.608**	**1. 318**	**1.969**
	3.00	0.50	0.496	1.093	1.478
	Control		0.382	0.670	0.982
LSD at 5%			0.053	0.053	0.053

Table 2. Effects of plant growth regulators on the fresh weight of shoots with calli and number of shoots developed from calli of internode and leaf explants

Explants	Concentration of BAP (mgl^{-1})	Fresh weight(g) of shoots with callus at days after culture			Number of shoots at days after culture		
		15 days	30 days	45 days	15days	30 days	45 days
Internode	0.50	0.532	1.232	1.982	-	1.25	2.00
	1.00	**0.579**	**1.404**	**2.131**	-	2.00	3.11
	2.00	0.613	1.238	2.026	-	1.78	2.75
	Control	0.367	0.657	0.962	-	-	-
Leaf	0.50	0.569	1.158	1.620	-	2.00	2.25
	1.00	0.574	1.374	2.106	-	**3.00**	**3.25**
	2.00	0.471	1.268	1.891	-	2.25	3.00
	Control	0.357	0.646	0.921	-	-	-
LSD at 5%		0.055	0.055	0.253	-	1.179	0.783



1A. 1B. 2A. 2B. 3A. 3B.

Fig. Different explants cultured on MS media supplemented with 3.00 mgl^{-1} BAP+0.25 mgl^{-1}NAA, for callus formation; 1.00 mgl^{-1} BAP for shoot production and 0.25 mgl^{-1} IAA for root initiation respectively. **1A.**Callus from internodes **1B.**Callus from leaf **2A.**Shoot from internode initiated callus **2B.**Shoot proliferation from leaf initiated callus **3A.**Root production from internode initiated shoots **3B.**Root production from leaf initiated shoots

Shoots(1.5 cm in length) growing on the medium containing 1.0 mgl^{-1} of BAP were isolated from the culture vessels and transferred to MS medium used as control and also media containing different levels of auxin (IAA) only for rooting. The shoots did not produce roots on the medium without containing growth regulator. The highest number of roots were observed from the calli containing 0.25 mgl^{-1} IAA in the medium (Table-3). The present result is in agreement with that of earlier authors Liu *et al.* (2003). Both the explants produced the highest number of roots on the medium supplemented with 0.25 mgl^{-1} IAA as compared to other concentrations of IAA. The roots attained a length of 4-12cm within 45 days of culture (Fig-3A, 3B). After rooting both shoots and roots continued to grow until complete plantlets were established.

Table 3. Effects of plant growth regulators on number of roots and length of plantlets developed from excised shoots

Explants	Initial length of shoots(cm)	Concentration of IAA (mgl^{-1})	Average number of roots $plantlets^{-1}$			Average length of plantlets (cm) at days after culture		
			15 days	30 days	45 days	15 days	30 days	45 days
Internode	1.5	0.15	15.46	20.71	27.00	0.440	1.200	3.650
		0.25	**18.00**	**25.00**	**29.50**	**0.610**	**1.420**	**4.910**
		0.50	16.17	21.15	22.78	0.470	1.410	3.990
		1.0	7.95	11.86	13.75	0.440	1.300	3.740
		Control	-	-	-	0.240	0.520	0.860
Leaf	1.5	0.15	15.00	18.61	22.23	0.430	1.340	3.710
		0.25	**17.32**	**23.96**	**27.82**	**0.610**	**1.620**	**4.710**
		0.50	16.30	19.96	22.25	0.450	1.340	3.730
		1.0	6.37	8.77	10.70	0.400	1.310	3.200
		Control	-	-	-	0.220	0.490	0.780
LSD at 5%			4.007	4.139	2.955	0.162	0.132	0.093

Conclusion

Finally it was clear that the internode explants produced maximum amount of calli with 3.00 mgl^{-1} BAP plus 0.25 mgl^{-1} NAA. Calli from leaf explants produced highest number of shoots with 1.00 mgl^{-1} BAP and shoots from internode calli produced maximum number of roots containing 0.25 mgl^{-1} IAA in the medium

References

Capote, R.A., Fundora, M.Z. and Perez, D.O. 2000. Effects of different factors on invitro plant regeneration from leaflets of five genotypes of tomato (*Lycopersicon esculentum* Mill.). Revista-del-Jardin-Botanica-Nacional, 21(1): 71-76.

FAO. 2002. Quaterly Bullentin of Statistics. Food and Agriculture Organization. Rome. Vol.20(1/4):87-88.

Geetha, N., Venkatachalam, P., Reddy, P.S. and Rajaseger, G. 1998. *In vitro* plant regeneration from leaf callus cultures of tomato (*Lycopersicon esculentum* Mill.). Advan. Plant Sci., 11(1): 253-257.

Liu, Qingbo, Zhao, Yan, Caj, Nerg, Liu, Q.B., Zhao,Yan and Cai, N. 2003. Studies on the rapid propagation of "peral" tomato. J. Hunan Agril. University. 29 (2); 128-126.

Locy, R.D. 1983. Callus formation and organogenesis by explants of six *Lycopersicon* species. Can. J. Bot., 61: 1072-1079.

Selvi, D.T. and Khader, M.A. 1993. In vitro morphogenetic capacity of tomato (*Lycopersicon esculentum* Mill.). var. PKM.1. South Indian Hort.,41(5):251-258.

Singh, R. and Bezai, T. S. 2002. Effects of PGRs on high rate of callus proliferation of tomato cv. Roma. VP. Can. J.Botany. 80:928-931.

Soniya,E .V.; Bakeries, N.S. and Das, M. R. 2001. Genetic analysis of somaclonal variation among callus-derived plants of tomato. Current Science (9): 1213-1215.

Venkatachalam, P., Geetha, N., Priya, P., Ajaseger, G. and Jayagbalan, N. 2000. High frequency plantlet regeneration from hypocotyls explants of tomato (*Lycopersicon esculentum* Mill.). via organogenesis. Plant cell Biotech. Molecular Biol., 1(3-4):95-100.