Academic Journal of Plant Sciences 5 (4): 114-118, 2012
ISSN 1995-8986

DOI: 10.5829/idosi.ajps.2012.5.4.323

Optimization of Growth Regulators for Shoot Induction and Regeneration of Tomato (*Lycopersicum esculentum* Mill.)

[1]*Pritesh Parmar,* [1]*R.B. Subramanian* and [2]*A.K.K. Achakzai*

[1]BRD School of Biosciences, Sardar Patel University,
Post Box No. 39, Vallbh Vidya Nagar-388120 (Gujarat) India
[2]Department of Botany, University of Balochistan, Quetta - Pakistan

Abstract: Until now numbers of efforts have been made to deduce the optimized conditions for the *in vitro* regeneration of tomato plant. The optimized conditions deduced are contradictory to each other and to validate the optimum conditions the present investigation was carried out which directs the use of 1.5 mg/l IAA + 1.5 mg/l Zeatin with full strength Murashige and Skoog's (MS) medium for the development of callus, which was regenerated in to complete plants by shoot development at 1.5mg/l of Zeatin and 1.5 mg/l of IAA and rhizogenesis in the full strength MS medium supplemented with 1.5 mg/l IAA.

Key words: Tomato · Tissue culture

INTRODUCTION

Tomato (*Lycopersicum esculentum* Mill.) is the world most important vegetable crop after potato, being cultivated in many regions of the world: in the Americas, Southern Europe, the Middle East and North Africa, India, China, Japan and Southeast Asia. The peoples like to have it in many forms such as raw vegetable, food additives or as processed products such as paste, juice, whole peeled, sauces, diced and soups. In terms of medical science it encompass antioxidant lycopene (alkaloids) a health promoting compounds whose consumption reduces the incidence of many types of cancer [1, 2]. Besides these characteristics, it is a crucial model systems for basic and applied research and can be easily propagated using either seeds, or clonally by tip or shoot cuttings. In present scenario, tomato was used as bioreactor in biopharming for the production and oral delivery of vaccines [3] and as functional food for cancer prevention [4]. Currently, tomato has become the subject of new areas of intensive research such as: functional genomics, proteomics and metabolomics.

Plant tissue culture has progressed immensely after its inception in the year 1930s, it was the time when scientists used this technique to grow cells in culture. Currently, it is applied for many different aspects such as callus induction, anther culture, protoplast culture and somatic embryogenesis amongst the others. Plant tissue/cell culture is a key step in genetic transformations using *Agrobacterium tumefaciens* for the development of transgenic plant. The development of a cost effective and efficient protocol for mass propagation of high quality seedlings via tomato tissue culture could help lower the price per seedling. A good *in vitro* plant regeneration system may also assist in further improvement of the commercially important varieties for disease resistance via genetic engineering [5].

In this study we focus on optimization of hormonal content for the regeneration of Pusa ruby, tomato cultivar by tissue culture techniques,

MATERIALS AND METHODS

Plant Material: 'Pusa ruby' a tomato variety which is known to be highly susceptible against *Fusarium oxysporum* f. sp. *lycopaersici* race 1 [6] was optimized for the *in vitro* regeneration.

Selection of Media: Basal MS nutrient medium [7] was selected and used during the course of present study. A comparative account of the inorganic and organic constituents of the above medium is tabulated in Table 1. Several Plant Growth Regulators (PGRs) were used from Sigma, USA or Himedia, Mumbai. PGRs like IAA, IBA, BA, KN, NAA, 2,4-D etc. (Table 2) were used in different concentrations and combinations to find out suitable media combinations for the growth and differentiation of different explants for selected plant species.

Corresponding Author: R.B. Subramanian, BRD School of Biosciences, Sardar Patel University,
Post Box no. 39, Vallbh Vidya Nagar-388120 (Gujarat) India.

Table 1: Composition of Murashige and Skoog's (1962) medium

Stock Solution	Chemical Constituents	Final Conc.^{n} in Medium (mg/l)	Conc.^{n} of Stock Sol.^{n} (gm/500ml)	Volume of Stock Sol.^{n} in Final medium (ml/l)
Stock-I(20 X)	KNO_3	1900.00	19.00	
	NH_4NO_3	1650.00	16.50	
	$CaCl_2.2H_2O$	440.00	4.40	
	$MgSO_4.7H_2O$	370.00	3.70	
	KH_2PO_4	170.00	1.70	50.00
Stock-II (200 X)	$MnSO_4.4H_2O$	22.300	2.230	
	$ZnSO_4.7H_20$	8.600	0.860	
	H_3BO_3	6.200	0.620	
	KI	0.830	0.083	
	$Na_2MoO_4.2H_2O$	0.250	0.025	
	$CuSO_4.5H_2O$	0.025	0.0025	
	$CoCl_2.6H_2O$	0.025	0.0025	5.00
Stock-III (200 X)	Na_2EDTA	37.30	3.73	
	$FeSO_4.7H_2O$	27.80	2.78	5.00
Stock-IV (200 X)	Myo-inositol	100.00	10.00	
	Glycine	2.00	0.20	
	Pyridoxine Hcl	0.50	0.05	
	Nicotinic Acid	0.50	0.05	
	Thiamine HCl	0.10	0.01	5.00

Addendum: Carbon Source= Sucrose (3.0 %)

Gelling Agent= Agar-Agar (0.8 %)

pH= 5.8

PGRs = As per requirements

Table 2: Plant Growth Regulators (PGRs) used in the present study

Plant Growth Regulators	Mol. Wt.	Solvent	Liquid Storage	Sterili.^{n}
Auxins				
Indole-3-acetic acid (IAA)	175.19	1N NaOH	0° C	CA/F
Indole-3-butyric acid (IBA)	203.24	-Do-	0° C	CA/F
2,4-Dichlorophenoxy acetic acid (2,4-D)	221.04	-Do-	2°-8° C	CA
α-Naphthalene acetic acid (NAA)	186.21	-Do-	2°-8° C	CA
Cytokinins				
6-Furfuryl amino purine (Kinetin)	215.21	1N Hcl	0° C	CA/F
6-Benzyl amino purine (BA)	225.26	-Do-	2°-8° C	CA/F

CA = Co-autoclavable with other media components.

CA/F = Co-autoclavable with other media components.

However, some loss of activity may occur. This can be compensated by increasing the concentration or may be filter sterilized.

Media Preparation: Different media combinations were prepared by using stock solutions of MS (Table 1) and PGRs. (Table 2) After all stock solutions were added and dissolved (except gelling agent); the pH of the medium was checked and adjusted to 5.8. Medium volume was adjusted to final volume using double distilled water (DDW). To gel the medium, 8 gm/l of Agar (Himedia, Mumbai) was mixed with the medium. The medium was boiled to dissolve the gelling agent and distributed into sugar tubes and was autoclaved at 121°C at 1.05 kg cm^{-2} for 20 min. in a vertical autoclave. After autoclaving, the container was put in a slanting position to make slants and allowed to cool for solidification.

Callus Regeneration and Selection of Effective Surface Sterilizing Agent: Different plant parts of *Lycopersicon esculentum*, like leaf, stem and roots were selected for callus formation. Explants were carefully excised and then surface sterilized using different surface sterilizing agents (Table 3) under laminar air flow. After the treatment with sterilant, the explants were thoroughly rinsed with sterile double distilled water for 5-6 times to remove traces of sterilant and blot dried with the help of sterile Whatman No. 1 filter paper before being cut into pieces and transferred to the nutrient culture medium. Each experiment was repeated thrice with

Table 3: Surface sterilizing agents with different combinations and concentration used for the study

Sr. no	Sterilizing agents	Concentration	Time duration	Optimum
01	Mercuric chloride	0.1 to 1.5%	1 to 10 minutes	(0.1% for 2 minutes)
02	Ethanol	70% to 100%	1 to 10 minutes	
03	Sodium hypochlorite	1 to 2%	1 to 10 minutes	
		Combination		
04	Hypochlorite + Tween 20	--	1 to 10 minutes	
05	Ethanol + Hypochlorite	--	1 to 10 minutes	
06	Mercuric chloride + Ethanol	--	1 to 10 minutes	
07	Mercuric chloride + hypochlorite	--	1 to 10 minutes	

Table 4: PGRs used for the callus initiation and multiple shoot formation

Sr. no	Cytokines	Concentration (mg/l)	Optimum
01	Benzyl adenine	0.2 to 2.0	
02	Kinetin	Do	
03	BA + IBA	Do	
04	NAA + BA	Do	
05	IAA + BA	Do	
06	Zeatin + IAA	Do	(0.5mg/l)
07	Zeatin + NAA	Do	
08	Zeatin	Do	
08	2-4 D	Do	

Table 5: PGRs used for the regeneration of roots

Sr. no	Auxins	Concentration (mg/l)	Optimum
01	IAA + Zeatin	0.2 to 2.0	
02	IBA	do	
03	IBA + Kinetin	do	
04	NAA + Kinetin	do	
05	Half MS medium	---	
06	One fourth MS medium	---	
07	NAA	do	
08	IAA	do	(0.5mg/l)
09	2-4 D	do	
10	Half Modified white root culture (Hi-media)	---	
11	One fourth Modified white root culture (Hi-media)	---	

15 replicates per treatment. All the tubes were incubated at 25 ± 2°C under 16/8 hours (day /night) photoperiod (Light intensity 50-60µmol/m^2 S).

Culture Media for Regeneration of Plants from Callus: For the regeneration of plantlets, callus was cultured on MS medium supplemented with different strengths of plant growth regulators alone or in various combinations (Table 4). The age of callus and hormonal conditions in culture media were variable for plantlet regeneration. All the tubes were incubated at 25 ± 2°C under 16/8 hours (day /night) photoperiod (Light intensity 50-60µmol/m^2 S). For *in vitro* root formation, the callus with multiple shoot development was inoculated in the root induction medium (Table 5). Each experiment was repeated thrice with 15 replicates per treatment. All the tubes were incubated at 25 ± 2°C under 16/8 hours (day /night) photoperiod (Light intensity 50-60µmol/m^2 S).

Establishment of Plantlets in Soil: After the development of roots, plantlets were taken out from the culture media, rinsed thoroughly with distilled water for removal of agar adhering to the plantlets, followed by Ampicillin and 0.5 % (w/v) Em-Carb (12% Carbendazime and 63% Mancozeb) treatment for about 20-25 min in distilled water or 20 ppm of biovistin solution treatment for 10 minutes to control microbial infection. Initially these plantlets were transferred to polycups containing sterilized cocopeat and 0.1% farmyard organic manure (FYM) supplemented with hormone and sucrose free sterile half-strength (1/2) MS basal medium for 2-3 weeks under aseptic conditions. Subsequently, plantlets were transferred to pots having a sterilized mixture of garden soil: farmyard organic manure [2:1 (w/w)] for about 4-6 weeks for acclimatization in green house and finally transferred to the field condition to their own natural habitat.

RESULTS

Callus Induction: Callus induction was initiated with use of different explants in combination of different surface sterilizing agents. Table 3 shows the different surface sterilizing agents tried in the study. Of all the explants tried for callus induction, it was observed that leaf explants showed excellent response while the seed and root explants showed slow response. Incase of surface sterilizing agents 0.1% mercuric chloride for 2 minutes proved to be the best among all other combinations.

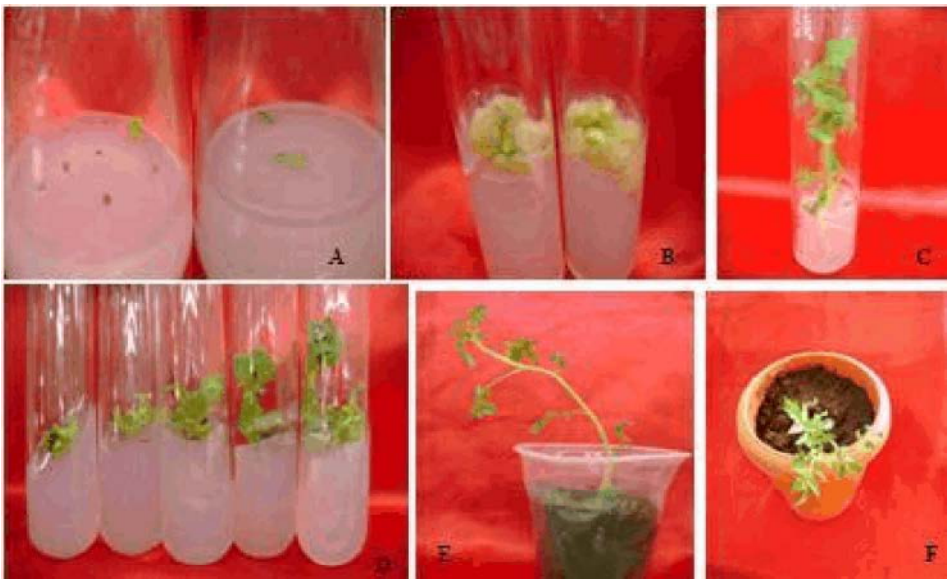

Fig. 1: Tissue culture study. A: Tomato explants used for regeneration, B: Green compact callus obtained from the leaf explant, C: Regeneration of roots, D: Regeneration of multiple shoots from the callus, E: Acclimatization of tomato plant in Cocopeat: soil mixture (1:1), F: Transfer of tomato plant to pot for acclimatization.

Fig. 1(A) shows the different explants that were used to initiate the callus. The leaf explants gave rise to compact, light green colored and regenerative type of callus in the MS medium augmented with auxin (IAA) and cytokinin (Zeatin) in different combinations. High frequency, compact, greenish white, regenerative callus was obtained from the leaf explants on full strength MS media supplemented with 30 gm/l sucrose and 0.5 mg/l IAA + 0.5 mg/l Zeatin [Fig. 1(B)].

Regeneration of Plantlets from Callus: Shoot formation was induced from the callus using different concentrations and combinations of cytokinins and auxins like BA, KN or 2-4D, Indole acetic acid, Indole butyric acid, Zeatin etc (Table 4) in MS full and half strength media. Shoot regeneration started after 4-6 weeks of inoculation and was observed at low frequency with 2.0mg/l of BA and 0.5 mg/l of 2-4D and 2.0mg/l of BA and 0.5 mg/l of KN. Shoot regeneration was found to be better with the combination 0.5mg/l of Zeatin and 0.5 mg/l of IAA [Fig. 1(D)]. Regenerated shoots were transferred to MS medium with different strengths as well as MS medium supplemented with different hormonal combinations (Table 5). The plantlets showed a high frequency of rhizogenesis in the full strength MS medium supplemented with 0.5 mg/l IAA [Fig. 1(C)]. Complete plantlets were obtained along with shoots and roots after 45 days.

Acclimatization: The *in vitro* regenerated plantlets of tomato were transferred to polycups containing a mixture of sterile moist cocopeat and sterile soil (1:1), sterile cocopeat, Hoagland's medium and sterile soil for acclimatization. Best response was observed in cocopeat: soil (1:1) mixture at 25 ± 2°C for 10 days [Fig. 1(E)]. Later, these plantlets were acclimatized to environmental conditions by taking the cups out of culture laboratory gradually initiating from 1 to 24 hrs. The *in vitro* grown plants exhibited a survival rate of 60% in field conditions [Fig. 1(F)]. During the acclimatization period, plantlets were provided with ½ strength MS basal medium without vitamins, sucrose and PGRs.

DISCUSSION

Plant tissue culture is a practice used to propagate plants under sterile conditions, often to produce clones of a plant. Development of protocols for *in vitro* selection can provide new advances for the production of stress tolerant varieties. Attempts have also been made to transfer the higher regenerative ability of wild varieties to cultivated tomatoes. Although, some information is available on the morphogenesis of tomato, the techniques have not been developed to a level at which they can be utilized in large-scale multiplication of commercially important varieties. The morphogenesis response seems to be highly dependent PGRs (plant growth hormones) used in the media.

In the present study, high yielding tomato variety, Pusa ruby variety was chosen for transformation, as it showed the best response during standardization of the regeneration protocol and also because it is highly sensitive to Fusarium wilt under natural conditions.

Roy *et al.* [8] regenerated callus from the leaf explants in the medium supplemented with hormones IAA (0.5 mg/l) and (0.5 mg/l). Similar combination was used for shoot regeneration and it was found to be optimum for the selected variety. After regeneration of shoot, rhizogenesis was achieved with (0.5 mg/l) IAA and the whole plants were transformed to garden soil mixture for acclimatization. Raj *et al.* [9] deduce similar findings with the same combination of growth hormones for the shoot initiation and the callus development but the concentration was 2.0 mg/l zeatin + 0.1 mg/l IAA for callus and 0.1 mg/l zeatin + 0.1 mg/l IAA for shoot initiation. The plant was raised in MS medium supplemented with Gamborg's B5 and ascorbic acid vitamin (vitamin c). Another hormone IBA with 0.05 mg/l and Hoagland's medium was found to be optimum for the root regeneration and acclimatization of 'Pusa ruby' variety. Accumulating evidences suggest that *in vitro* tissue culture and micropropagation are necessary for transformation experiments to remodel the aesthetic and growth characteristics of the plants [10-12].

CONCLUSION

In view of the variable optimized conditions for the regeneration of tomato plant in present study it was rechecked and validated with optimum conditions.

ACKNOWLEDGEMENTS

This work was supported by grants for scholarship to meritorious students from University Grants commission (UGC), Government of India. We would like to thank Ms. Laura Bassolino, Dr. S. Gonzalli, Plant Lab, Scuola Superiore Sant'Anna Via Mariscoglio, Pisa, Italy, for assisting in generation of tomato plant through tissue culture technique.

REFERENCES

1. Rao, A. and S. Agarwal, 2000. Role of antioxidant lycopene in cancer and heart disease. J. Amer. College Nutr., 19: 563-569.
2. Pohar, K.S., M.C. Gong, R. Bahnson, E.C. Miller and S.K. Clinton, 2003. Tomatoes, lycopene and prostate cancer: a clinician's guide for counselling those at risk for prostate cancer. World J. Urol., 21: 9-14.
3. Jiang, X.L., Z.M. He, Z.Q. Peng, Y. Qi, Q. Chen and S.Y. Yu, 2007. Cholera toxin B protein in transgenic tomato fruit induces systemic immune response in mice. Transgenic Res., 16: 169-175.
4. Butelli, E., L. Titta, M. Gorgio, H.S. Mock, A. Mantos, S. Peterek, E.G.W.M. Schijjlen, R.D. Hall, A.G. Bovy, J. Luo and C. Martin, 2008. Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. Nature. Biotechnology. Doi:10.1038/ntb.1506.
5. Bhatia, P., N. Ashwath, T. Senaratna and D. Midmore, 2004. Tissue culture studies of tomato (*Lycopersicon esculentum*). Plant Cell, Tissue and Organ Culture, 78: 1-21.
6. Parmar, P.P., V.P. Oza, A.D. Patel, K.B. Kathiria and R.B. Subramanian, 2011. Development of a rapid and reliable bioassay to discriminate between susceptible and resistant cultivars of tomato against Fusarium wilt. Int. J. Biosci. Agric. and Technol., 3(2): 6-11.
7. Murashige, T. and F. Skoog, 1962. A revised médium for rapid growth and bio assays with tobacco tissue cultures. Phy. Plantarum, 15(3): 473-497.
8. Roy, R., R. Purty, V. Agrawal and S.C. Gupta, 2006. Transformation of Tomato cultivar 'Pusa Ruby' with bsp A gene from *Populus tremula* for drought tolerance. Plant Cell Tissue and Organ Culture, 84: 55-67.
9. Raj, S.K., R. Singh, S.K. Pandey and B.P. Singh, 2005. Agrobacterium mediated tomato transformation and regeneration of transgenic lines expressing Tomato leaf curl virus coat protein gene for resistant against TLCV infection. Current Sci., 88(10): 1674-1679.
10. Jaime, A and D.S. Teixeira, 2003. Chrysanthemum: advances in tissue culture, cryopreservation, postharvest technology, genetics and transgenic biotechnology. Biotechnol. Adv., 21(8): 715-766.
11. Tang, K., P. Tinjuangjun, Y. Xu, X. Sun, J.A. Gatehouse, P. Ronald, H. Qi, X. Lu, P. Christou and A. Kohli, 1999. Particle-bombardment-mediated co-transformation of elite Chinese rice cultivars with genes conferring resistance to bacterial blight and sap sucking insect pests. Planta, 208: 552-563.
12. Liasu, M.O. and A.K.K. Achakzai, 2007. Influence of Tithonia diversifolia leaf mulch and fertilizer application on the growth and yield of potted tomato plants. American-Eurasian J. Agric. and Environ. Sci., 2(4): 335-340.