http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 7 (2): 269-272, 2004
ISSN 1028-8880

Regeneration from Various Explants of *in vitro* Seedling of Tomato (*Lycopersicon esculentum* L., c.v. Roma)

Zubeda Chaudhry, Darima Habib, Hamid Rashid and [1]Asfari Sharif Qureshi
Agricultural Biotechnology Programme, Institute of Agricultural Biotech and Genetic Resources (IABGR)
National Agricultural Research Centre, Park Road Islamabad, Pakistan
[1]Department of Biological Sciences, Q.A.U., Islamabad, Pakistan

Abstract: The *in vitro* response of Roma was tested using hypocotyls and leaf discs as an explant source for callus induction and regeneration. Leaf discs and hypocotyl showed variable response by use of different growth regulators. Formation of calli from leaf discs and hypocotyls were obtained on MS medium with IAA 2 mg l^{-1}, BAP 2 mg l^{-1}, NAA 2 mg l^{-1} and kinetin 4 mg l^{-1}. Callus formation was 82.5% from hypocotyl and 57% from leaf discs. Maximum percentages of shoot formation from hypocotyl and leaf discs derived calli was 45.8% and 30.8%, respectively on MS medium with IAA 2 mg l^{-1}, BAP 5 mg l^{-1}, NAA 2 mg l^{-1} and kinetin 4 mg l^{-1}.

Key words: Roma, MS medium, leaf disc, hypocotyl, regeneration

INTRODUCTION

Tomato is an important food crop and plays an important role in Pakistan's economy. There are many commercial varieties of tomatoes grown all over the country. Roma is one of them, it is widely grown in Pakistan. It is a warm season crop and is sown in spring and summer. Its production is affected by various stresses which include diseases. Hence, there is a need to improve this cultivar using biotechnological approaches. As we know that tomato is a favourable crop for genetic improvement and many genes for different traits have been introduced into this crop. Taking this approach, we want to develop a transformation system for introduction of gene in Roma. Before that, there is a need to develop a high frequency regeneration system in this cultivar.

Previously, various explant sources are reported for callus induction and regeneration on different media in tomato. Tomato explants from many sources of tissue have been successfully grown in culture[1]. Explants like hypocotyl segments, leaf discs, roots, shoot tips, cotyledons and anthers are also reported for callusing and regeneration[2]. Earlier, development of high frequency regeneration system was achieved in Feston and Nagina[3] but no evidence is available on tissue culture studies in Roma.

The experimental work was conducted to establish a reproducible protocol for callus induction and regeneration in tomato c.v. Roma by using specific combinations of growth regulators.

MATERIALS AND METHODS

Seeds of tomato c.v. Roma were obtained from Horticultural Research Programme, National Agricultural Research Centre, Islamabad. Overnight soaked seeds of tomato cv. Roma were surface sterilized with clorox (sodium hypochlorite 5.25%) for 10 min. and rinsed 4-5 times with autoclaved water. Then washed with Tween-20 for 2-3 min. and rinsed with sterilized water till the foam was completely removed. The seeds were inoculated on MS medium[4] and later transferred to conditions with 16h. photoperiod of a light intensity of 1500 lux. It was noticed that seeds started growing in dark and later they were transferred to light. It was observed that germination was possible after 12-18 days from Roma variety.

Leaf discs and hypocotyl segments from 12-18 days old *in vitro* plants were excised under aseptic conditions. The size of leaf disc was almost 5x5 cm^2 and that of hypocotyl was 3-4 cm. These explants were cultured on callus induction media. The different hormonal combinations which were used in callus induction media as shown in (Table 1).

The pH of the medium was adjusted at 5.8 after the addition of hormones. Every medium was sterilized at 120°C for 20 min.

After 2-3 weeks, callus were formed and these calli were then transferred to the maintenance medium for 3 weeks. The maintained calli were then transferred to the regeneration medium (Table 2).

Corresponding Author: Hamid Rashid, Agricultural Biotechnology Programme, Institute of Agricultural Biotech and Genetic Resources (IABGR) National Agricultural Research Centre, Park Road Islamabad, Pakistan

Table 1: Different hormonal combinations used in callus induction media of tomato

S. No	Media Composition
CIM_1	MS salts and vitamins, sucrose 30 g l^{-1}, IAA (2 mg l^{-1}), BAP (2 mg l^{-1}), NAA (2 mg l^{-1}), Kinetin (4 mg l^{-1}) and Agar 6 g l^{-1}.
CIM_2	MS salts and vitamins, sucrose 30 g l^{-1}, IAA (2 mg l^{-1}), BAP (5 mg l^{-1}), NAA (2 mg l^{-1}), Kinetin (4 mg l^{-1}) and Agar 6 g l^{-1}.
CIM_3	MS salts and vitamins, sucrose 30 g l^{-1}, IAA (0.5 mg l^{-1}), BAP (2 mg l^{-1}), NAA (0.5 mg l^{-1}), Kinetin (2 mg l^{-1}) and Agar 6 g l^{-1}.

Table 2: Different hormonal combinations used in regeneration medium of tomato

S. No	Media Composition
RM_1	MS salts and vitamins, sucrose 30 g l^{-1}, IAA (2 mg l^{-1}), BAP (5 mg l^{-1}), NAA (2 mg l^{-1}), Kinetin (4 mg l^{-1}) and Gelrite 2 g l^{-1}.
RM_2	MS salts and vitamins, sucrose 30 g l^{-1}, BAP (2 mg l^{-1}), NAA (1 mg l^{-1}), Kinetin (4 mg l^{-1}) and Gelrite 2 g l^{-1}.
RM_3	MS salts and vitamins, sucrose 30 g l^{-1}, IAA (1 mg l^{-1}), BAP (1 mg l^{-1}), NAA (1 mg l^{-1}), Kinetin (2 mg l^{-1}) and Gelrite 2 g l^{-1}.

Table 3: Effect of different concentrations of clorox on *in vitro* plant germination of tomato

C:W (Clorox:Water)	Total culture	Germinated	Germination (%)
1:3	72	15	20.8
1:5	72	21	29.1
1:8	72	61	84.7

Table 4: Callus induction response from hypocotyl and leaf discs of *in vitro* seedling of tomato cv. Roma

		Callus formed		Callus formation (%)	
Medium	Total cultures	Hypocotyls	Leaf discs	Hypocotyls	Leaf discs
CIM_1	120	99	69	82.5	57.5
CIM_2	120	50	41	41.6	34.1
CIM_3	120	35	19	29.1	15.8

Table 5: Percentage of maintained calli on maintenance medium of tomato

Explants	No. of calli	Browning	Growth	Maintained calli (%)
Hypocotyls	100	30	70	70
Leaf discs	100	74	26	26

Thirty to sixty cultures were raised for each experiment and each experiment was conducted thrice. Visual observations was taken every week and the effect of different treatment on explants showing the response for plant development, callus induction and regeneration of shoots/roots.

RESULTS AND DISCUSSION

The seeds of Roma were cultured on MS medium. For sterilization various strengthen of clorox were used to optimize the level of clorox suitable for *in vitro* germination. High concentration had an inhibitory effect and the seed became dead whereas when the concentration was diluted from 1:0 to 1:8, the percentage germination of seeds was increased. The clorox becomes diluted by the addition of water and 1:8 was the most suitable for the germination of seeds and maximum percentage germination of seed i.e. 84.7% was observed at this ratio. However strong concentration i.e. 1:3 does not show very much positive response and minimum percentage germination i.e. 20.8% was observed at this ratio. (Table 3) Fig. 1.

Two explant sources i.e. leaf discs and hypocotyls were used. Many workers are reported to use such explant sources[5]. Leaf discs and hypocotyls showed variable response by use of different growth regulators. Their response was dependent on the different combinations of growth regulators. Hypocotyl showed 82.5% of callus induction at the hormonal combination of IAA 2 mg l^{-1}, BAP 2 mg l^{-1}, NAA 2 mg l^{-1}, Kn 4 mg l^{-1}, whereas leaf discs exhibited 57% at the same hormonal combinations (Table 4) Fig. 2a and 2b. Earlier, Chaudhry *et al.*[3] obtained 84.8% of hypocotyl calli and 76.6% of leaf discs calli on MS medium containing BAP and Zeatin (1-5 mg l^{-1}) combined with IAA (0.1-0.5 mg l^{-1}).

Calli which were produced on CIM_2 and CIM_3 are very weak and developed very slowly. Our results are contrary as reported by Hill *et al.*[6] observed that callus is generally induced on medium with high cytokinins content and to moderate level of auxins. The calli which were formed were then transferred to the maintenance medium having the same hormonal combinations which were used in CIM_1 (Table 5) Fig. 2c and 2d.

Shoot formation from calli of hypocotyls and leaf discs were successfully obtained on different hormonal combinations. It was observed that maximum shoot formation was observed at the hormonal combination of IAA 2 mg l^{-1}, BAP 5 mg l^{-1}, NAA 2 mg l^{-1}, Kn 4 mg l^{-1}, leaf disc derived calli 30.8% regeneration whereas

Fig. 1: *In vitro* plant germination from sterilized seed of tomato (*Lycopersicon esculentum* M.) c.v. Roma

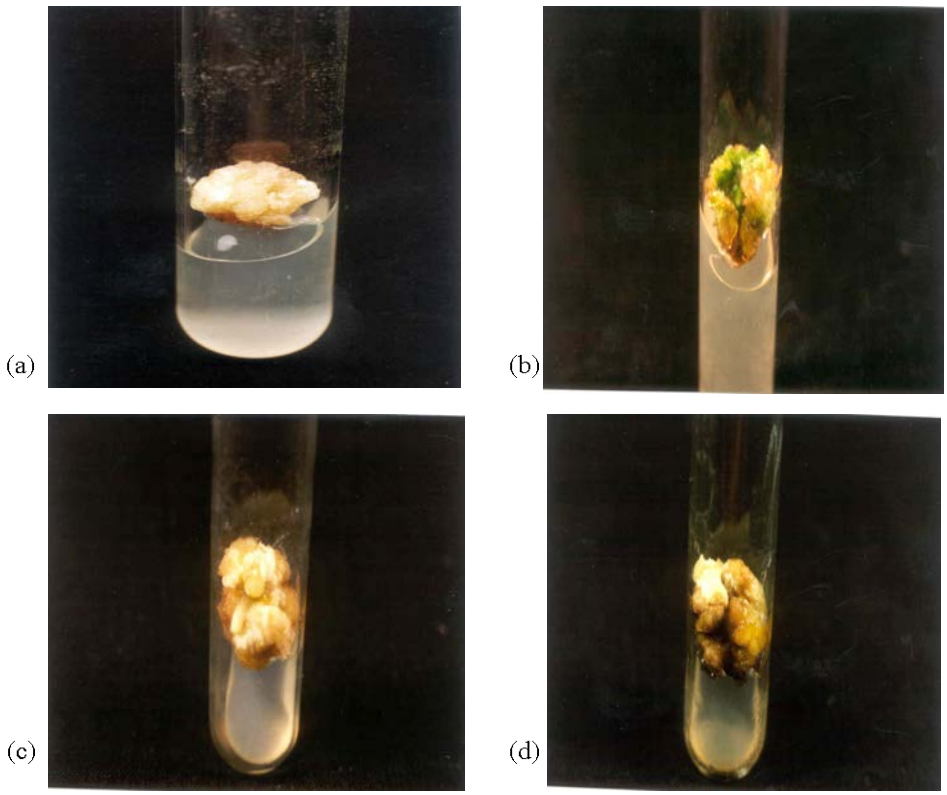

Fig. 2: Callus formation and maintenance of hypocotyls and leaf discs
(a) Callus formation form hypocotyls on CIM_1. (b) Callus formation form leaf disc on CIM_1. (c) Maintained Hypocotyl calli on maintenance medium. (d) Maintained leaf disc calli on maintenance medium

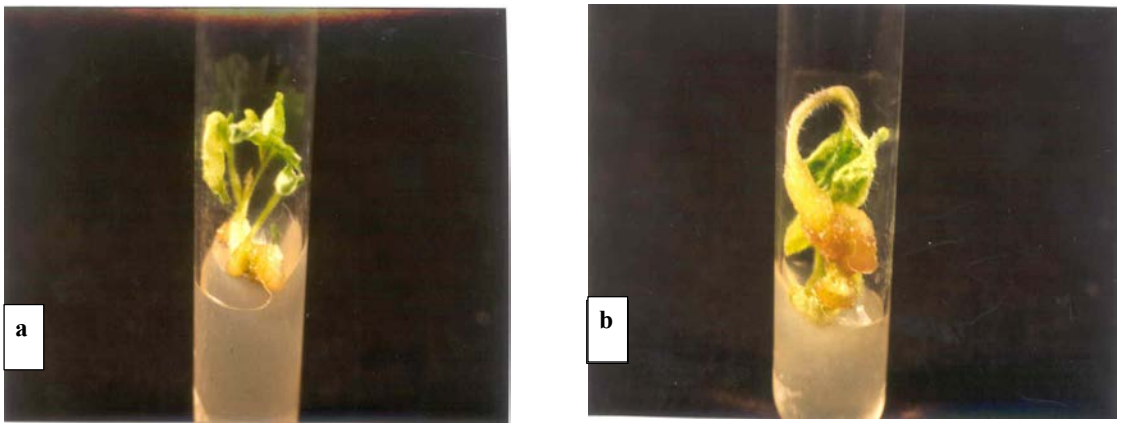

Fig. 3 (a): Shoot formation from Hypocotyl calli on RM_2 (b): Shoot formation from leaf disc calli on RM_2

hypocotyl derived calli 45.8% regeneration (Table 6, Fig. 3). Duzvaman *et al.*[7] achieved maximum shoot formation on medium containing BAP 3.0 mg l^{-1} and IAA 0.20 mg l^{-1}.

Shoot formation is also affected by the age of seedling. Minimum %age of shoot formation for hypocotyl derived calli was 18% and for leaf discs derived calli was 8.5% from 5 days old *in vitro* plants. However, with the increase in the age of seedling the %age of shoot formation was also increased. Maximum shoot formation for hypocotyl 38.1% and for leaf discs 40% was noticed on 15-20 days old *in vitro* seedling. More than 20 days old seedlings showed less %age of shoot formation (Table 7).

Table 6: Effect of different hormonal combination on the shoot regeneration percentage of callus from hypocotyls and leaf discs

		Shoot regeneration		Shoot formation (%)	
Medium	Total cultures	hypocotyls	Leaf discs	hypocotyls	Leaf discs
RM_1	120	55	37	45.8	30.8
RM_2	120	22	18	18.5	15.8
RM_3	120	15	15	12.5	12.5

Table 7: Effect of seedlings age on regeneration efficiency for hypocotyl of tomato (*Lycopersicon esculentum* L.) cv. Roma

Age of seedling (days)	Total explants cultured	Shoot formation from calli	Shoot formation (%)
Hypocotyls			
5	55	10	18.0
10	55	13	23.6
15	55	18	32.7
20	55	21	38.1
25	55	16	29.0
Leaf discs			
5	35	3	8.5
10	35	7	20.0
15	35	9	25.7
20	35	14	40.0
25	35	8	22.8

To compare the *in vitro* performance of explants used i.e. leaf discs and hypocotyl. Hypocotyl appeared to be better explant source as compared to leaf discs. Hypocotyl showed 82.5, 45.8% as compared to leaf discs 57.55, 30.8% in callogenesis and shoot formation, respectively.

All the study of hormonal combinations are quite close with other workers and different may be due to the varietal different explants of tomato and differences in composition of media used.

In summary, this study has shown that hypocotyl is a better explant source for callus formation and regeneration which is a pre-requisite for *Agrobacterium*-mediated transformation to improve this cultivar for disease resistance.

REFERENCES

1. Newman, P.O., S. Krishnasaj and P.K. Saxena, 1996. Regeneration of tomato (*Lycopersicon esculentum* L.) somatic embryogensis and shoot organogene from hypocotyl segments induced with Benzylaminopurine. Intr. J. Plant Sci., 157: 554-560.
2. Costa, M.G.C., F.I.S. Nogueira, M.L. Figueira, W.C. Otoni, S.H. Brommonschenkel and P.R. Cecon, 2000. Influence of an antibiotic timentin on plant regeneration of tomato (*Lycopersicon esculentum*, L.) cultivars. Revista brasileria de Fisiologia Vegetal., 19: 327-323.
3. Chaudhry, Z., I. Feroz, W. Ahmed, H. Rashid, B. Mirza and A. Quraishi, 2001. Varietal response of *Lycopersicon esculentum* L. to callogenesis and regeneration. OnLine J. Biol. Sci., 1: 1138-1140.
4. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plantarum, 15: 437-497.
5. Davis, D.G., K.A. Breiland, D.S. Frear and G.A. Sector, 1994. Callus induction of regeneration of tomato (*Lycopersicon esculentum* L.) cultivars with different sensitivities to meterbuzim, Plant growth. Regulatory Society of America Quarterly, 22: 65-73.
6. Hill, T., M. Koornneef, M.S. Remanna and P. Zabel, 1989. Tomato: a crop species amenable to improvement by cellular and molecular method. Euphytica, 42: 1-23.
7. Duzvaman, E., A. Taurisever, G. Gunver, K.E. Cockskull, Y. Tuzel and A. Gul, 1994. Comparative studies on regeneration of different tissues of tomato *in vitro*. Acta Horticulturae, 366: 235-242.