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In Vitro Multiple Shoot, Root and Callus Induction from Different Explants in Tomato (Lycopersicon Esculentum Mill)

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Abstract
Tomato *(Lycopersicon esculentum* Mill*)* is an important crop that plays an important role in Pakistan economy. There are three commercial varieties of tomato i.e. Roma, Moneymaker and Nagina grown all over the country. *In vitro* culture response was assessed in these three varieties for optimum callus induction and plantlet regeneration. Results showed that under *in vitro* condition seed germination increased with the passage of time. Statistical analysis of data regarding callus induction from cotyledon leaf and hypocotyls explants showed highly significant results for three varieties. When cotyledon was used as explants, highest callus induction percentage was observed in cultivar Moneymaker (91.4%) containing MS medium supplemented with NAA+BAP 1+ 2 mg/L. Variety Moneymaker was found better for callus induction when cotyledon was used as explant.

Introduction

Tomato (*Lycopersicon esculentum* Mill) is an important member of family solanaceae. It plays an important role in Pakistan's economy. It is a warm season crop and is sown in spring and summer. Tomato is also popular because of its high nutritive value and diversified uses. . There are many commercial varieties of tomatoes e.g. Roma, Moneymaker and Nagina which are grown widely in Pakistan. Tomato is dealt as a model plant in conventional plant breeding and biotechnology for the transfer of agronomically important genes in dicots (Augusta & Mary, 1992). Production of tomato is affected by various stresses which includes diseases. The susceptibility of tomato cultivars to different pathogens has reduced the yield. Viruses like tomato mosaic virus is the major cause for this decline and vectors like white fly and aphid transmit viruses from diseased plant to healthy ones (Mark, 1986). Fusarium wilt and anthracnose also seriously limit its production. In recent years pathogen have not only reduced its production but also destroyed the whole crop or make the yield totally unmarketable (Plana *et al*., 2005). Tissue culture offers the novel solution of such problems. Research in biotechnology makes use of Genetic Engineering and tissue culture (Chaudary *et al*., 2001). Establishment of an efficient tissue culture protocol is an essential prerequisite in harnessing the advantage of cell and tissue culture for genetic improvement (Sheeja *et al.,* 2004). Various explants sources are reported for callus induction and regeneration on different media in tomato (Sheeja *et al*., 2004). Tomato explants from many sources of tissue have been successfully grown in tissue culture. Explants like hypocotyls segments, leaf discs, roots, shoot tips, cotyledons and anthers are also reported for callusing and regeneration (Chaudhary *et al.,* 2004). *In vitro* plant regeneration has been found to depend on many factors, of which most important are: composition of the basic medium, growth regulators, gelling agent, light intensity and quality, photoperiod and temperature (Gubis *et al.,* 2003). The parental line needed for inbreeding takes 6 to 8 years for variety development whereas tissue culture offers the mass production of virus free plants of many crops and helps in rapid propagation of selected plants with desirable characteristics in shortest possible time and variety development by somatic hybridization. The seed of commercial hybrid cultivars is highly expensive. The development of an efficient micro propagation protocol will also be helpful in the production of disease free hybrid plants at a faster and cheaper rate. Keeping in view these factors the aim of our study was to explore different explants from *in vitro* sources for shoot induction and subsequent root regeneration for rapid micro propagation in commercial tomato cultivars Roma, Moneymaker and Nagina by using different combinations of growth regulators.

Materials and Methods

Present study was conducted in Plant Tissue Culture Cell, Institute of Horticultural Sciences, University of Agriculture, Faisalabad during the year 2005.

Media Preparation: The basal medium for shoot proliferation, callusing and further regeneration of tomato *w*as constituted according to Murashige and Skoog (1962) salt mixture supplemented with various plant growth regulators. Stock solutions of growth regulators and major salts were prepared in distilled water. Required concentrations of the solutions were obtained to prepare the medium. Sugar (30 g/L) was added as carbohydrates source, pH of the medium was adjusted at 5.6 and 8g of agar was added as a solidifying agent in the media. 10

ml of medium was dispensed in each test tube necked with plastic sheet and autoclaved for 15 minutes at 121^{o}C under pressure 1.5 kg/m^2 for sterilization. Treatments detail and different combination of growth regulators i.e., NAA (Naphthalene acetic acid) and BAP (Benzyl amino purine) used are given in Table-1.

Explants Used: Regeneration behavior of *in vitro* explants was noted. Plant parts (seed, cotyledon leaf, hypocotyls) excised from seed germinated *in vitro* and used as explants for further propagation of tomato cultivars Roma, Moneymaker and Nagina.

Explant Sterilization: Explants were surface disinfected with 70% ethanol for 3 minutes followed by rinsing with sterilized double distilled water for three times. Seeds were kept in 5% sodium hypochlorite solution for 2-3 minutes followed by 3 rinses with sterilized distilled water.

Explant Isolation and Culture Procedures: Seeds were cultured after sterilization on MS medium for germination. Explants type as cotyledon leaf, hypocotyls were excised according to their respective dimensions from *in vitro* grown seedlings and were sub- cultured on MS medium supplemented with growth regulators for the development of callus and then complete plants. The cultures were kept in growth condition with temperatures at 25^{o}C and fluorescent light intensity of 2500lux. The effect of different growth regulators used in different combinations (Table-1) on different varieties was observed for seed germination %age, callus induction %age, shoot induction %age, root induction %age.

Results

Germination %age: Results regarding germination percentage of three tomato varieties showed that under *in vitro* condition it increased with the passage of time. Germination of 15%, 17% and 19% occurred on 2nd day of culture which increased up to 88%, 90% and 93% after 35 days in Roma, Moneymaker and Nagina respectively. Maximum germination was observed in cultivar Nagina (Figure 1). Sterile plants parts (Cotyledonary leaf, hypocotyls) were excised from *in vitro* grown seedling for further study. Cotyledonary leaf and hypocotyl was used to detect the callus, shoot and root induction %age.

Callus induction %age: The statistical analysis of data regarding callus induction from cotyledonary leaf and hypocotyls explants showed highly significant differences among three tomato varieties. It is obvious from results that callus induction from cotyledon significantly increased with higher concentration of BAP and lower concentration of NAA while minimum callus induction was observed when no hormone was added to MS media (control). When cotyledon used as explants, highest callus induction percentage was observed in cv. Moneymaker (91.3%) at treatment NAA 1mg/L+BAP 2mg/L (Table 2A). On average, variety Moneymaker (62.5%) was found better for callus induction than Roma (58.1%) and Nagina (33.5%) when cotyledon was used as explants. However, the highest callus induction from hypocotyls was observed in Nagina (72.3%) at treatment of NAA and BAP 1+ 2 mg/L (Table 2B). It is evident from the given results that hormone works best in combination than alone so it is concluded from data that cotyledon was found to be the better explants material for callus induction as compare to hypocotyls and hormonal concentration of NAA and BAP 1 + 2 mg/L seemed to be the optimum for callus induction in tomato.

Shoot induction %age: Significant difference was observed for shoot induction %age for various hormonal treatment, varieties and type of explants used. Data (Table 3A & 3B) showed that NAA has a positive effect on shoot induction i.e. higher the concentration of NAA higher will be the shoot induction percentage and when is used with BAP its effects more positively for both the explants (cotyledon and hypocotyls). Highest shoot induction %age for cotyledon explant was observed while Cv. Moneymaker gave the maximum shoot induction (21.7%) at concentration of NAA 1mg/L+BAP 2 mg/L in all three varieties.

Root induction %age: The statistical analysis of data regarding root induction from cotyledon and hypocotyls explants at various media formulation showed highly significant differences for treatment, varieties and their interactions. Root induction on MS media supplement with different levels of NAA was higher as compared to control; however, when NAA was used in combination with BAP better results were observed. When cotyledon was used as explant, maximum root induction was observed for cultivar Roma (18%) at the concentration NAA 1 mg/L+BAP 2 mg/L, while the overall, lowest root induction was found in Moneymaker. Roma was found significantly better for root induction than Moneymaker and Nagina. In case of hypocotyls explant root induction % was maximum in cv. Nagina (37.6%) at the concentration of NAA1 mg/L+BAP 2 mg/L followed by Moneymaker (21.3%) and Roma (16%) at the same concentration.

Among cultivars Nagina showed highest root induction %age than Roma and Moneymaker. On overall basis, hypocotyls explant was found better explant as compared to cotyledon for root induction in tomato (Table

4A & B).

Discussion

In tomato various explants tissues like cotyledon, hypocotyls, embryo, ovules protoplast and leaf have been used for various biotechnological application (Koblitz, & Koblitz 1982; Uddin & Berry, 1988; Chen & Adachi, 1998; Gill *et al.,* 1995; Newman *et al*., 1996; Ling *et al.,* 2000; Ruf *et al.,* 2001). It includes production of virus free plants, transgenic plant resistant to various biotic and abiotic stresses. In present study two different explants sources from three varieties were used to evaluate their ability to produce callus, shoot and root induction percentage and time taken to regenerate shoot and root at various concentration of growth regulators (NAA and BAP). Among tomato varieties used, Moneymaker was recognized as one of the best variety around the world which responded positively towards various tissue culture techniques (Van Rockel *et al*., 1993; Frary and Earle, 1996; Hanus-Fajerska, 2001). Our results showed that Moneymaker was best for callus induction from cotyledon explant while Nagina variety stood first when explant was hypocotyls. The results indicated that both the explants could be used for callus induction and in accordance with the result of Van Rocket *et al*., (1993), Frary and Earle (1996), Hanus-Fajerska (2001) and Ruf *et al.,* (2001). These explants were used to check root and shoot induction percentage. For shoot induction, the variety Moneymaker was considered best among the three varieties used. Same results were also reported by Hanus-Fajerska (2001), Ruf *et al.*, (2001) and Khan *et al*., (2006). In case of root induction percentage maximum roots were developed in variety Nagina as compared to Roma and Moneymaker when hypocotyls explant was used. The hypocotyl was found to be better explants as compared to cotyledon for root induction in tomato at various concentration of NAA alone or in combination with BAP. Chaudhry *et al*., (2004) also reported that hypocotyls showed variable responses by use of different combination of growth regulators.

Table 1. Detail of different treatments of NAA (Naphthalene acetic acid) alone and in combination with different concentrations of BAP (Benzyl amino purine)

Treatments	NAA (mg/L)	BAP (mg/L)
T_0 (control)	0	0
T_1	1.0	0
T_2	2.0	0
T_3	3.0	0
T_4	1.0	1.0
T_5	2.0	2.0
T_6	3.0	3.0
T_7	2.0	1.0
T_8	1.0	2.0

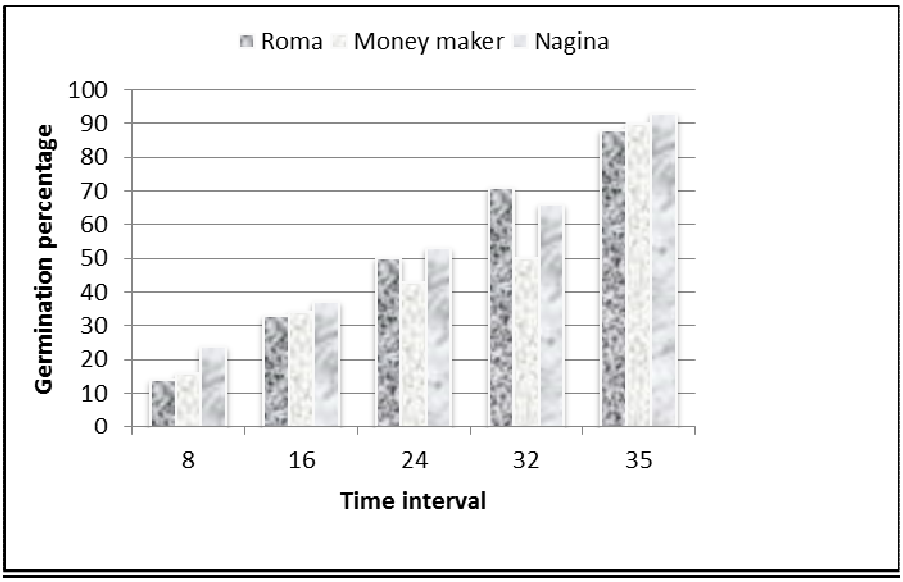

Fig.1 Micropropagation (%) of tomato cultivars during different time intervals

Table 2A. Response of cotyledon leaf explants of tomato cultivars for callus induction (%age) on different levels on NAA and BAP

Treatments (mg/L)	Roma	Moneymaker	Nagina	Treatment Means
Control	31.4	30.0	17.7	26.4
1	40.7	42.0	23.7	35.5
2	41.0	45.7	29.0	38.6
3	50.4	54.0	33.7	46.0
1+1	60.0	61.7	40.4	54.0
2+2	71.0	75.0	47.7	64.6
3+3	73.7	80.4	35.0	63.0
2+1	77.0	82.7	41.0	66.9
1+2	81.7	91.4	34.0	69.0
Cultivar Means	58.6	62.6	35.6	

LSD for Cultivar (V) =0.8787
LSD for Treatment (T) =1.522
LSD for V x T =2.636

Table 2B. Response of hypocotyl explant of tomato cultivars for callus induction (%age) on different levels of NAA and BAP

Treatments (mg/L)	Roma	Moneymaker	Nagina	Treatment Means
Control	24.0	21.3	25.3	23.5
1	32.0	30.3	30.0	30.7
2	36.3	37.0	34.3	35.8
3	36.0	42.3	41.3	39.8
1+1	40.0	42.3	46.0	42.7
2+2	42.0	50.0	51.3	47.7
3+3	41.0	57.6	56.3	51.6
2+1	48.0	64.0	64.0	58.6
1+2	53.0	70.0	72.3	65.1
Cultivar Means	39.1	46.1	46.7	

LSD for Cultivar (V) =0.727
LSD for Treatment (T) =1.260
LSD for V x T =2.183

Table 3A. Response of cotyledon leaf explant of tomato cultivars for shoot induction (%) on different level of NAA & BAP

Treatments (mg/L)	Roma	Moneymaker	Nagina	Treatment Means
Control	6.5	8.3	6.8	6.8
1	9.2	6.4	7.5	8.6
2	13.0	9.7	10.1	11.3
3	15.9	13.3	10.4	13.5
1+1	5.0	17.7	10.0	9.9
2+2	15.7	5.9	6.8	10.5
3+3	16.2	17.2	16.7	13.7
2+1	16.0	16.0	17.0	16.3
1+2	20.2	21.7	20.1	18.9
Cultivar Means	12.2	13.3	13.2	

LSD for Cultivar (V) =3.075
LSD for Treatment (T) =2.097
LSD for V x T =3.632

Table 3B. Response of hypocotyl explant of tomato cultivars for shoot induction (%age) on different levels of NAA and BAP

Treatments (mg/L)	Roma	Moneymaker	Nagina	Treatment Means
Control	6.0	14.0	6.0	6.0
1	24.0	6.0	20.0	18.3
2	21.0	11.0	23.6	21.2
3	16.0	19.0	21.3	19.8
1+1	11.0	22.3	16.3	14.1
2+2	9.0	15.0	8.6	12.7
3+3	10.3	20.6	16.0	18.1
2+1	13.3	28.0	16.0	21.7
1+2	14.0	36.0	21.0	25.3
Cultivar Means	13.8	22.1	16.5	

LSD for Cultivar (V) =0.804
LSD for Treatment (T) =1.473
LSD for V x T =2.643

Table 4A. Response of cotyledon leaf explants of tomato cultivars for root induction (%age) on different levels on NAA and BAP

Treatments (mg/L)	Roma	Moneymaker	Nagina	Treatment Means
Control	3.4	2.4	3.0	2.9
1	7.4	3.4	4.4	5.0
2	10.0	2.0	4.0	5.4
3	16.4	3.0	6.0	8.5
1+1	15.4	6.0	7.7	9.7
2+2	18.7	9.0	8.0	11.9
3+3	15.4	12.0	8.0	11.8
2+1	15.7	13.7	14.0	14.5
1+2	18.0	15.0	16.0	16.4
Cultivar Means	13.4	7.4	7.9	

LSD for Cultivar (V) =0.727
LSD for Treatment (T) =1.260
LSD for V x T =2.183

Table 4B. Response of hypocotyl explant of tomato cultivars for root induction (%age) on different levels of NAA and BAP

Treatments (mg/L)	Roma	Moneymaker	Nagina	Treatment Means
Control	4.0	3.0	8.3	5.1
1	7.0	6.3	12.3	8.5
2	9.0	6.6	13.3	9.6
3	12.0	11.0	16.6	13.2
1+1	14.0	14.0	20.6	16.2
2+2	18.0	16.3	11.3	15.2
3+3	18.0	13.3	17.6	16.3
2+1	11.0	18.0	28.3	19.1
1+2	16.0	21.3	37.6	25.0
Cultivar Means	12.1	12.2	18.4	

LSD for Cultivar (V) =0.181
LSD for Treatment (T) =1.417
LSD for V x T =2.454

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