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TISSUE CULTURE STUDIES IN TOMATO (*LYCOPERSICON ESCULENTUM*) VAR. MONEYMAKER

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Abstract

A protocol was developed for callus induction and regeneration in tomato (*Lycopersicon esculentum*) var. Moneymaker. Hypocotyl and leaf disc explants of tomato were used as a starting material for callus induction. Explants were cultured on MS medium having different concentrations of hormones. Maximum callogenesis from hypocotyls was obtained on MS medium supplemented with IAA (2 mg/l), NAA (2 mg/l), BAP (5 mg/l) and Kin (4 mg/l). It was 65.2% for hypocotyls. For leaf discs maximum callogenesis was achieved on MS medium supplemented with IAA (2 mg/l), NAA (2 mg/l), BAP (2 mg/l) and Kin (4 mg/l). It was 81.3% for leaf discs. Calli were cultured on MS medium having concentrations of Zeatin (1 mg/l) and IAA (1 mg/l) for regeneration. They showed maximum regeneration of 69.2% from hypocotyls. Minimum regeneration of 2.8% was obtained on MS medium supplemented with IAA (2 mg/l), NAA (2 mg/l), BAP (2 mg/l) and Kin (4 mg/l). Tomato shoots were shifted to ½ MS medium containing IBA (0.1 mg/l) and BAP (0.0025 mg/l) for rooting and all responded positively to rooting.

Introduction

Tomato is one of the most important Solanaceae crop grown throughout the world (Rick, 1980). It is recognized as a highly valuable and nutritious food. Nowadays tomato is one of the major vegetable throughout the world. It is grown in tropical, sub-tropical and temperate areas (Atherton & Rudich, 1986). Several *In vitro* investigations have been conducted on tomato based on its relationship with tobacco, and in account of its consequently expected good workability (Koblitz, 1982). Tomato is a favorable food crop for *In vitro* studies due to its low chromosomal no i.e., 2n=2x=24 and due to comprehensive knowledge of tomato genetics (Chaudhry *et al*., 2001).

Productivity of tomato has been low due to many biotic and abiotic stresses. There are several common diseases of tomato crops viz., bacterial wilt caused by *Pseudomonas solanacearum* and bacterial scab, which is caused by *Xanthomonas campestris*. Fungal diseases have resulted in decreased trend of its yield/acre, powdery mildew caused by *Leveillula taurica*. Other main diseases are early blight, leaf spot, leaf mold and wilts etc. Moreover changes in insect's biotype and disease resistance are becoming a continuing threat to increased production (Anon., 1983).

Different explant sources can be used for callogenesis and regeneration. **Studies about the effect of variety and plant growth regulators on callus proliferation and regeneration of three tomato cultivars has been reported (Chaudhry *et al*., 2007).** Shoot apex, nodal segments and root segments were successfully used for callus induction and regeneration (Jatoi *et al*., 2001). Various hormonal combinations are used to induce callus and regeneration like BAP and IAA, IAA and Kin (Chen *et al*., 1999). The present study was conducted to explore the callogenic and regeneration potential of hypocotyls and leaf disc

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segments so as to establish a reproducible protocol for callus induction and regeneration from tomato cv. Moneymaker by using different combination of growth regulators.

Material and Methods

Seeds of moneymaker were kindly provided by vegetable program of National Agricultural Research Center (NARC), Islamabad. Surface sterilization of seeds was done by soaking into the solution of 1ml Clorox (5.25% Sodium hypochlorite) and 8 ml distilled water for 10 minutes, followed by three times rinsing with sterilized distilled water (5 minutes each). Ethanol was sprayed on the seed and left for 15-20 seconds. Traces of spirit were removed by washing with autoclaved distilled water (thrice). The sterilized seeds were transferred in sterilized petri plates.

Seeds were inoculated in test tubes containing MS medium and were transferred in dark room for germination. Germinated seedlings served as explants source for tissue culture experiments. Hypocotyl segments (1-2 cm) and leaf discs ($5\times5cm^2$) of 18-21 day old *In vitro* plants were excised under aseptic conditions. The excised explants were cultured on callus induction media (Table 1). All the cultures were transferred to growth room for a period of almost 4-6 weeks. Compact callus was selected and used for regeneration at 25˚C ± 2˚C in growth room (Table 1).

Calli induced from the hypocotyls and leaf discs were shifted to different regeneration media. Certain hypocotyls and leaf discs were directly regenerated on the same medium. As the tomato shoots began to regenerate either directly from hypocotyls or from calli, they were transferred to rooting media.

Results and Discussion

After surface sterilization, 363 seeds were inoculated on plain MS medium. Germination rate was 68.8%, 3.0% seeds were contaminated and remaining were unable to grow as given in Table 2 and Fig. 1.

Fig. 1. Different growth stages of *In vitro* seedlings of tomato cv. Moneymaker.

Table 1. MS media in combination with different hormones used for callogenesis and regeneration from *Lycopersicon esculentum* var. Moneymaker.

Treatment no.	BAP (mg/l)	Kin (mg/l)	NAA (mg/l)	IAA (mg/l)	Zeatin (mg/l)	IBA (mg/l)
T_0	0.0	0.0	0.0	0.0	0.0	0.0
T_1	2.0	4.0	2.0	2.0	0.0	0.0
T_2	5.0	4.0	2.0	0.0	0.0	0.0
T_3	1.0	0.0	1.0	0.0	0.0	0.0
T_4	0.0	0.0	0.0	1.0	1.0	0.0
T_5	5.0	4.0	2.0	2.0	0.0	0.0
T_6	0.0025	0.0	0.0	0.0	0.0	0.1

Table 2. Germination from seeds of *Lycopersicon esculentum* var Moneymaker.

No of seeds	Germinated	Non-germinated	Contamination
363	250 (68.8%)	102 (53.1%)	11 (3.0%)

Table 3. Percentage Callogenesis from hypocotyls and leaf discs of *Lycopersicon esculentum* var. Moneymaker on different hormonal combinations.

Treatment no.	Callogenesis (%)	
	Hypocotyls	Leaf discs
T_0	0.0	0.0
T_1	56.3	81.3
T_2	38.4	30.0
T_3	24.3	0.0
T_4	30.7	0.0
T_5	65.2	57.1

Hypocotyls and leaf discs were used for callus induction. In present research callus induction was observed from both leaf disc segments and hypocotyls. Hypocotyl and leaf disc explants of Moneymaker were cultured on different concentration of hormones. The callus growth was remarkably affected by hormone treatment (Table 3). About 10.8% hypocotyls exhibited no callus induction. Necrosis was observed in 6.9% calli. Good callus was obtained in 39.8% hypocotyls (Table 4). The highest callus induction frequency was obtained in T_5 given in Table 1 and Fig. 4. Callus obtained were bulky and of pale green in color. Whereas by using T_2 hypocotyls showed good initial swelling but callus developed slowly. Callus obtained on T_3 were weak and developed very slowly.

In 25.7% of leaf disc there was no callus induction. Good callus were obtained in 22% leaf disc segments. Whereas necrosis was observed in 20.7% segments (Table 4;

Figs. 2 and 3). Maximum callus induction was achieved with combination T_1 (Table 3). None of leaf discs showed shoot formation (Table 5).

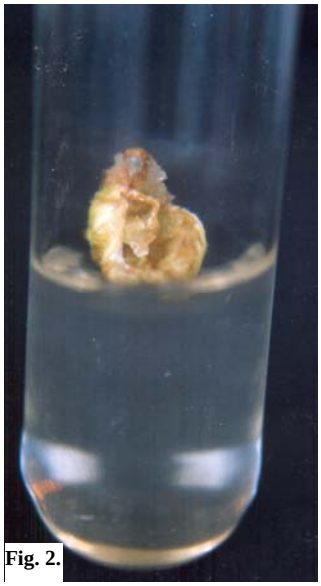
Fig. 2.

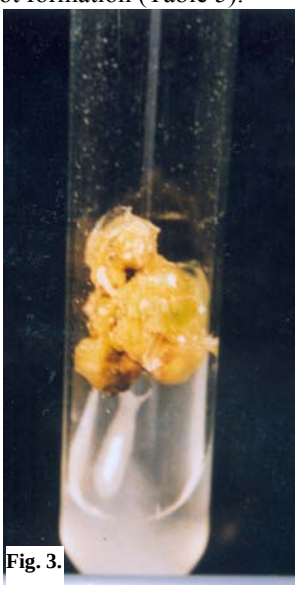
Fig. 3.


Fig. 4.

Fig. 2. Initiation of callogenesis from leaf disc segment on MS medium containing NAA (2 mg/l), IAA (2 mg/l) and Kin (4 mg/l).

Fig. 3. Two-week-old callus from leaf disc cultured on MS medium supplemented with NAA (2 mg/l), BAP (2 mg/l) and Kin (4 mg/l).

Fig. 4. Hypocotyl derived callus obtained on MS medium containing different concentrations of NAA (2 mg/l), IAA (2 mg/l), BAP (5 mg/l) and Kin (4 mg/l).

Table 4. Overall callogenesis from hypocotyls and leaf disc of *Lycopersicon esculentum* var. Moneymaker.

Exp. no.	Total cultures	No. callus	Good callus	Medium callus	Poor callus	Necrosis	Powdery callus
	Overall callogenesis from hypocotyls						
1.	192	24 (12.5%)	60 (31.2%)	35 (18.2%)	27 (14.0%)	19 (9.8%)	27 (14.0%)
2.	165	16 (9.6%)	72 (43.3%)	33 (20%)	15 (9.0%)	8 (4.8%)	20 (12.1%)
3.	187	19 (10.1%)	85 (45.4%)	30 (16.0%)	8 (4.2%)	11 (5.8%)	30 (16.0%)
Total	544	59 (10.8%)	217 (39.8%)	98 (18.0%)	50 (9.1%)	38 (6.9%)	77 (14.1%)
	Overall callogenesis from leaf disc						
1.	59	16 (27.1%)	15 (25.4%)	6 (10.1%)	6 (10.1%)	11 (18.6%)	5 (8.4%)
2.	75	14 (18.6%)	18 (24.0%)	11 (14.6%)	12 (16.0%)	17 (22.6%)	3 (4.0%)
3.	64	21 (32.8%)	12 (18.7%)	7 (10.9%)	4 (6.25%)	13 (20.3%)	7 (10.9%)
Total	198	51 (25.7%)	45 (22.7%)	24 (12.1%)	22 (11.1%)	41 (20.7%)	15 (7.5%)

Table 5. Percentage regeneration frequency from hypocotyls and leaf discs of *Lycopersicon esculentum* var. Moneymaker on different hormonal combinations.

Treatment no.	Hypocotyls		Leaf discs	
	DR Regeneration	IDR regeneration	DR Regeneration	IDR regeneration
T_0	0.0	0.0	0.0	0.0
T_1	2.8	0.0	0.0	0.0
T_2	5.7	0.0	0.0	0.0
T_3	5.4	0.0	0.0	0.0
T_4	69.2	0.0	0.0	0.0
T_5	0.0	7.24	0.0	0.0

T = Treatment
DR= Direct regeneration = Where explants become green and new shoot is produced.
IDR = Indirect regeneration = Where explant first give callus

In general highest callus induction about 65% was obtained on MS medium supplemented with NAA, BAP, IAA and Kin in T_5, 9.85% hypocotyls were unable to induce callus and with increase in BAP concentration from 3 mg/l (T_5) an increase in callus induction was observed in both explant sources. Jatoi *et al*., (2001) observed an increased callogenesis in two tomato hybrids cvs. Bornia and Royesta with increase in BAP from 5, 10, 20 and 30 µM. It appeared that the presence of a high concentration of BAP enhanced the growth of the plant. Although this combination was used for regeneration but in this particular variety it produced good callus with fresh green color. Root formation was observed in only one callus. Combination of NAA, BAP and Kin (T_2) produced 38.4% good callus, 13.4% hypocotyls showed no callogenesis and shoot regeneration plus callus formation was observed in 5.7% hypocotyls (Table 5; Fig. 5). In this particular variety BAP and NAA (T_3) produced only 24.3% good callus (Table 3). Shoot formation was observed in 5.4% hypocotyls.

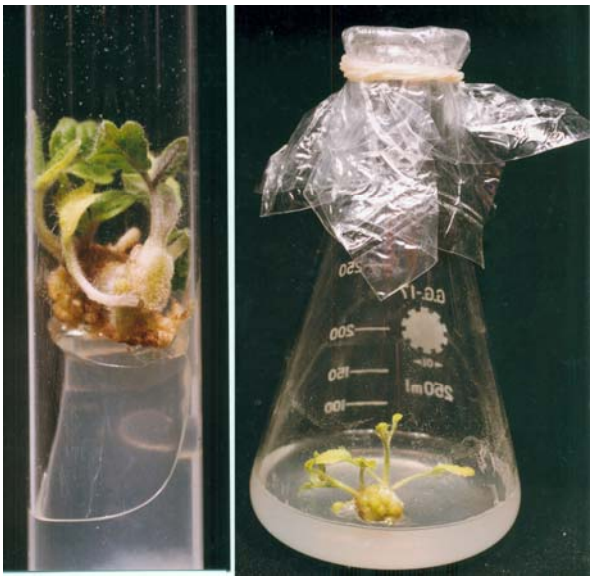

Fig. 5. Shoot regeneration from hpocotyl derived callus of tomato cv. Moneymaker on MS medium containing NAA (2 mg/l), BAP (5 mg/l) and Kin (4 mg/l).

For regeneration, callus was sub cultured and shifted to regeneration medium. Some hypocotyls had directly shown regeneration. T_1 exhibited no indirect regeneration and direct regeneration was observed in 2.8% hypocotyls. Whereas 4 hypocotyls cultured on T_2 and T_3 containing different levels of IAA, NAA, BAP and Kin showed shoot emergence (Table 5). Different researcher in addition to BAP, Kin and NAA used IAA and Zeatin for callusing and regeneration. Lu *et al*., (1997) used IAA and Zeatin for callus induction and for regeneration of two tomato cultivars. **A rapid high frequency regeneration system was established by using GA3 in the treatments for three tomato cultivars. The time to regenerate the plants is reduced to half in all tested varieties (Afroz *et al*., 2009).**

In the present study also, best results were obtained by using Zeatin and IAA (T_4). About 69% hypocotyls exhibited regeneration (Fig. 6). Callus as well as leaf disc segments were transferred to regeneration medium. None of them showed any regeneration (Table 5). Costa *et al*., (2000) used cotyledonary-derived explants of two processing tomato cultivars (*L. esculentum* cvs. IPA-5 and IPA-6). After three weeks, 'IPA-5' and 'IPA-6' cultivars presented shoot regeneration with (average of 97 and 80%, respectively) when cultured on MS medium supplemented with 1 mg zeatin and 0.1 mg IAA. Ichimura *et al*., (1995) successfully cultured cotyledon segments of tomato on several kinds of supporting materials made from polyester, ceramic, wood pulp and cotton fiber. Callus was induced by using 0.1 mg/l IAA and 1.0 mg/l Zeatin.

Fig. 6. Shoot regeneration from hpocotyl callus culture of tomato cv. Moneymaker obtained on MS medium supplemented with Zeatin (1 mg/l) and IAA (1 mg/l).

Table 6. Sub culturing of calli obtained from hypocotyls and leaf disc of *Lycopersicon esculentum* var. Moneymaker.

Exp. no.	No of sub cultured calli	Proliferation			Necrosis	Powdery callus
		Good	Average	Poor		
	Sub culturing of calli obtained from hypocotyls					
1.	69	38 (55.3%)	11 (15.9%)	4 (5.7%)	7 (10.1%)	9 (13.0%)
2.	54	35 (64.8%)	6 (11.1%)	2 (3.7%)	4 (7.4%)	5 (9.2%)
3.	74	43 (58.1%)	9 (12.1%)	7 (9.4%)	3 (4.0%)	12 (16.2%)
Total	197	116 (58.8%)	26 (13.1%)	13 (6.5%)	14 (7.1%)	26 (13.1%)
	Sub culturing of calli obtained from Leaf disc					
1.	42	24 (57.1%)	6 (14.2%)	3 (7.1%)	9 (21.4%)	0.0
2.	61	31 (50.8%)	9 (14.7%)	11 (18.0%)	7 (11.4%)	3 (4.9%)
3.	58	17 (29.3%)	15 (25.8%)	14 (24.1%)	11 (18.9%)	1 (1.7%)
Total	161	72 (44.7%)	30 (18.6%)	28 (17.3%)	27 (16.7%)	4 (2.4%)

Table 7. Plant regeneration from *Lycopersicon esculentum* var. Moneymaker.

Exp. no.	Explant source	Total no.	Growth differentiation	Shoot formation
	Hypocotyls			
1.		192	30 (15.6%)	29 (15.1%)
2.		165	43 (26.0%)	23 (14.0%)
3.		187	51 (27.2%)	31 (16.5%)
	Total	544	124 (22.8%)	83 (15.2%)
	Leaf discs			
1.		59	11 (18.6%)	0.0
2.		75	19 (25.3%)	0.0
3.		64	14 (21.8%)	0.0
	Total	198	44 (22.2%)	0.0

Fresh and green calli obtained from hypocotyls were subcultured on T_5 having different concentrations of Kin, IAA, NAA, and BAP. After 6 weeks of observation 58.8% calli showed rapid proliferation and 6.5% showed poor proliferation. Necrosis was observed in 7.1% calli (Table 6), whereas shoot regeneration was achieved in 7.24% calli (Table 5). Only 3% hypocotyls exhibited root regeneration (Fig. 7). Calli obtained from leaf disc segments were sub-cultured on T_5. Rapid proliferation was observed in 44.7% calli. Necrosis was in 16.7% calli (Table 6). None of calli showed any root or shoot regeneration (Table 5).

Fig. 7. Regeneration from hypocotyls of tomato cv Moneymaker.

About 22.8% hypocotyls produced embryogenic calli. In 15.2% hypocotyls shoot regeneration was observed. About 22% leaf discs showed differentiation (Table 7). Tomato shoots obtained were shifted to ½ MS medium containing IBA (0.1 mg/l) and BAP (0.0025 mg/l) for rooting in T_6 (Fig. 8). Multiple shoot formation with roots was observed (Fig. 9).

Fig. 8. Root formation.

Fig.9. Multiple shoot formation.

Conclusion

Callus induction was observed in both hypocotyl and leaf disc explants. Hypocotyls showed to be better explants for callogenesis and regeneration. Maximum callogenesis was noted on MS medium supplemented with NAA (2 mg/l), IAA (2 mg/l), BAP (5 mg/l) and Kin (4 mg/l). Maximum regeneration was observed on MS medium containing Zeatin (1 mg/l) and IAA (1 mg/l). This study is a baseline to carry further research on this tomato variety for improvement by using gene transfer technology.

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