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Effect of Variety and Plant Growth Regulators in MS Medium on Callus Proliferation from Virus Infected Tomato Plant

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Abstract: The experiment was conducted in the Tissue Culture Laboratory of the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh for micropropagation of tomato plant to evaluate the effect of variety and Plant Growth Regulators (PGRs) in MS medium on callus proliferation from infected tomato plant. Three tomato varieties namely, Bahar, Binatomato-2 and Binatomato-3 were used as plant materials in the present study. Virus infected young internode segments of tomato plants were cultured on MS medium supplemented with different concentrations and combinations of Plant Growth Regulators (PGRs) for callus proliferation. The combination of 0.5 mg L^{-1} NAA + 2.0 mg L^{-1} BAP in MS medium was found the best for inducing healthy and light yellow callus after 15 days culture and callus turned into yellow at 30 Days After Inoculation (DAI) in all varieties. Calli were fully virus infected which was confirmed by ELISA test. These calli can be used for the production of virus free tomato plant by shoot derived meristem culture.

Key words: Plant growth regulators, NAA, BAP, callus, tomato

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) belongs to the family Solanaceae is one of the most important and nutritious vegetable in the world. With wide range of adaptability of soil and climate[1] tomato is cultivated almost all over the world. It is popular because of its high nutritive value and diversified uses[2]. Hundred grams of edible parts of tomato contains 0.9 g protein, 0.1 g fat, 3.5 g carbohydrates, 15-20 calories energy, 500-1500 IU vitamin "A", 0.1 mg thiamin, 0.02 mg riboflavin, 0.6 mg niacin, 20-25 mg vitamin "C", 6-9 mg calcium and 0.1-0.3 mg iron[3]. Tomato is widely grown in Bangladesh usually in winter season. The demand of tomato is increasing day by day in the agro and food industries of Bangladesh. Thus, it has become cash crop in this country. Recent statistics shows that tomato was grown in 14338.06 ha of land and the production was 97565 metric tons during 1998-99 in Bangladesh. The average yield was 1.113 metric tons per ha[4], which was very low, compared to other leading tomato producing countries[5].

There are many factors behind the low yield of tomato in our country. Among the various reasons, infection by viruses, fungi, bacteria, nematodes and parasitic weeds play an important role[6]. Over 200 diseases have been reported to affect tomato plants in the world[7]. Tomato leaf curl disease caused by TLCV is a limiting factor for a successful cultivation of tomato in Bangladesh. This disease can infect at any growth stages of the plant. Dwarfing, puckering, twisting and curling towards dorsal side of the leaves, vein clearing, mottling and excessive branching along with stunting of the plant growth and partial or complete sterility are the main symptoms of tomato leaf curl viral disease[8]. It seriously affects the growth and yield of crop. The loss due to leaf curl amounts up to 93.3% when the tomato is infected at early stage[9]. Virus free plant may be obtained by meristem culture. Virus free seed production is significantly important by different methods of tissue culture, likewise meristem culture. In 1952, Morel and Martin first obtained virus free dahlias and potatoes with the help of meristem culture. Meristem culture is especially applied in horticulture but some agricultural crops, such as potatoes, Trifolium, Lolium and tobacco are included in this regard[10].

A very little attempt has been made in the production of virus free tomato plants for establishing *in vitro* plant regeneration media in Bangladesh. Tomato seed production program in Bangladesh is being practiced with imported virus free seeds that are expensive. It is possible

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to bring down the cost of production by developing virus free seeds through tissue culture technique. Moreover, maintenance of valuable germplasm in disease free condition may be obtained by meristem culture. Callus production is one of the important step of meristem culture. Different plant growth regulators (PGRs) in MS medium affect callus proliferation. Geetha *et al.*[11] initiated callus by the culture of tomato explant on MS medium supplemented with different combinations of NAA (3 or 6 μM) and BAP (2, 4, 6 or 8 μM). In view of above facts, the present research was designed to evaluate the effect of variety and plant growth regulators in MS medium on callus proliferation from infected tomato plant. These calli can be used for the production of virus free tomato plant by meristem culture.

MATERIALS AND METHODS

The experiment was conducted at the Plant Tissue Culture Laboratory, Department of Crop Botany, Bangladesh Agricultural University, Mymensingh during July, 2001 to November, 2002. Three tomato varieties, one is winter variety namely, Bahar and the rest two are summer variety namely, Binatomato-2 and Binatomato-3, were used as plant materials in the present study. Stock solutions were prepared prior to medium preparation. Five stock solutions namely, macronutrients, micronutrients, Fe-EDTA, vitamins/organics and PGRs were prepared.

One litre of MS medium was prepared. For preparation of one litre of MS medium, 30 g sucrose was dissolved in 500 mL of distilled water. Stock solution of 100 mL of macro-nutrients, 10 mL stock solution of micronutrients, 10 mL stock solution of Fe-EDTA, 20 mL myoinositol and 10 mL of stock solution of vitamins were added to the aforementioned 500 mL sucrose solution and mixed well. Different concentrations of hormonal supplements as required were added either in single or in different combinations to this solution and were mixed thoroughly. The mixture was then made up to 1000 mL by adding of dw. pH of the medium was adjusted to 5.8 with a digital pH meter with the help of 0.1N NaOH or 0.1N HCl, whichever was necessary. After adjusting the pH, eight g Difco-Brand Bacto Agar was added to solidify the medium. The mixture was then gently heated with continuous stirring still complete dissolution of agar. Required volume of hot medium was dispensed into 100 mL conical flasks. Then the conical flasks were sealed with aluminum foil and marked with different codes with the help of a glass marker to indicate specific hormonal combinations.

The conical flasks containing the medium were autoclaved with 1.16 kg cm^{-2}, pressure at 121°C for 20 min. The medium was then cooled before use. To investigate the best concentration and combination of auxin (NAA, 2, 4-D) and cytokinin (BAP, kinetin) on *in vitro* callus proliferation of virus infected three tomato cultivars, Bahar, Binatomato-2 and Binatomato-3. Three concentrations and combinations of PGRs were used for callus proliferation such as: I) 0.5 mg L^{-1} NAA+2.0 mg L^{-1} BAP, ii) 0.5 mg L^{-1} NAA + 3.0 mg L^{-1} kinetin and iii) 0.5 mg L^{-1} 2,4-D + 2.0 mg L^{-1} BAP.

Virus infected immature internode segments (stem) of 75 days old plants of three varieties were used. Stem segments about five cm long, having leaf buds were cut and surface sterilized for 30 seconds in 70% alcohol and then for 10-15 min in 1.5% (a.i.) solution of sodium hypochlorite. Finally, the hypochlorite was removed by washing with sterilized distilled water in a laminar airflow cabinet. Then five cm long stem segments were cut by a sharp knife and 1 or ½ mm stem segments were used as explants. Calli initiation took place from immature cultured stem segments mentioned above. These calli were proliferated by sub-culturing for two months on fresh BM supplemented with PGRs. Calli were fully virus infected which was confirmed by ELISA test. Fresh weight of callus, dry weight of callus and relative colour change of explants (stem) from infected tomato plants were recorded at 15, 30, 45 and 60 days after inoculation (DAI). The experiment was laid out in Completely Randomized Design with five replications. The data of different parameters were subjected to statistical analysis using the analysis of variance to find out the variation resulting from experimental treatments. Treatments were compared by Duncans Multiple Range Test Gomez and Gomez[12].

RESULTS AND DISCUSSION

The fresh weight of calli was recorded at 15, 30, 45 and 60 days after inoculation (DAI) of explants. The fresh weight varied significantly among varieties at sampling dates except 60 DAI (Table 1). The maximum fresh weight of calli was produced in Bahar (0.590, 1.733, 2.239 and 3.993 g at 15, 30, 45 and 60 DAI, respectively) and the minimum fresh weight of calli was found in of Binatomato-2 (Fig. 1-3). This might be due to the variation in genetical make up of the varieties. There was significant difference among the different concentrations and combinations of PGRs in MS medium in respect of fresh weight of calli at all sampling dates (Table 2). The maximum fresh weight of calli (0.608, 1.860, 2.493 and 4.369 g at 15, 30, 45 and 60 DAI, respectively) was produced by the concentrations and combinations of 0.5 mg L^{-1} NAA+2.0 mg L^{-1} BAP and the minimum fresh weight of calli was produced by 0.5 mg L^{-1} NAA+3.0 mg L^{-1}

Table 1: Effect of different varieties on callus proliferation from virus infected stem of tomato at different days after inoculation

Variety	Fresh weight of inoculated explants (g)	Fresh weight of callus at different days after inoculation (g)				Dry weight of callus at DAI (g)
		15	30	45	60	60
Bahar	0.25	0.590a	1.733a	2.239a	3.993	0.382a
Binatomato-2	0.25	0.542b	1.587b	2.142b	3.930	0.345c
Binatomato-3	0.25	0.563ab	1.632b	2.200ab	3.952	0.368b
$LSD_{0.05}$		0.033	0.094	0.078	0.251	0.007

Figures with same letter(s) do not differ significantly at 5% level of significance

Table 2: Effect of plant growth regulators (PGRs) in MS medium on callus proliferation from virus infected tomato plant at different days after inoculation

Concentrations and combination of PGR	Fresh weight of inoculated explants (g)	Fresh weight of callus at different days after inoculation (g)				Dry weight of callus at DAI (g)
		15	30	45	60	60
0.5 mg L^{-1} NAA+2.0 mg L^{-1} BAP	0.25	0.608a	1.860a	2.493a	4.369a	0.385a
0.5 mg L^{-1} NAA +3.0 mg L^{-1} Kinetin	0.25	0.492b	1.336c	1.835c	3.504c	0.347c
0.5 mg L^{-1} 2, 4-D+2.0 mg L^{-1} BAP	0.25	0.497b	1.755b	2.254b	4.002b	0.363b
$LSD_{0.05}$		0.033	0.094	0.078	0.251	0.007

Figures with same letter(s) do not differ significantly at 5% level of significance

Table 3: Interaction effect of variety and plant growth regulators in MS medium on callus proliferation from virus infected tomato plant at different days after inoculation

Interaction (Variety concentrations and combination of PGRs)	Fresh weight of inoculated explants (g)	Fresh weight of callus at different days after inoculation (g)				Dry weight of callus at DAI (g)
		15	30	45	60	60
Bahar x 0.5 mg L^{-1} NAA + 2.0 mg L^{-1} BAP	0.25	0.635	1.957	2.576	4.421	0.403
Bahar x 0.5 mg L^{-1} NAA + 3.0 mg L^{-1} Kinetin	0.25	0.509	1.369	1.856	3.531	0.357
Bahar x 0.5 mg L^{-1} 2, 4-D + 2.0 mg L^{-1} BAP	0.25	0.626	1.873	2.287	4.029	0.385
Binatomato-20 x 0.5 mg L^{-1} NAA + 2.0 mg L^{-1} BAP	0.25	0.586	1.817	2.439	4.338	0.364
Binatomato-2 x 0.5 mg L^{-1} NAA + 3.0 mg L^{-1} Kinetin	0.25	0.474	1.333	1.810	3.478	0.332
Binatomato-2 x 0.5 mg L^{-1} 2, 4-D + 2.0 mg L^{-1} BAP	0.25	0.571	1.609	2.178	3.975	0.340
Binatomato-3 x 0.5 mg L^{-1} NAA + 2.0 mg L^{-1} BAP	0.25	0.603	1.806	2.465	4.349	0.388
Binatomato-3 x 0.5 mg L^{-1} NAA + 3.0 mg L^{-1} Kinetin	0.25	0.493	1.307	1.839	3.502	0.351
Binatomato-3 x 0.5 mg L^{-1} 2, 4-D + 2.0 mg L^{-1} BAP	0.25	0.593	1.784	2.297	4.004	0.365
$LSD_{0.05}$		0.057	0.163	0.135	0.435	0.004

Figures with same letter(s) do not differ significantly at 5% level of significance

Table 4: Relative colour change of the explants (stem) from infected tomato plants at different concentrations and combinations of plant growth regulators

Concentration and combination of PGRs	Tomato variety											
	Bahar at DAI				Binatomato-2 at DAI				Binatomato-3 at DAI			
	15	30	45	60	15	30	45	60	15	30	45	60
0.5 mg L^{-1} NAA + 2.0 mg L^{-1} BAP	LYe	Ye	Ye	Ye	LYe	Ye	Ye	Ye	LYe	Ye	Ye	Ye
0.5 mg L^{-1} NAA + 3.0 mg L^{-1} Kinetin	LYe	LYe	LYe	LYe	LYe	LYe	LYe	LYe	LYe	LYe	LYe	LYe
0.5 mg L^{-1} 2, 4-D + 2.0 mg L^{-1} BAP	LYe	Wh	LGr	LGr	LYe	Wh	LGr	LGr	LYe	Wh	LGr	Lgr

Ye-Yellow, LYe-Light yellow, Wh-White and LGr-Light grey

kinetin at all sampling dates. Balancing in hormones is an important factor for enhancing the cell division. In the present experiment, 0.5 mg L^{-1} NAA+2.0 mg L^{-1} BAP might be more balanced than other treatments. Geetha *et al.*[11] found maximum calli from leaflet explants of tomato Cv. CO-1 on MS medium supplemented with different combinations of NAA (3 or 6 μM) and BAP (2, 4, 6 or 8 μM). Capote *et al.*[13] also obtained maximum amount of calli by using 0.175 mg L^{-1} NAA and 1.5 mg L^{-1} BAP. The interaction effect of the concentrations and combinations of PGRs and tomato variety was insignificant on fresh weight of calli at all sampling dates. Although the maximum fresh weight of callus (0.635, 1.957, 2.576 and 4.421 g at 15, 30, 45 and 60 days after inoculation, respectively) was obtained from the interaction of Bahar with 0.5 mg L^{-1} NAA+2.0 mg L^{-1} BAP at 15, 30, 45 and 60 days after inoculation, respectively and minimum fresh weight was obtained from Binatomato-2 with 0.5 mg L^{-1} NAA+3.0 mg L^{-1} kinetin at the respective sampling dates (Table 3). In the present experiment, among the concentrations and combinations MS medium supplemented with 0.5 mg L^{-1} NAA+2.0 mg L^{-1} BAP exhibited better callusing ability. The variety Bahar showed the highest callus induction

Fig. 1: The effect of 0.5 mg L^{-1} NAA + 2.0 mg L^{-1} BAP (A), 0.5 mg L^{-1} NAA + 3.0 mg L^{-1} kinetin (B) and 0.5 mg L^{-1} 2, 4-D + 2.0 mg L^{-1} BAP (C), in MS medium on the proliferation of callus from virus infected stems of tomato variety Bahar at 60 days after inoculation. Scale bar = 0.5 inch

Fig. 2: The effect of 0.5 mg L^{-1} NAA + 2.0 mg L^{-1} BAP (A), 0.5 mg L^{-1} NAA + 3.0 mg L^{-1} kinetin (B) and 0.5 mg L^{-1} 2, 4-D + 2.0 mg L^{-1} BAP (C), in MS medium on the proliferation of callus from virus infected stems of tomato variety Binatomato-2 at 60 days after inoculation. Scale bar = 0.5 inch

Fig. 3: The effect of 0.5 mg L^{-1} NAA + 2.0 mg L^{-1} BAP (A), 0.5 mg L^{-1} NAA + 3.0 mg L^{-1} kinetin (B) and 0.5 mg L^{-1} 2, 4-D + 2.0 mg L^{-1} BAP (C), in MS medium on the proliferation of callus from virus infected stems of tomato variety Binatomato-3 at 60 days after inoculation. Scale bar = 0.5 inch

ability followed by Binatomato-3 and Binatomato-2, respectively. The minimum callus induction was observed at minimum concentration of the said growth regulators. Calli were fully virus infected which was confirmed by ELISA test.

The dry weight of calli varied significantly due to different varieties at 60 DAI. The maximum dry weight (0.382 g) was produced by the calli of Bahar and the minimum (0.345 g) by the calli of Binatomato-2 (Table 1). The dry weight of calli varied significantly due to different concentrations and combinations of the PGRs in MS medium (Table 2). The maximum dry weight (0.385 g) was obtained from 0.5 mg L^{-1} NAA+2.0 mg L^{-1} BAP followed by 0.5 mg L^{-1} 2, 4-D+3.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA+3.0 mg L^{-1} kinetin. Capote *et al.*[13] and Srivastava *et al.*[14] reported similar results while working with leaf tissue and stem segments of different cultivars with NAA + BAP combination of PGRs. The effect of interaction of variety and different concentrations and combinations of PGRs in MS medium was insignificant on dry weight of calli (Table 3). However, the maximum dry weight (0.403 g) was produced by the interaction of Bahar x 0.5 mg L^{-1} NAA+2.0 mg L^{-1} and the lowest dry weight (0.332 g) by the interaction of Binatomato-2x0.5 mg L^{-1} NAA+3.0 mg L^{-1} kinetin.

After inoculation of explants to culture media, stem segment showed light yellow appearance at first sight and gradually become yellow, white and light grey on MS medium supplemented with different concentrations and combinations of NAA, 2, 4-D, BAP and kinetin and change of colour was observed regularly. The extent of colour change was recorded at 15, 30, 45 and 60 DAI. The response of colour change of the explants of Bahar varied in respect of time, concentrations and combinations of NAA, 2, 4-D, BAP and kinetin used (Table 4). The explants of Bahar become light yellow at 0.5 mg L^{-1} NAA+2.0 mg L^{-1} BAP, 0.5 mg L^{-1} NAA+3.0 mg L^{-1} kinetin and 0.5 mg L^{-1} 2, 4-D+2.0 mg L^{-1} BAP after 15 days of inoculation. After 30, 45 and 60 days of inoculation, the explants of Bahar become yellow at 0.5 mg L^{-1} NAA+2.0 mg L^{-1} BAP. Changes of colour of the explants were not observed at 0.5 mg L^{-1} NAA+3.0 mg L^{-1} kinetin till 60 DAI. At 30, 45 and 60 DAI, the explants turned white, light grey, light grey, respectively at 0.5 mg L^{-1} 2,4-D+2.0 mg L^{-1} BAP. The response of colour change for the explants of variety Binatomato-2 and Binatomato-3 was similar to Bahar in respect of time, concentrations and combinations of NAA, 2, 4-D, BAP and kinetin used. This colour change of inoculated explants showed clear variation in the

different PGRs treatments. This result was closed to the treatments of Le *et al.*[15] in respect of callus proliferation at different concentrations of NAA, 2, 4-D and BAP.

It can be concluded that initiation of stem segment derived callus was influenced by different varieties at different days after inoculation (DAI). The fresh weight of callus increased gradually with increasing DAI in all varieties. Among them Bahar performed better in respect to produce both fresh and dry weight of callus. On the other hand, fresh weight of callus increased gradually in MS medium supplemented with different concentrations and combinations of plant growth regulators (PGRs). In all sampling dates, 0.5 mg L^{-1} NAA + 2.0 mg L^{-1} BAP produced higher fresh and dry weight compared to other concentrations and combinations of PGRs. The quality of callus was measured by its relative colour. The relative colour of callus of the tomato varieties changed as the inoculation days proceeded in MS medium supplemented with different concentration and combination of PGRs. Explants cultured on 0.5 mg L^{-1} NAA+2.0 mg L^{-1} BAP supplemented MS medium grew well for callus proliferation and remained yellow at 60 DAI. At 60 DAI, explants were light yellow in 0.5 mg L^{-1} NAA+3.0 mg L^{-1} kinetin and light grey in 0.5 mg L^{-1} 2, 4-D + 2.0 mg L^{-1} BAP.

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