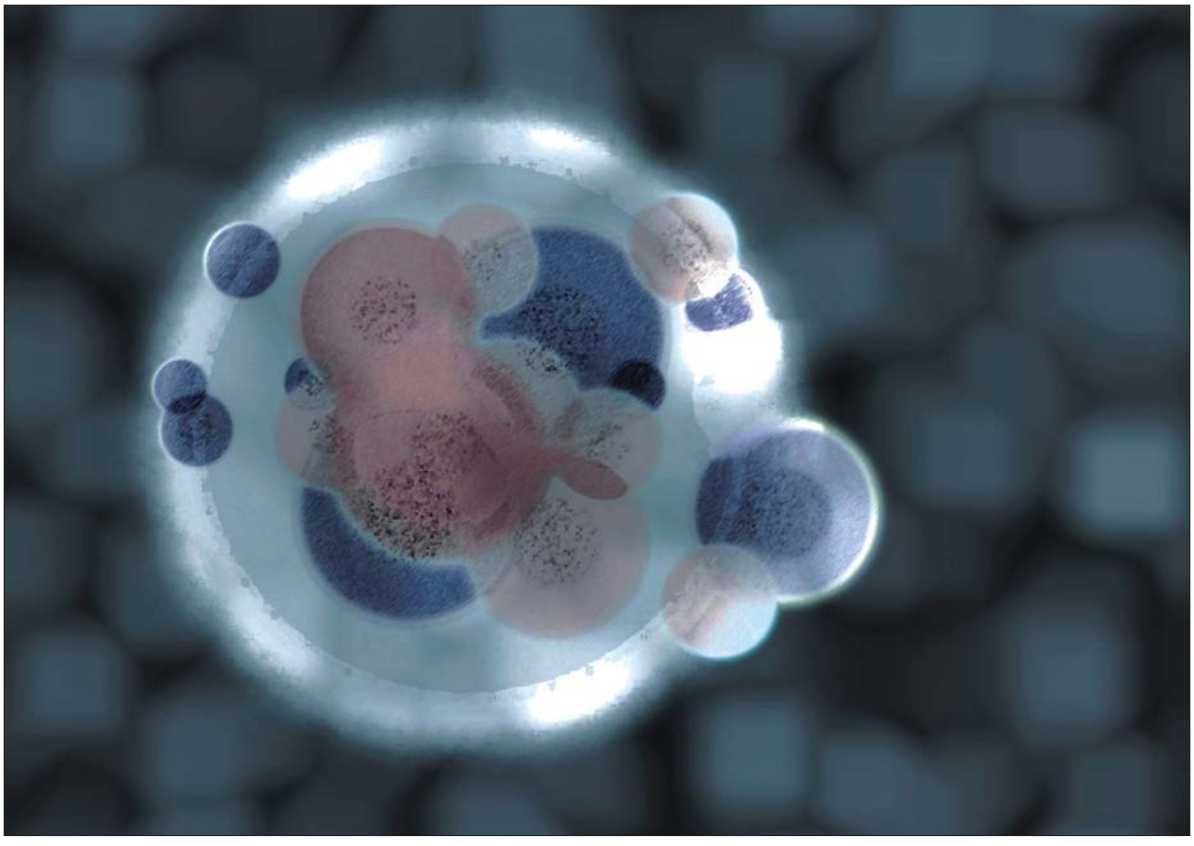

Journal of Biological Sciences

ISSN 1727-3048

science alert

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Journal of Biological Sciences 4 (4): 521-526, 2004
ISSN 1727-3048

Effect of Variety and Plant Growth Regulators in MS Medium on Shoot Induction from Virus Infected Calli of Tomato

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Abstract: The experiment was conducted in the Tissue Culture Laboratory of the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh, Bangladesh for micropropagation of tomato to evaluate the effect of variety and plant growth regulators in MS medium on shoot induction from virus infected calli of tomato plants. Three tomato varieties namely Bahar, Binatomato-2 and Binatomato-3 were used as plant materials in the present study. Callus derived shoots were induced on MS medium supplemented with different concentrations and combinations of plant growth regulators (PGRs). The combination of 0.2 mg L^{-1} IAA+4.0 mg L^{-1} BAP in MS medium was the best for inducing shoots which turned green to dark green after 15 days of culture. Callus derived shoots were fully virus infected which was confirmed by ELISA test. Meristem of plantlet can be used for the production of virus free tomato plant by meristem culture.

Key words: Plant growth regulators, NAA, BAP, shoot, 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]. Ripe tomato is used mostly in salad and in many other forms of fresh vegetables. Tomato is widely grown in Bangladesh usually in winter season. In Bangladesh, tomato was grown in 14338.06 ha of land and the production was 97565 metric tons during 1998-99. The average yield was 1.11 metric tons ha^{-1}[3], which was very low, compared to other leading tomato producing countries[4]. 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[5]. Over 200 diseases have been reported to affect tomato plants in the world[6]. Tomato leaf curl disease caused by TLCV is a limiting factor for a successful cultivation of tomato in Bangladesh. 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[7]. 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. Tomato seed production program in Bangladesh is being practiced with imported virus free seeds that are expensive. It is possible to bring down the cost of production by developing virus free seeds through tissue culture technique. Meristem of plantlet can be used for producing virus free tomato plant by meristem culture. Different plant growth regulators in MS medium affect shoot induction from callus. Costa *et al.*[8] observed higher shoot regeneration when cultured on MS medium supplemented with 1.0 mg L^{-1} zeatin and 0.1 mg L^{-1} IAA or 2.5 mg L^{-1} BAP and 0.2 mg L^{-1} IAA. In view of above facts, the present research was designed to evaluate the effect of variety and plant growth regulators in MS medium on shoot induction from tomato leaf curl virus infected calli of tomato.

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

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were used as plant materials in the present study. Stock solutions were prepared prior to medium preparation. Five stock solutions were prepared such as macronutrients, micronutrients, Fe-EDTA, vitamins/organics and PGRs.

One litre of MS medium was prepared. For preparation of 1 L 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 distilled water. pH of the medium was adjusted to 5.8 with a digital pH meter with the help of 0.1 N NaOH or 0.1N HCl, whichever was necessary. After adjusting the pH, 8 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. For callus proliferation, 0.5 mg L^{-1} NAA+2.0 mg L^{-1} BAP were used as PGRs. To investigate the best concentration and combination of auxin (IAA) and cytokinin (BAP) on *in vitro* tomato callus derived shoot production, four concentrations and combinations of PGRs were used such as: (I) 0.2 mg L^{-1} IAA+3.0 mg L^{-1} BAP, (ii) 0.2 mg L^{-1} IAA+4.0 mg L^{-1} BAP, (iii) 0.5 mg L^{-1} IAA+3.0 mg L^{-1} BAP and (iv) 1.0 mg L^{-1} IAA+3.0 mg L^{-1} BAP. Virus infected immature internodes segment (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 sec 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 5 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. Shoot obtained from callus by sub-culturing for two months in the same way with different supplements. Different size of shoots (larger than 1-4 cm) were used in meristem culture on 25 mL MS medium supplemented with IAA and BAP for eight weeks to investigate their effects on the growth and development of shoot. The produced shoots were virus infected, as it obtained from virus infected plants via calli and confirmed by ELISA test. Fresh weights of shoot with callus, number of shoot and relative colour change of shoot with callus from calli 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 by using the analysis of variance to find out the variation resulting from experimental treatments. Treatments were compared by Duncans Multiple Range Test[9].

RESULTS AND DISCUSSION

Total fresh matter is the sum of fresh weight of shoot with calli. The variation among different varieties of tomato was significant in their ability to proliferate fresh weight of shoot with calli at all the sampling dates (Table 1). The maximum fresh weight of shoot with calli (0.574, 1.27, 2.61 and 3.98 g at 15, 30, 45 and 60 days after inoculation, respectively) was produced by the shoots with calli of Bahar and the minimum fresh weight of shoot with calli was produced by Binatomato-2 (Table 1). In the present experiment, explants of Bahar were superior in proliferation of fresh weight of shoot with calli than Binatomato-2 and Binatomato-3 at all sampling dates and the shoot with calli of Binatomato-2 produced the minimum fresh weight at all the sampling dates. 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 shoot with calli at all sampling dates (Table 2). The highest fresh weight of shoot with calli (0.602, 1.34, 2.77 and 4.23 g at 15, 30, 45 and 60 DAI, respectively) was produced by 1.0 mg L^{-1} IAA+3.0 mg L^{-1} BAP and the minimum was produced by 0.2 mg L^{-1} IAA+3.0 mg L^{-1} BAP. Many researchers showed that different concentrations of IAA alone with BAP increase fresh weight of shoot with callus. Chande and Katiyar[10] observed that formation of shoot buds from 8-12 weeks old callus in MS medium with 1.5 mg L^{-1} BAP and 1.5 mg L^{-1} IAA was the most appropriate. The effect of interaction of variety and concentrations and combinations was not significant on induction of shoot with calli at 15, 30, 45 and 60 DAI (Table 3). However, the maximum fresh weight of shoot with calli (0.619, 1.36, 2.88 and 4.29 g at 15, 30, 45 and 60 DAI, respectively) was obtained from the interaction of Bahar with 1.0 mg L^{-1} IAA+3.0 mg L^{-1} BAP (Fig. 1) and the minimum was obtained from the interaction of Binatomato-2 with 0.2 mg L^{-1} IAA+3.0 mg L^{-1} BAP (Fig. 2).

Table 1: Effect of tomato varieties on the induction of shoot from virus infected calli at different days after inoculation

Varieties	Fresh weight of inoculated explants (g)	Fresh weight of shoot with callus at different days after inoculation (g)				Number of shoot at DAI			
		15	30	45	60	15	30	45	60
Bahar	0.2	0.574a	1.27a	2.61a	3.98a	-	2.80a	8.25a	13.35a
Binatomato-2	0.2	0.540b	1.22c	2.53b	3.84b	-	1.80c	7.50b	12.45c
Binatomato-3	0.2	0.559ab	1.25b	2.55ab	3.96a	-	2.40b	7.75b	12.90b
$LSD_{0.05}$		0.024	0.02	0.063	0.075	-	0.282	0.334	0.313

Table 2: Effect of different concentrations of IAA and BAP in MS medium on shoot induction from virus infected calli at different days after inoculation

Concentrations and combinations of IAA and BAP	Fresh weight of inoculated explants (g)	Fresh weight of shoot with callus at different days after inoculation (g)				Number of shoot at DAI			
		15	30	45	60	15	30	45	60
0.2 mg L^{-1} IAA+ 3.0 mg L^{-1} BAP	0.2	0.504c	1.13d	2.33d	3.55d	-	0.33d	3.00d	6.66d
0.2 mg L^{-1} IAA+ 4.0 mg L^{-1} BAP	0.2	0.573b	1.31b	2.69b	4.13b	-	1.26c	4.53c	8.53c
0.5 mg L^{-1} IAA+ 3.0 mg L^{-1} BAP	0.2	0.551b	1.20c	2.45c	3.79c	-	3.53b	10.33b	16.33b
1.0 mg L^{-1} IAA+ 3.0 mg L^{-1} BAP	0.2	0.602a	1.34a	2.77a	4.23a	-	4.20a	13.26a	20.06a
$LSD_{0.05}$		0.027	0.027	0.072	0.087	-	0.325	0.386	0.361

Table 3: Interaction effect of variety and IAA and BAP in MS medium on shoot induction from virus infected calli at different days after inoculation

Interaction variety ×concentrations and combinations of IAA and BAP	Fresh weight of inoculated explants (g)	Fresh weight of shoot with callus at different days after inoculation (g)				Number of shoot at DAI			
		15	30	45	60	15	30	45	60
Bahar×0.2 mg L^{-1} IAA+3.0 mg L^{-1} BAP	0.2	0.516	1.15	2.36	3.61	-	1.00f	3.20e	7.00f
Bahar×0.2 mg L^{-1} IAA+4.0 mg L^{-1} BAP	0.2	0.591	1.34	2.72	4.17	-	1.60e	4.80d	8.80e
Bahar×0.5 mg L^{-1} IAA+3.0 mg L^{-1} BAP	0.2	0.570	1.23	2.48	3.84	-	4.00bc	10.80c	16.60d
Bahar×1.0 mg L^{-1} IAA+3.0 mg L^{-1} BAP	0.2	0.619	1.36	2.88	4.29	-	4.60a	14.20a	21.00a
Binatomato-2×0.2 mg L^{-1} IAA +3.0 mg L^{-1} BAP	0.2	0.493	1.11	2.31	3.46	-	0.00	2.80e	6.40f
Binatomato-2×0.2 mg L^{-1} IAA +4.0 mg L^{-1} BAP	0.2	0.555	1.27	2.67	4.07	-	1.00f	4.20d	8.20e
Binatomato-2×0.5 mg L^{-1} IAA +3.0 mg L^{-1} BAP	0.2	0.528	1.18	2.42	3.70	-	2.60d	10.20c	16.00d
Binatomato-2×1.0 mg L^{-1} IAA +3.0 mg L^{-1} BAP	0.2	0.584	1.31	2.71	4.13	-	3.60c	12.80b	19.20c
Binatomato-3×0.2 mg L^{-1} IAA +3.0 mg L^{-1} BAP	0.2	0.504	1.13	2.33	3.58	-	0.00	3.00e	6.60f
Binatomato-3×0.2 mg L^{-1} IAA +4.0 mg L^{-1} BAP	0.2	0.573	1.32	2.69	4.14	-	1.20ef	4.60d	8.60e
Binatomato-3×0.5 mg L^{-1} IAA +3.0 mg L^{-1} BAP	0.2	0.555	1.20	2.44	3.83	-	4.00bc	10.60c	16.40d
Binatomato-3×1.0 mg L^{-1} IAA+3.0 mg L^{-1} BAP	0.2	0.602	1.34	2.73	4.28	-	4.40ab	12.80b	20.00b
$LSD_{0.05}$		0.014	0.014	0.106	0.151	-	0.564	0.669	0.626

In column, figures with same letter(s) do not differ significantly at 5% level of significance

Table 4: Relative colour change of shoot with callus from calli in different concentrations and combinations of PGRs at different days after inoculation

Concentration and combination of PGRs	Tomato varieties											
	Bahar at DAI				Binatomato-2 at DAI				Binatomato-3 at DAI			
	15	30	45	60	15	30	45	60	15	30	45	60
0.2 mg L^{-1}IAA+ 3.0 mg L^{-1} BAP	Gr	Gr	DGr	DGr	Gr	Gr	DGr	DGr	Gr	Gr	DGr	DGr
0.2 mg L^{-1} IAA+ 4.0 mg L^{-1} BAP	Gr	Gr	DGr	DGr	Gr	Gr	DGr	DGr	Gr	Gr	DGr	DGr
0.5 mg L^{-1} IAA+ 3.0 mg L^{-1} BAP	Gr	Gr	Gr	LBr	Gr	Gr	Gr	LBr	Gr	Gr	Gr	LBr
1.0 mg L^{-1} IAA+ 3.0 mg L^{-1} BAP	Gr	Gr	LBr	LBr	Gr	Gr	LBr	LBr	Gr	Gr	LBr	LBr

Gr-Green, DGr-Dark green and LBr-Light brown

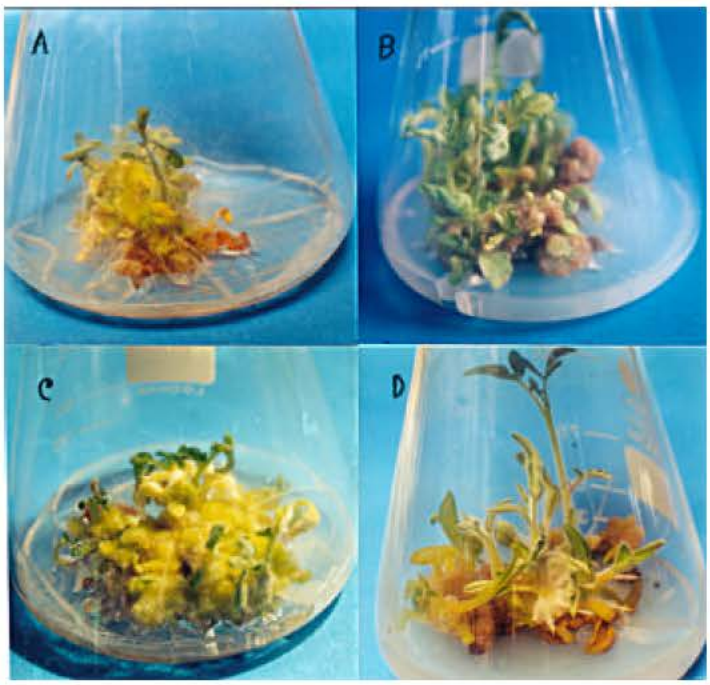

Fig. 1: The effect of 0.2 mg L^{-1} IAA+3.0 mg L^{-1} BAP (A), 0.2 mg L^{-1} IAA+4.0 mg L^{-1} BAP (B), 0.5 mg L^{-1} IAA+3.0 mg L^{-1} BAP (C) and 1.0 mg L^{-1} IAA+3.0 mg L^{-1} BAP (D) in MS medium on virus infected shoot induction from prepared callus of tomato variety Bahar at 60 days after inoculation. Scale bar = 0.5 inch

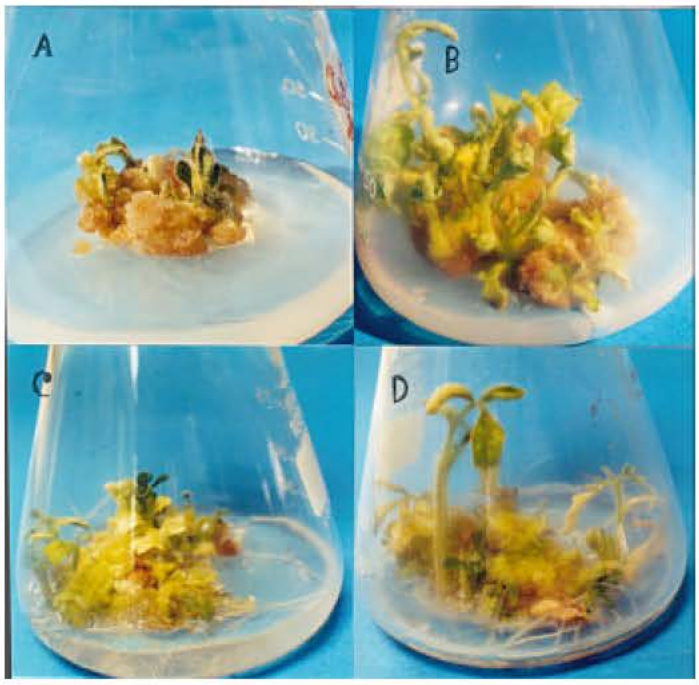

Fig. 2: The effect of 0.2 mg L^{-1} IAA+3.0 mg L^{-1} BAP (A), 0.2 mg L^{-1} IAA+4.0 mg L^{-1} BAP (B), 0.5 mg L^{-1} IAA+3.0 mg L^{-1} BAP (C) and 1.0 mg L^{-1} IAA+3.0 mg L^{-1} BAP (D) in MS medium on virus infected shoot induction from prepared callus of tomato variety Binatomato-2 at 60 days after inoculation. Scale bar = 0.5 inch

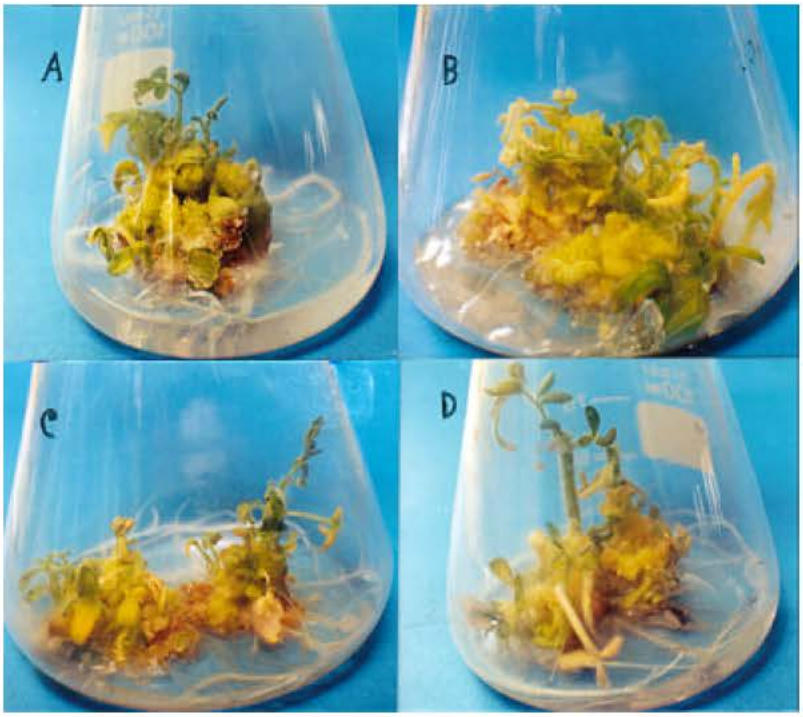

Fig. 3: The effect of 0.2 mg L^{-1} IAA+3.0 mg L^{-1} BAP (A), 0.2 mg L^{-1} IAA+4.0 mg L^{-1} BAP (B), 0.5 mg L^{-1} IAA+3.0 mg L^{-1} BAP (C) and 1.0 mg L^{-1} IAA+3.0 mg L^{-1} BAP (D) in MS medium on virus infected shoot induction from prepared callus of tomato variety Binatomato-3 at 60 days after inoculation. Scale bar = 0.5 inch

The number of shoots varied significantly due to variation of varieties at different DAI (Table 1). At 30, 45 and 60 DAI, explants of Bahar produced the maximum number of shoots (2.80, 8.25 and 13.35, respectively) while Binatomato-2 produced the minimum number of shoots (1.80, 7.50 and 12.45, respectively). In the present experiment, the number of shoots increased gradually with the increase of time. None of the varieties produced shoot at 15 DAI. There was significant difference among the different concentrations and combinations of plant growth regulators in respect of number of shoots at all sampling dates (Table 2). The highest number of shoots (4.20, 13.26 and 20.06) was produced by the 1.0 mg L^{-1} IAA+3.0 mg L^{-1} BAP at 30, 45 and 60 DAI, respectively. While 0.2 mg L^{-1} IAA+3.0 mg L^{-1} BAP produced the minimum number of shoots (0.333, 3.00 and 6.66). At 15 DAI, shoot was not found. Venkatachalam *et al.*[11] found that BAP was more suitable compared to kinetin from the maximum shoot bud differentiation as well as multiple shoot induction. Costa *et al.*[8] observed higher shoot regeneration when cultured on MS medium supplemented with 1.0 mg L^{-1} zeatin and 0.1 mg L^{-1} IAA or 2.5 mg L^{-1} BAP and 0.2 mg L^{-1} IAA. Interaction effect of variety and PGRs in MS medium on number of shoot was also significant at all DAI except 15 DAI (Table 3). The highest number of shoot (4.60, 14.20 and 21.00) was obtained from the interaction of Bahar×1.0 mg L^{-1} IAA+3.0 mg L^{-1} BAP at 30, 45 and 60 DAI, respectively (Fig. 1). At 45 and 60 DAI, the minimum number of shoot (2.80 and 6.40, respectively) was obtained from Binatomato-2×0.2 mg L^{-1} IAA+3.0 mg L^{-1} BAP (Fig. 2). It was clear that all the cultivars highly responded with MS+1.0 mg L^{-1} IAA+3.0 mg L^{-1} BAP for shoot induction. It could be the best combination for multiple shoot induction. Chen *et al.*[12] and Moghaieb *et al.*[13] found the similar result on MS medium supplemented with the present concentrations and combinations of IAA and BAP.

After inoculation to shoot induction culture media, the callus explants showed green appearance at first sight and gradually become dark green to light brown on MS medium supplemented with different concentrations and combinations of IAA and BAP. Colour of the shoot with calli was observed at 15, 30, 45 and 60 DAI (Table 4). The response of colour of Bahar, Binatomato-2 and Binatomato-3 varied in respect of time and concentrations and combinations of PGRs used in MS medium. All the varieties become green in 0.2 mg L^{-1} IAA+3.0 mg L^{-1} BAP, 0.2 mg L^{-1} IAA+4.0 mg L^{-1} BAP, 0.5 mg L^{-1} IAA+3.0 mg L^{-1} BAP and 1.0 mg L^{-1} IAA+3.0 mg L^{-1}

BAP after 15 and 30 DAI. Callus derived shoots were fully virus infected which was confirmed by ELISA test.

From the above findings, initiation of callus derived shoots found to be different in the tomato varieties. Variety Bahar produced the maximum fresh weight of shoot with callus and number of shoot in MS media supplemented with PGRs. Fresh weight of shoot with callus and number of shoot increased gradually as the inoculation days proceeded in different concentrations and combinations of PGRs. Among them, 1.0 mg L^{-1} IAA+3.0 mg L^{-1} BAP produced the maximum fresh weight and number of shoot in all sampling dates compared to other concentrations. The relative colour of shoot with callus of the tomato varieties changed as the inoculation days preceded in MS medium. So 1.0 mg L^{-1} IAA+3.0 mg L^{-1} BAP was the best concentration and combination for shoot induction from virus infected calli. Meristem of these callus dried shoot can be used for the production of virus free tomato plant by meristem culture.

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