

PLANTLET REGENERATION FROM SEEDLING EXPLANTS OF SWEET GOURD

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ABSTRACT

To regenerate plantlets both directly and indirectly, different explants (cotyledon, hypocotyl, root tip and shoot tip) of sweet gourd (*Cucurbita moschata*) were cultured on medium supplemented with different concentrations and combinations of NAA (0.0, 0.2, 0.5 and 1.0 mg/L) and BAP (0.0, 0.5, 2.0 and 5.0 mg/L). Cotyledon explants performed best in callus induction. The combination of 5 mg/L BAP and 0.2 mg/L NAA produced the highest callus frequency in cotyledon (90.0%). The calli derived from cotyledon, hypocotyl and root tip were cultured in MS medium supplemented with different concentrations of kinetin, BAP and/or NAA for shoot induction. Regeneration via callus was achieved only from cotyledon calli at a frequency of 65.0% on 2 mg/L BAP and 0.2 mg/L NAA. Shoot tips cultured for direct regeneration, in the same media containing 1 mg/L BAP or 1 mg/L BAP + 0.2 mg/L NAA resulted in 100.0% shoot differentiation. The regenerated shoots rooted on MS medium with or without 0.1 mg/L NAA (100%).

Key Words : Callus, Plantlet, Regeneration, Seedling explants, Sweet gourd

INTRODUCTION

Sweet gourd (*Cucurbita moschata* Duchex Poir) is an important vegetable in Bangladesh. It is a seed propagated, day-neutral, annual, monoecious vine crop. It is grown in all districts of Bangladesh round the year. However, its production is concentrated during summer season. It is a good source of carbohydrates, soluble fibre and β -carotinoid. It also contains protein, oils and vitamin E in the seeds. Among all the cucurbits, sweet gourd is the richest source of carotene. Immature fleshy green leaves, tender shoots and male flowers are also used as vegetables, and contain appreciable amount of vitamins and minerals (Robinson and Walters, 1997). This crop is, therefore, thought to have potentiality to solve malnutrition problem of Bangladesh to certain extent particularly of the vulnerable groups in respect of vitamin A requirement.

Improvement of sweet gourd and development of variety are obviously necessary. Tissue culture could be potentially applied for the purpose. Unfortunately there have been only few attempts to regenerate sweet gourd via tissue culture (Zhao, 1999; Hegazi, 1999). The

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present study was therefore, undertaken to find out the suitable explant and medium for rapid *in vitro* regeneration protocol of sweet gourd for possible utilization in genetic improvement in future studies.

MATERIALS AND METHODS

Healthy seeds were collected and washed in tap water. The seeds were then sterilized with 70% ethanol for one minute and then rinsed with sterile distilled water. Afterwards, the seeds were soaked in 0.1% HgCl₂ plus two drops of a liquid detergent (Tween-20) solution and then agitated gently for 15-20 minutes, followed by 4 rinses in sterilized distilled water. The seeds were aseptically germinated on half MS medium. Cotyledons, hypocotyls and root tips were excised aseptically from 10-12 days old seedlings and cultured on MS medium containing different concentrations and combinations of BAP (0.0, 0.5, 2.0 and 5.0 mg/L) and NAA (0.0, 0.2, 0.5 and 1.0 mg/L). Subcultures were carried out at 21 days interval and finally the calli were transferred to MS medium with different concentrations of cytokinins (BAP and Kinetin) and auxin (NAA) singly or in combination. Shoot tips were also cultured on the same media. The regenerated shoots were rooted in hormone free ½ MS media, hormone free MS media and MS media with different concentrations of NAA (0.1, 0.2, 0.3, 0.4 and 0.5 mg/L).

The pH of the medium was adjusted to 5.8 prior to the addition of agar @ 8.0g/l and the medium was autoclaved at 121°C for 30 minutes. Twenty explants were cultured per treatment and each treatment was replicated 4 times. All cultures were maintained at 26 ± 1°C under white fluorescent tubes with a 16h photoperiod.

RESULTS AND DISCUSSION

The results revealed that a high variation in callus induction ability was demonstrated by the explants and treatments under present study. Among the various explants, cotyledon performed best for callus induction. Cotyledon explants had the highest frequency (53.44%) of callus and required minimum number of days (12.81) (Table 1).

NAA and BAP combinations had significant effect on callus induction. The minimum number of days (18.0) required for callusing and the highest percentage (83.33%) were observed at 5 mg/L BAP combined with 0.2 mg/L NAA. The callus weight (1.48g) was also highest at this combination (Table 1). The combined effects of growth regulators and explants revealed that, cotyledon explants showed the best performance in percentage of callus induction (90.0%) at 5 mg/L BAP and 0.2 mg/L NAA (Fig. 1A). The combination of 4.44 µM BA + 2.69 µM NAA was reported to be the most suitable for callus induction from cotyledon explants of cucumber (Selvaraj *et al.*, 2007). Urbanek *et al.* (2005) reported callus formation from cotyledon of styrian pumpkin with 26.85 µM NAA and 13.32 µM BA. The results of the present study also showed that a combination of auxin and cytokinin effectively induced callus from cotyledon explants of sweet gourd.

Different concentrations of BAP or kinetin alone and BAP in combination with NAA were used to investigate the shoot induction capability of calli or different explants.

Among the explants used, shoot tip explants exhibited best performance (76.56%) followed by cotyledon (17.19%). It was also evident that shoot regeneration was not achieved from hypocotyl and root tip derived calli. The highest percentage (80.0%) of shoots regenerated at the combination of 2 mg/L BAP and 0.2 mg/L NAA (Fig. 1B). Considering the combined effect of explants and kinetin, BAP and/or NAA, 100.0% shoot was regenerated at 1 mg/L BAP as well as 1 mg/L BAP and 0.2 mg/L NAA from shoot tip explants via direct regeneration (Table 2). Similar result was also reported by Awal *et al.* (2005). The least number of days (8.25) and highest number of shoots per explants (13.25) were evident at BAP 1 mg/L. Shoot tip explants also had the tallest shoot (4.45 cm) with 1 mg/L BAP and 0.2 mg/L NAA. The present finding agrees well with that of Vasudevan *et al.* (2001). The regeneration of shoots from cotyledon derived callus was not so satisfactory. The percentage of shoot regenerated from cotyledon derived callus was 65.0 and it required 50.50 days to initiate shoot (Table 2).

Table 1. Main effect of hormone concentrations on callus induction

Hormone concentration (mg/L) NAA + BAP	% Callus induction	Days required for callus induction	Weight of callus (g)	Nature of callus
0.0 + 0.0 (T ₁)	0.00 j	0.00 f	0.00 h	-
0.0 + 0.5 (T ₂)	33.75 i	22.50 a	0.37 g	Friable
0.0 + 2 (T ₃)	37.50 h	22.83 a	0.34 g	Friable
0.0 + 5 (T ₄)	39.17 h	23.17 a	0.34 g	Friable
0.2 + 0.0 (T ₅)	0.00 j	0.00 f	0.00 h	-
0.2 + 0.5 (T ₆)	59.58 g	21.50 b	0.42 g	Friable
0.2 + 2 (T ₇)	76.67 c	20.67 c	1.47 a	Compact
0.2 + 5 (T ₈)	83.33 a	18.00 e	1.48 a	Compact
0.5 + 0.0 (T ₉)	0.00 j	0.00 f	0.00 h	-
0.5 + 0.5 (T ₁₀)	69.58 f	21.58 b	1.06 d	Friable
0.5 + 2 (T ₁₁)	75.00 c	20.42 c	0.57 f	Friable
0.5 + 5 (T ₁₂)	76.67 bc	20.17 c	0.73 e	Friable
1.0 + 0.0 (T ₁₃)	0.00 j	0.00 f	0.00 h	-
1.0 + 0.5 (T ₁₄)	74.17 d	18.50 de	1.35 b	Compact
1.0 + 2 (T ₁₅)	78.75 b	18.67 de	1.41 ab	Compact
1.0 + 5 (T ₁₆)	72.08 e	18.92 d	1.20 c	Compact

In a column, the figures followed by same letter(s) do not differ significantly

For root initiation the regenerated shoots were cultured on hormone free MS media and MS media with NAA (0.1, 0.2, 0.3, 0.4, 0.5 mg/L). There was no statistical difference for rooting among the explants. However, different concentrations of NAA showed highly significant variation in their effects on the rooting parameters under study. The rooting frequency reached up to 100.0% in MS medium without hormones and it required minimum number of days (6.63). The number of roots (6.88) per plant was also highest

(Table 3) in this treatment. With the increase of NAA concentration, there was a gradual decrease in root induction frequency and number of roots per explant with an increase in the days required for root induction. A contradictory result was earlier reported by Wei *et al.* (2006); Sultana *et al.* (2004) and Lixin and HuaiMing (1998). They observed the highest frequency of root in medium supplemented with 0.1 mg/L NAA.

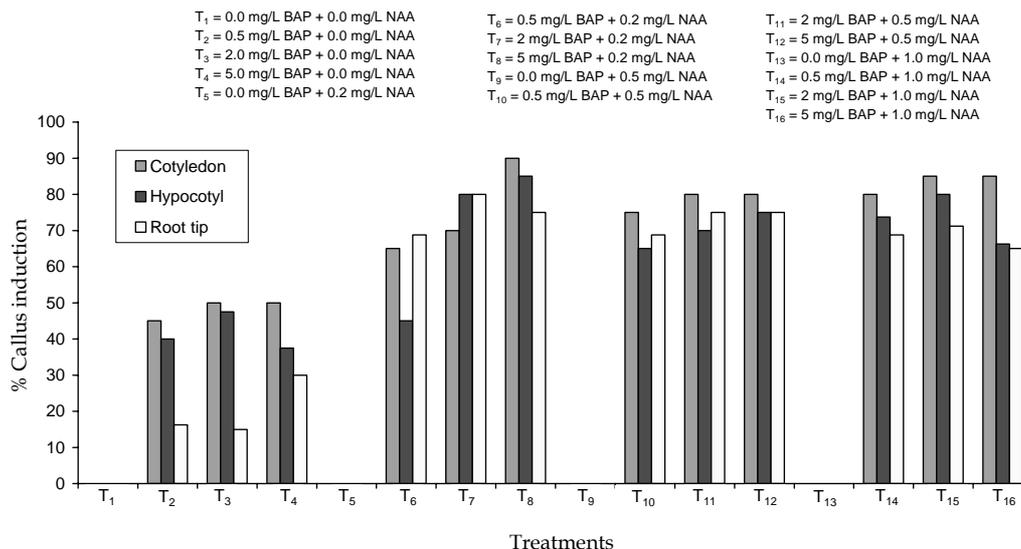


Fig. 1. Combined effect of explants and hormone concentrations on callus induction

Table 2. Combined effect of explants and hormone concentrations on shoot induction

Explants	Hormone concentration (mg/L)	% Shoot induction	Days required for shoot induction	No. of shoot per explants	Shoot length (cm)
Cotyledon	Control (T ₁)	0.00 h	0.00 c	0.00 h	0.00 d
	1 BAP (T ₂)	0.00 h	0.00 c	0.00 h	0.00 d
	5 BAP (T ₃)	0.00 h	0.00 c	0.00 h	0.00 d
	1 Kinetin (T ₄)	0.00 h	0.00 c	0.00 h	0.00 d
	5 Kinetin (T ₅)	0.00 h	0.00 c	0.00 h	0.00 d
	1 BAP+0.2NAA (T ₆)	10.00 g	42.25 a	1.75 g	2.13 c
	2 BAP + 0.2 NAA (T ₇)	65.00 c	45.75 a	4.25 f	3.67 b
	5 BAP + 0.2 NAA (T ₈)	62.50 cd	50.50 a	4.00 f	3.50 b
Shoot tip	Control (T ₁)	57.50 de	10.50 bc	6.50 d	3.70 b
	1 BAP (T ₂)	100.00 a	8.25 bc	13.25 a	4.43 a
	5 BAP (T ₃)	55.00e	11.75 bc	5.50 e	3.55 b
	1 Kinetin (T ₄)	65.00 c	11.50 bc	5.25 e	3.25 b
	5 Kinetin (T ₅)	47.50 f	14.75 b	4.75 ef	3.22 b
	1 BAP + 0.2 NAA (T ₆)	100.00 a	8.50 bc	13.50 a	4.45 a
	2 BAP + 0.2 NAA (T ₇)	95.00 ab	11.75 bc	9.75 b	3.85 b
	5 BAP + 0.2 NAA (T ₈)	92.50 b	10.75 bc	8.50 c	3.60 b
LSD _(0.05)	6.805	10.030	0.7813	0.5470	

In a column, the figures followed by the same letter(s) do not differ significantly

Table 3. Main effect of hormone concentrations on root induction

Hormone concentration (mg/L)	% root induction	Days required for root initiation	No. of roots/shoot
MS without hormone (T ₁)	100.00 a	6.63 e	6.88 a
1/2 MS (T ₂)	90.00 ab	8.38 d	3.88 b
0.1 NAA (T ₃)	97.50 a	6.75 e	6.50 a
0.2 NAA (T ₄)	80.00 b	10.50 c	3.25 c
0.3 NAA (T ₅)	67.50 c	12.25 b	2.75 c
0.4 NAA (T ₆)	42.50 d	14.38 a	1.75 d
0.5 NAA (T ₇)	35.00 d	14.38 a	1.50 d
LSD _(0.05)	5.878	0.5823	0.5396

In a column, the figures followed by the same letter(s) do not differ significantly

From the results obtained from the present research work as described above, it may be concluded that cotyledon explants are best for callus induction. However, shoot tip explants were better for *in vitro* regeneration than cotyledon explants. The direct regeneration was more rapid process than indirect regeneration via callus. Among the hormone combinations used, a combination of BAP at 5 mg/L and NAA at 0.2 mg/L was best for callus induction. On the other hand, 1 mg/L BAP or 1 mg/L BAP in addition to 0.2 mg/L NAA was best for shoot induction and hormone free MS media was found best for rooting of *in vitro* regenerated shoots of sweet gourd (Fig. 1C).

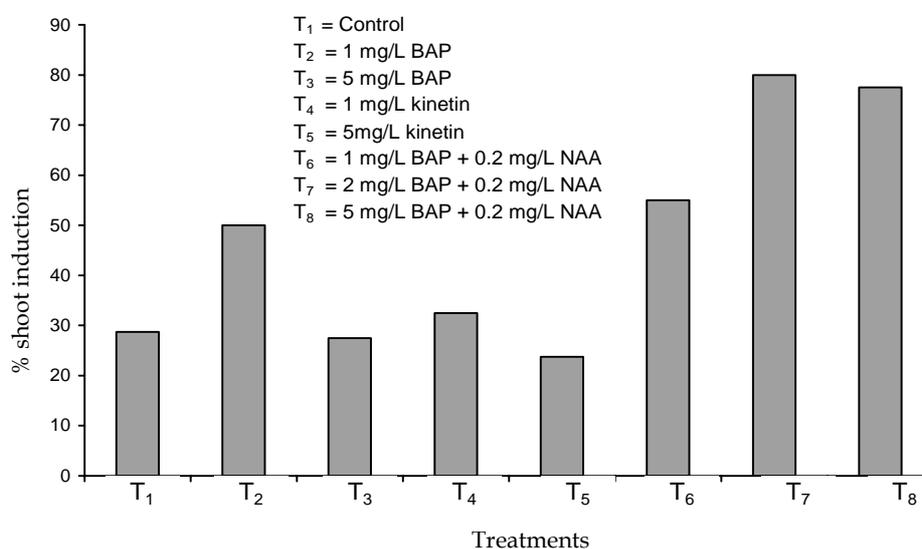


Fig. 2. Main effect of hormone concentrations on % shoot induction

In conclusion, the callus formation and regeneration ability of the explants have the potential for future biotechnological studies for the improvement of sweet gourd. Here a

direct *in vitro* regeneration protocol was developed. However, further study is needed with different explants to standardize the protocol of regeneration via callus.



Fig. 1. A) Callus proliferation from cotyledon explants in 5 mg/L BAP + 1.0 mg/L NAA at 42 days after inoculation. B) Direct shoot regeneration from shoot tip explants on 1 mg/L BAP at 45 days after inoculation. C) Root formation from regenerated shoot in hormone free MS medium at 80 days after inoculation.

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