In Vitro Propagation of Eggplant through Meristem Culture

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Summary

Meristem culture was done for developing an efficient protocol of production of eggplant clones. Shoot tips of 30-35 days old field grown eggplants were used for meristem isolation. Three cultivars viz. 'Islampuri', 'Khatkhatia' and 'Katabegun' were used in the present investigation as explants source. Surface sterilization of shoot tips was found to be the best in 0.1% $HgCl_2$ solution for 3 minutes. For primary establishment of isolated apical meristem in MS liquid medium containing 2.0 mg l^{-1} BAP was found the best in cv Islampuri. BAP was also proved to be best for the primary establishment of isolated apical meristem in all the cultivars. Subsequent development of meristem derived shoot was achieved in MS semisolid medium containing either 2.0 mg l^{-1} BAP and 1.0 mg l^{-1} NAA or 1.0 mg l^{-1} BAP. For root development from meristem derived shoots, 1.0 mg l^{-1} IBA was found most responsive in cv. 'Islampuri' and 'Khatkhatia'. After transplantation, the *in vitro* plants showed normal growth.

Key words

shoot-tip, acclimatization, *Solanum melongena* L.

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Introduction

Solanum melongena L. (eggplant) is one of the important horticultural plants of *Solanaceae* family. It is also one of the world most important crops by virtue of volume produced and its contribution to our national economy. It is a cheap source of vitamins and minerals particularly iron and its total nutritional value is comparable with tomato (Choudhury, 1976). It has significant medicinal values (Khan, 1979). Eggplant germplasm resources and collections have been well documented, evaluated and conserved throughout the world (Sarathbabu et al., 1999). Generally eggplants have been preferred for large scale production and for door yard plants as a vegetable crop in tropical countries. It is an economically important crop in Asia, Africa and sub-tropics and it is also cultivated in some warm temperate regions of the Mediterranean and South America (Sihachakr et al., 1993). Various diseases cause yield loss of this crop. A number of viral, bacterial, fungal and nematode disease attack eggplant. Many viral diseases such as tobacco mosaic virus, cucumber mosaic virus (CMV), tobacco ring spot virus (TRSV) is common in *Solanum melongena*. Bacterial disease like bacterial wilt, damping off, early blight and leaf spot are also reported (Daunay et al., 1991).

Conventional breeding methods have limitation to protect against diseases. Meristem culture is unique technique to produce virus free plants. It has been extensively used for quick vegetative propagation of a large numbers of plant species. Tissue culture techniques have been demonstrated in a wide variety of agronomically momentous plants such as *Solanum melongena* and other species (Johri et al., 1980; Taha and Tijan, 2002). Otherwise, virus elimination through meristem culture is a popular horticulture practice nowadays (Bhojwani and Razdan, 1983). The application of meristem culture is either to eliminate virus infection in clonal plant or its large scale production of asexual seedling. Other horticulture benefits have been discussed in many different publications (Djurdjina, et al., 1997; Edriss et al., 1996; Islam and Chowdhury, 1998; Zaman et al., 2001, Mohammad, 2002; Nagib et al., 2003). The aim of this investigation was to develop protocol for large scale production of virus free eggplant plantlets through meristem culture.

Materials and methods

Three eggplant (*Solanum melongena* L.) cultivars such as 'Islampuri', 'Khatkhatia' and 'Katabegun' were used in the present investigation as explants source. Seedlings were grown in the Botanical Garden, Department of Botany, Rajshahi University for explants collecting. Shoot tips from 30-35 days old plants were used as explants for meristem isolation and culture. At first, shoot tips were cut from the 30-35 days old field grown three cultivars ('Islampuri', 'Khatkhatia', 'Katabegun') of egg plant and collected in a conical flask. The shoot tips were thoroughly washed under running tap water for a few minutes to reduce the dust and then transferred in another conical flask containing distilled water adding 2-3 drops of tween 80 and a few drops of savlon (chlorhexidine gluconate plus cetrimide). Inside the laminar air flow cabinet surface disinfection was done with 0.1% $HgCl_2$ solution by gently shaking for five minutes. After exposure to the sterilant, the shoot tips were washed several times with double distilled water to remove all traces of $HgCl_2$.

After sterilization, the immature leaves and leaf primordial were snapped of with slight pressure of the needle. Then the exposed meristem (0.1-0.2 mm) which appeared as a shiny dome was gently isolated with a sharp blade.

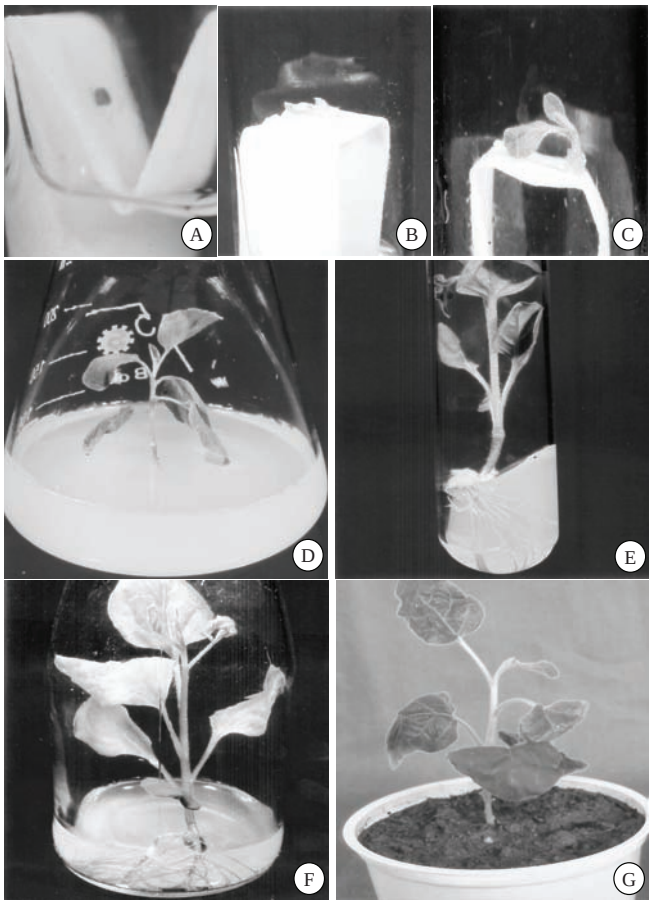

Figure 1. Plantlets development through meristem culture (A: Isolated meristem on filter paper bridge in liquid MS medium; B & C: Primary shoot initiatioin in liquid medium for isolated meristem; D: Development of shoots in semisolid medium; E & F: Shoot and root development from primary established meristem; G: Acclimatization of plantlets in soil).

Table 1. Effect of different concentrations of BAP, GA_3, KIN and MS_0 control medium for primary establishment of apical meristems isolated from 30-35 days old field grown plants

Treatment (mg/l)		Days to response			Number of primary established meristem (%)		
		Islampuri	Khatkhatia	Katabegun	Islampuri	Khatkhatia	Katabegun
BAP	0.1	12-16	12-17	12-16	14.28	15.00	14.13
	0.5	12-16	11-16	11-15	28.57	30.30	29.00
	1.0	10-18	9-18	9-17	50.00	45.20	44.10
	1.5	10-18	8-17	9-17	85.70	80.50	82.50
	2.0	6-12	9-16	8-18	93.85	85.80	85.00
	2.5	9-16	8-17	9-16	56.15	55.36	57.27
	3.0	12-18	13-18	13-18	42.87	41.17	43.17
	4.0	12-18	12-18	10-16	35.71	32.34	34.04
	5.0	12-18	11-18	10-18	14.27	16.07	15.13
KIN	0.1	10-16	10-16	10-14	14.20	10.28	14.28
	0.5	9-16	8-16	8-16	42.85	40.87	46.51
	1.0	9-19	9-17	7-15	21.43	18.01	20.71
	2.0	10-16	9-16	10-17	14.28	10.38	17.50
GA_3	0.1	8-15	8-16	9-17	35.70	33.00	37.07
	0.5	8-14	8-16	8-13	60.50	55.17	56.14
	1.0	7-15	8-15	7-14	28.52	25.04	26.57
	2.0	7-14	8-14	7-12	14.25	10.11	11.26
MS_0 (control)		12-16	13-15	12-15	12.00	9.20	8.50

Then the excised meristems were carefully placed on the filter paper bridge in the culture tubes containing liquid MS medium (Murashige and Skoog, 1962). All cultures were grown in the growth chamber illuminated by 40 watts white fluorescent tubes fitted at a distance of 30-40 cm on the culture shelves. The cultures were maintained at $25^0 \pm 2^0$C with light intensity varied from 2000-3000 lux. The photoperiod was maintained generally 16 hours light and 8 hours dark.

For establishment of primary meristem, MS liquid medium containing different concentrations of KIN, BAP, GA_3 singly (Table 1) or in combinations of KIN and GA_3 (Table 2) were used. The medium was solidified with 6.2% agar. After three to four weeks of inoculation of meristem, the primary established meristems that showed morphogenic responses were removed aseptically from the culture tubes and transferred into tubes containing agar gelled MS medium supplemented with BAP, KIN and IBA (Table 3) individually or in different combinations of BAP, NAA, KIN and GA_3 (Table 4). During inoculation, special care was taken that the explants touch the medium equally and do not dip into the medium. Percentage of explants (meristem) that responded was calculated. After 35 days of culture shoot length was measured in cm scales for each plantlet and average of shoot length was calculated and noted. Number of shoots per plantlet was computed after required days of culture.

When the plantlets attained 5-8 cm height with few leaves and well-developed root system, they were ready for transplantation into beds. Numbers of roots were counted for each plantlet at 35 day of subcultures of five randomly selected cultures and then mean values were recorded. Before transfer, the plantlets were taken out from the controlled environment of growth chamber and were kept in room temperature for three to five days to bring them in contact with normal temperature for acclimatization. After three to five days of hardening the plantlets were taken out from the test tube and the roots were washed under running tap water to remove medium. Then the plantlets were ready for transfer to the field.

Results and discussion

When the tips were treated in 0.1% $HgCl_2$ solution for one and two minutes, the cultures were contaminated completely. About 90%-100% of explant was found contamination free when the shoot tips were treated in 0.1% $HgCl_2$ solution for three to eight minutes. So surface sterilization of shoot tips, treatment with 0.1% $HgCl_2$ solution for three minutes duration was found most effective. This concentration and duration of $HgCl_2$ treatment were used by other workers (Das et al., 2001; Banu, 2001; Ahmad, 2002; Nagib et al., 2003; Alam et al., 2004).

Establishment of isolated apical meristem was influenced by the different growth regulators. Liquid culture methods were also used in eggplant and other crops (Balukiewicz and Kryczyski, 2001; Zhang, 2002; Faccioli and Colalongo, 2002; Nagib et al., 2003; Alam et al., 2004). Liquid medium is more conductive than semisolid for quick supply of nutrient to tiny meristem (Banu, 2001; Ahmad, 2002). The cultured apical meristems

Table 2. Effect of different combination and concentration of GA_3 + KIN in MS medium for primary establishment of apical meristems isolated from 30-35 days old field grown plants

Treatment (mg/l)		Days to response			Number of primary established meristem		
		Islampuri	Khatkhatia	Katabegun	Islampuri	Khatkhatia	Katabegun
GA_3	0.01+0.01	12-18	10-17	9-16	14.10	14.28	16.00
+	0.1+0.01	12-18	10-17	10-16	21.50	21.42	22.00
KIN	0.5+0.1	9-16	9-17	9-16	57.40	45.07	50.04
	0.5+0.5	8-16	7-15	7-14	86.72	80.55	78.82
	1.0+0.5	10-18	10-15	9-16	35.71	30.71	37.09
	1.0+1.0	9-18	9-18	9-16	30.61	30.71	35.71
	1.5+1.0	12-18	10-15	10-17	21.42	20.19	18.22
	2.0+1.5	12-17	10-17	12-16	14.00	13.07	12.92

Table 3. Effect of MS_0 and different concentration of BAP, KIN and IBA in MS medium on shoot and root development from primary established meristems. Data was recorded five weeks after inoculation

Treatment (mg/l)		Cultivar	Number of shoot	Number of root	Shoot length (cm.)	Root formation (%)
BAP	0.1	Islampuri	1.10±0.10	-	5.40±0.47	-
	0.5		1.71±0.49	-	8.00±0.62	-
	1.0		2.12±0.41	-	10.00±0.45	-
	2.0		2.20±0.70	-	9.00±0.39	-
	3.0		1.11±0.51	-	7.20±0.32	-
	0.1	Khatkhatia	1.20±0.24	-	3.09±0.08	-
	0.5		1.53±0.13	-	8.12±0.51	-
	1.0		2.11±0.14	-	9.54±0.08	-
	2.0		1.45±0.21	-	9.32±0.25	-
	3.0		1.01±0.91	-	6.90±0.98	-
	0.1	Katabegun	1.12±0.015	-	5.21±0.14	-
	0.5		1.34±0.22	-	8.23±0.76	-
	1.0		2.20±0.21	-	9.80±0.16	-
	2.0		1.42±0.72	-	9.42±0.17	-
	3.0		1.12±0.92	-	6.95±0.29	-
KIN	0.5	Islampuri	1.01±0.79	2.00±0.37	3.21±0.17	12
	1.0		1.17±0.52	1.23±0.42	5.91±0.95	9
	0.5	Khatkhatia	1.15±0.32	3.11±0.24	3.82±0.04	17
	1.0		1.41±0.35	2.50±0.33	4.71±0.46	12
	0.5	Katabegun	1.21±0.28	3.69±0.11	3.50±0.40	20
	1.0		1.50±0.88	3.00±0.29	6.90±0.42	12
IBA	0.5	Islampuri	1.40±0.81	13.60±0.37	6.12±0.34	93
	1.0		1.41±0.71	12.40±0.33	3.44±0.13	97
	0.5	Khatkhatia	1.35±0.27	14.38±0.19	5.10±0.33	95
	1.0		1.10±0.67	10.14±0.31	3.50±0.23	97
	0.5	Katabegun	2.00±0.22	15.21±0.38	4.33±0.52	96
	1.0		1.09±0.58	12.30±0.52	3.61±0.44	96

showed their first growth response by increasing in size and became greenish white in colour. They continued their growth and development of shoot. Among the different concentrations of BAP, 2.0 mg l^{-1} BAP showed better performance than other treatments (Table 1 and 2). Responses of meristem started from six to12 days. About 93.85% (the highest percentage) of meristems of cv. Islampuri in 2.0 mg l^{-1} BAP containing medium started responses after six days of inoculation (Table 1), whereas 14.13% (the lowest percentage) meristem showed responses in case of cv. Katabegun after 12 days of inoculation in media containing 0.1 mg l^{-1} BAP. This result is similar to the results of some other workers (Nagib et al., 2003; Alam et al., 2004). However the use of BAP, KIN, GA_3 + KIN for establishment of primary meristem was also reported by others (Merja and Stasa, 1997; Roksana et al., 2002; Nagib et al., 2003; Alam et al., 2004). Among the three cultivars 'Islampuri' was found the most responsive. Among the three cultivars 46.51% (the highest percentage) of the isolated meristem of cv. Katabegun responded in 0.5 mg/l KIN within 8-16 days. It was found that

Table 4. Effect of different concentration and combination of BAP + NAA, KIN + NAA, GA_3 + KIN in MS medium on shoot and root development from primary established meristems. Data was recorded five weeks after inoculation

Treatment (mg/l)		Cultivar	Number of shoot/plantlet	Number of root	Shoot length (cm.)	Root formation (%)
BAP	1.0+0.1	Islampuri	1.25±0.22	5.20±0.89	5.61±0.02	30
+	1.0+0.5		2.40±0.15	6.31±0.70	6.72±0.82	40
NAA	2.0+1.0		2.81±0.90	8.21±0.49	7.02±0.31	56
	1.0+0.1	Khathatia	1.13±0.27	4.82±0.91	5.94±0.99	45
	1.0+0.5		1.50±0.92	5.90±0.91	6.12±0.97	50
	2.0+1.0		2.00±0.24	7.90±0.98	6.51±0.85	63
	1.0+0.1	Katabegun	1.93±0.75	5.93±0.1	6.13±0.1	42
	1.0+0.5		2.30±0.33	6.03±0.79	6.81±0.09	49
	2.0+1.0		2.50±0.37	9.42±0.86	7.82±0.45	70
KIN	1.0+0.5	Islampuri	1.43±0.96	6.52±0.13	4.60±0.37	42
+	2.0+0.5		2.10±0.18	6.0±0.23	6.82±0.91	65
NAA	2.0+1.0		1.9±0.09	5.71±0.13	6.82±0.66	45
	1.0+0.5	Khatkhatia	1.32±0.83	7.11±0.27	5.32±0.38	40
	2.0+0.5		1.74±0.49	6.21±0.33	8.60±0.26	58
	2.0+1.0		1.5±0.19	5.91±0.94	8.20±0.67	40
	1.0+0.5	Katabegun	1.20±0.72	6.21±0.35	6.10±0.96	38
	2.0+0.5		2.12±0.19	7.21±0.72	7.22±0.43	55
	2.0+1.0		1.93±0.17	5.31±0.10	6.21±0.96	39
GA_3	0.5+0.5	Islampuri	2.20±0.20	4.20±0.30	6.82±0.53	50
+	0.5+1.0		1.05±0.25	2.26±0.15	8.12±0.51	18
KIN	0.5+2.0		1.60±0.24	3.51±0.22	6.31±0.03	10
	0.5+0.5	Khatkhatia	1.35±0.29	2.75±0.35	6.41±0.75	70
	0.5+1.0		2.00±0.50	3.25±0.05	7.90±0.93	75
	0.5+2.0		1.60±0.40	3.15±0.51	6.20±0.18	50
	0.5+0.5	Katabegun	1.75±0.48	3.75±0.05	6.02±0.41	35
	0.5+1.0		1.25±0.25	3.00±0.51	8.21±0.23	20
	0.5+2.0		1.25±0.39	3.00±0.12	7.12±0.32	10

the response of apical meristem was from seven to 12 days in GA_3. About 60.50% (the highest percentage) apical meristem cv. Islampuri started response after 8-16 days of inoculation in 0.5 mg l^{-1} GA_3 containing medium. It was evident that MS_0 (no growth regulators) showed poor result. Results suggested that the use of phytohormone is effective for quick responses and high establishment rate of inoculated meristem. The explants responded well when the excised meristems were cultured in MS liquid medium supplemented with combination of GA_3 and KIN (Table 2). Among the three cultivars of eggplant, about 86.72% of the meristems of cv. Islampuri showed the response after eight days of inoculation in media containing 0.5 mg l^{-1} GA_3 and 0.5 mg l^{-1} KIN. The medium having 2.0 mg l^{-1} BAP was found most effective for primary establishment for meristem and among the three cultivars Islampuri was the most responsive.

After 30-35 days of culture initiation, the primary established meristems formed shoots and roots. The tiny shoots developed from meristems were carefully rescued after 20-25 days of inoculation. MS medium containing 1.0 mg l^{-1} BAP showed the best response for shoot proliferation. In this medium the longest shoot length 10.0 ± 0.45 cm. was noted. Maximum number (2.20 ± 0.21) of shoots/explant was found in media containing 1.0 mg l^{-1} BAP for 'Katabegun' (Table 3). Effect of IBA in shoot proliferation was also notable. Combination of growth regulators was also suitable in shoot proliferation. Combination of 2.0 mg l^{-1} BAP and 1.0 mg l^{-1} NAA gave notable shoot proliferation. In this medium, the longest shoot length (7.82±0.45 cm) and maximum shoot/explant (2.81±0.90) was found in cv. Katabegun and Islampuri, respectively. The result is similar to the previous reports (Novak et al., 1986; Nagasawa and Finer, 1988; Roksana et al., 2002). It was observed that longest shoot 8.60±0.26 cm was found in cv. Khatkhatia in MS medium containing 2.0 mg l^{-1} KIN and 0.5 mg l^{-1} NAA. Combination of GA_3 and KIN was also treated. Cv. Katabegun produced longest shoot length 8.21±0.23 cm when they were cultured on 0.5 mg l^{-1} GA_3 and 1.0 mg l^{-1} KIN.

Shoots (97%) developed roots in 1.0 mg l^{-1} IBA containing medium in cv. Islampuri and cv. Khatikhatia. The highest number 15.21±0.38 of roots/shoot developed when the shoots of cv. Katabegun were cultured

in 0.5 mg l^{-1} IBA containing medium (Table 3). The maximum of 75% shoots induced root in 0.5 mg l^{-1} GA_3 and 1.0 mg l^{-1} KIN containing medium in cv. Khatkhatia (Table 4). The highest root formation was 96% in cv. Khatkhatia and cv. Katabegun in IBA containing medium. Considering all the treatments, 1.0 mg l^{-1} IBA was recommended for proper root development from primary established meristems (Table 3). For proper shoot and root development from meristem derived shoot use of same media was reported effective (Koppel and Butenko, 1992; Venkatahalam, et al., 2000; Zaman et al, 2001; Nagib et al., 2003; Alam et al., 2004). Langhe and Buijne (1993) also reported that 0.5 mg l^{-1} IBA was optimum for shoot elongation and root induction in *Musa* spp.

Conclusions

The present investigation was undertaken with a view to develop a standard method for establishment of meristem culture and subsequent regeneration in eggplant cultivars of Bangladesh. In order to raise meristem derived virus free eggplant clones, shoot tips were collected from 30-35 days old field grown plants. Among different hormonal treatments in MS liquid medium, 2.0 mg l^{-1} BAP was proved to be best medium for primary establishment of meristem culture in all the cultivars. Best shoot development was found in cv. Islampuri containing MS semisolid medium supplemented with 2.0 mg l^{-1} BAP and 1.0 mg l^{-1} NAA. Rooting of meristem derived shoots was found best by using MS medium containing 1.0 mg l^{-1} IBA in both cv. Islampuri and cv. Khatkhatia. The successful acclimatization of the *in vitro* grown plantlets proved the validity of the developed protocol of using biotechnological techniques for improvement of eggplant.

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