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Original article

Regeneration efficiency based on genotype, culture condition and growth regulators of eggplant (*Solanum melongena* L.)

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ABSTRACT

Several experiments were carried out to establish an efficient regenerating protocol for cultivated eggplant varieties. Among the five varieties cultured on Murashige and Skoog (MS) medium with free plant growth regulator (PGR), *Nayantara* performed better considering the number of shoots/explant (2.48). Considering explant types and culture conditions, better performance was observed (3.68 shoots/explant) when seed germination in the dark was proceeded by bottom hypocotyl segments cultured under dark conditions. A higher rate of shoot regeneration was observed in *Nayantara* when cultured in Zeatin Riboside (ZR) and Thidizuron (TDZ) supplemented MS medium. The highest number of shoots per explant was produced on MS medium supplemented with 2.0 mg/L ZR and 0.1 mg/L indole acetic acid (6.65 shoots/explant). Proliferation and elongation of the regenerated shoots were obtained in the MS medium with free PGR. The best rooting performance was observed in MS medium supplemented with 2.0 mg/L indole butyric acid. Plantlets with well developed roots and shoots were successfully transferred to soil.

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Introduction

Eggplant (*Solanum melongena* L.) (2n = 24), is an important vegetable crop of tropical and temperate regions and is a popular and principal vegetable crop in Bangladesh where it is the second most important vegetable crop after potato in terms of area under cultivation (Yearbook of Agricultural Statistics of Bangladesh, 2007). The crop is susceptible to several diseases (*Phomopsis* blight, southern wilt, anthracnose) and pests especially brinjal shoot and fruit borer that causes serious yield losses, but efforts to overcome these problems, using hybridization with wild *Solanum* species are limited by sexual incompatibilities (Collonier et al., 2001; Kashyap et al., 2003). In addition, traditional improvement methods are hampered by the scarcity of natural resistance sources. Introgression of desired traits such as parthenocarpy, improved nutritional value and post harvest qualities into the cultivated varieties is difficult to achieve due to the lack of appropriate sexually compatible varieties or species (Collonier et al., 2001).

Eggplant tissues show a high morphogenetic potential under *in vitro* culture conditions that could be useful for developmental studies as well as for establishing biotechnological approaches to produce improved varieties. Organogenesis from various explants such as leaf, cotyledon, hypocotyl, epicotyl, anther and isolated microspores has been reported previously (Isouard et al., 1979; Gleddie et al., 1983; Sharma and Rajam, 1995; Magioli et al., 1998). Furthermore, culture conditions affecting regeneration via organogenesis (Mukherjee et al., 1991) have been well documented. In those protocols, the regeneration efficiency has been reported to be affected by different factors, such as the combination of plant growth regulators, explant type and genotype. Such variability in the tissue culture response necessitates the optimization of culture protocols for particular, elite varieties in different geographical regions. Bangladesh being one of the centre of origins of this crop, having available efficient protocols for *in vitro* regeneration and genetic transformation will offer an excellent model system to investigate plant physiology *in vitro* and pave the way for the development of pest resistance and stress tolerance in this crop.

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Materials and methods

Plant materials

Seeds of five eggplant varieties—*Nayantara, Kazla, Islampuri, ISD-006* and *Uttara*—were collected from the Horticulture Research Center, Bangladesh Agricultural Research Institute, Gazipur. Seeds were first dipped into 100% alcohol for 20–30s followed by washing four times with sterile distilled water. After that Tween 20 (3–5 drops) was added into 100 mL 40% Clorox (2% sodium hypochlorite) and shaken gently. Seeds were dipped in a 50 mL conical flask containing the prepared solution for 20 min with frequent shaking followed by washing with sterile distilled water four times. Finally, 25–30 seeds were inoculated in each baby jar containing 30 mL agar-solidified MS medium with 3% sucrose for supporting seedling development. Culture conditions were maintained by germinating the seeds both in the dark and in the light followed by incubation in the dark and light according to the experimental objectives. After seed germination, incubation was maintained for 2 wk for both cases. The explants from the dark culture treatment were transferred to light after 2 wk to allow proper growth of the regenerated shoots.

Effects of genotypes on organogenesis

The first experiment evaluated genotypic variation among genotypes. Five varieties of eggplants—*Nayantara*, Kazla, Islampuri, ISD-006 and Uttara—were tested for their innate ability to regenerate multiple shoots on growth-regulator-free, MS medium using hypocotyl explants on the basis of a previous observation that hypocotyl segments of eggplant can produce shoots on growth-regulator-free, MS medium (Akhond and Bhuiyan, 2001). This experiment had four replications with nine explants plated for each replication. According to the results, *Nayantara* was the best variety to produce maximum effective shoots. Therefore, *Nayantara* was used in the subsequent experiment.

Effects of dark pretreatment and type of explant

This experiment aimed to determine the dark pretreatment effect (both in seedling raising and culturing) and to study the morphogenetic response of different regions of the hypocotyl in MS medium. Seeds of *Nayantara* were germinated under dark and light conditions and cultured under both dark and light conditions. In the dark treatment, after 2 wk of dark, the germinants were transferred to light conditions. The samples under the light conditions received normal culture as described below. Each hypocotyl was cut into 6–8 mm long top, middle and bottom segments that were cultured on growth-regulator-free, MS medium to determine the suitable culture conditions as well as the suitable explant type. Responses of bottom hypocotyl segments were found to be better compared to the top and middle parts. Furthermore, seed germinated in the dark followed by culture under dark conditions was found to be best among the treatments. This experiment had four replications with nine explants plated for each replication.

Regeneration response against plant growth regulator

This experiment was carried out to determine the effects of growth regulators on adventitious shoot development. Treatments were composed of PGR-free, MS medium, naphtaleneacetic acid (NAA; 0.0, 0.5 and 1.0 mg/L), 6-benzylaminopurine (BAP 0.0, 2.5 and 5.0 mg/L), thidizuron (TDZ; 0.1 and 0.2 mg/L), zeatin riboside (ZR; 1.0 and 2.0 mg/L) and indole acetic acid (IAA; 0.1 mg/L) either singly or in combinations. The combinations of these five PGR are shown in Table 3. Each treatment contained nine explants with four replicates.

Table 1
Regeneration efficiency of different eggplant varieties on MS medium.

Cultivar	Responsive explant (%)	Number of shoots/ explant	Rooted shoots (%)
Kajla	80.67^{ab}	1.63^{bc}	79.17^{ab}
Nayantara	84.67^{a}	2.48^{a}	95.00^{a}
ISD-006	67.00^{c}	2.05^{ab}	100.00^{a}
Islampuri	70.33^{bc}	1.27^{c}	60.00^{b}
Uttara	83.00^{ab}	1.47^{bc}	77.67^{ab}

Means followed by different letters within a column are significantly different at $p < 0.05$ (Duncan, 1955).

Table 2
Effect of culture condition and explants type on shoot formation of eggplant variety Nayantara

Germination conditions	Culture conditions	Number of shoots/explant		
		Top hypocotyl	Mid hypocotyl	Bottom hypocotyl
Dark	Dark	1.30	1.25	3.68
	Light	1.66	1.00	1.95
Light	Dark	1.00	1.20	1.74
	Light	1.00	1.00	1.48

Table 3
Effect of various concentration and combination of NAA, BAP, ZR, TDZ and IAA on callus, shoot and root formation of eggplant var. Nayantara using hypcotyl.

MS supplemented with growth regulators (mg/L)	Explant producing root (%)	Explant producing shoot (%)	Explant producing callus (%)	Number of shoots/ explant
NAA + BAP				
0.0 + 0.0	100.00^{a}	76.00^{a}	27.63+	1.40^{f}
0.0 + 2.5	0.00^{c}	24.00^{d}	100.00++	1.58^{e}
0.0 + 5.0	0.00^{c}	52.33^{c}	100.00++	1.42^{f}
0.5 + 0.0	0.00^{c}	100.00^{a}	100.00++	2.53^{b}
0.5 + 2.5	0.00^{c}	0.00^{e}	100.00++++	0.00^{g}
0.5 + 5.0	0.00^{c}	0.00^{e}	100.00++++	0.00^{g}
1.0 + 0.0	0.00^{c}	57.33^{c}	100.00++	2.20^{d}
1.0 + 2.5	0.00^{c}	0.00^{e}	100.00++++	0.00^{g}
1.0 + 5.0	24.00^{b}	0.00^{e}	100.00++++	0.00^{g}
ZR + IAA				
1.0 + 0.1	100.00^{a}	75.33^{b}	100.00++	2.27^{c}
2.0 + 0.1	6.33^{c}	100.00^{a}	100.00+++	6.65^{a}
TDZ + IAA				
0.1 + 0.1	0.00^{c}	100.00^{a}	100.00+	2.55^{b}
0.2 + 0.1	0.00^{c}	95.33^{a}	100.00++	2.49^{b}

NAA = naphtaleneacetic acid; BAP = 6-benzylaminopurine; ZR = zeatin riboside; TDZ = thidizuron; IAA = indole acetic acid.
Means followed by different letters within a column are significantly different at $p < 0.05$ (Duncan, 1955).
+ = poor, ++ = moderate, +++ = good, ++++ = very good.

Root development and plant establishment

For root induction, half-strength MS medium supplemented with different concentrations of IBA (0.0, 0.5, 1.0 and 2.0 mg/L) were used. Regenerated shoots from 2.0 mg/L ZR in combination with 0.1 mg/L IAA-treated media were carefully removed from the baby jar/Petri dish and each shoot was cut from the bottom end and transferred into growth-regulator-free, MS medium. Shoots were kept in this medium for up to 4 wk to allow sufficient elongation and to reduce any possible adverse effects of the growth regulators during regeneration. Shoots of 2.5–4.0 cm length were excised and cultured in rooting medium. The regenerated rooted plantlets at the 5–6 leaf stage were transferred from the culture room and kept at room temperature (28–30 °C) for 5 d. The plantlets were then

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