Micropropagation of black turmeric (C*urcuma caesia* Roxb.) through *in vitro* culture of rhizome bud explants

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Abstract

In the present study, preliminary *in vitro* shoots propagation of *Curcuma caesia* Roxb. was investigated. Rhizome buds were used as explants and were cultured on Murashige and Skoog (MS) medium containing 6-Benzyl adenine (BA) alone or in combination with α- Naphthalene acetic acid (NAA). The results showed that the optimum shoot proliferation was obtained from MS medium containing 3.0 µM BA + 0.5 µM NAA. In this growth regulator combination, maximum 99.97 % explants produced 10.38 shoots with 4.53 cm length after 8 weeks of culture. Although spontaneous rooting was observed after 4 weeks of cultivation with all treatments using half strength MS medium containing IBA and NAA at different concentrations, high frequency of rooting (89.76 %) was obtained in 3.0 µM IBA (Indole-3-butyric acid) containing medium. The plantlets, thus developed, were hardened and successfully established in natural soil.

Keywords: black turmeric, *Curcuma caesia* Roxb, *in vitro* culture, micropropagation, rhizome bud explants

Introduction

Black turmeric (*Curcuma caesia* Roxb.) is an important medicinal plant belonging to zingiberaceae family. Although this plant is native to North-East and Central India, this is also found in Bangladesh as a wild species. The rhizomes of black turmeric have a high economical importance owing to its putative medicinal properties. Rhizome of this plant is claimed to be useful in treating several disease like piles, leprosy, bronchitis, asthma, cancer, epilepsy, fever wounds, impotency, fertility tooth ache and vomiting etc (Flowers of India, 2005). Presently this plant is considered to be a threatened species since natural habitat is destroying widely through several human activities such as overexploitation of black turmeric for traditional medicine purposes, industrialization, urbanization etc. Nowadays, in vitro cell and tissue culture methodology is widely used as a mean for germplasm conservation to ensure the survival of endangered plant species, rapid mass propagation for large-scale revegetation, and for genetic manipulation studies (Nalawade et al., 2003). The present study, therefore, was carried out with a view to develop an effective *in vitro* method for clonal propagation of *C. caesia* using rhizome bud explants.

Materials and Methods

The stock plants for this study were collected from the naturally grown plant population of *C. caesia* in the botanical garden of Rajshahi University, Bangladesh. Rhizome buds about 1 to 2 cm long were selected as the initial explants. The fresh buds collected were cleaned of soil dirt and left under running tap water for 30–40 minutes. Then the buds were immersed in 75% (w/v) ethanol for one minute before transferring them into laminar air flow cabinet. Explants were then surface sterilized with 0.1% $HgCl_2$ for 10 minutes and washed thoroughly 3 to 4 times with sterile distilled water and soaked with sterile blotting paper under laminar air flow cabinet. The medium used was the MS (Murashige, Skoog, 1962) medium, gelled with 8.0g/L Agar and 30% sucrose as car-bon source. The pH was adjusted to 5.7 -5.8 with 1N KOH or 0.1N HCl prior to autoclaving at 1.2 kg*cm^2 pressure and 121°C temperature for 15–20 minutes. Rhizome buds were inoculated onto the medium fortified with 0.0, 1.0, 3.0, 5.0, 7.0 and 9.0 µM BA alone or 1.0 and 3.0 µM BA in combination with 0.5 and 1.5 µM NAA. For adventitious rooting, *in vitro* derived shoots were cultured onto half strength MS medium containing NAA and IBA at different concentrations (0.0, 1.0, 3.0, 3.5 4.0 and 4.5 µM). The cultures were illuminated with white fluorescent light (3000 lux), at 16 h photoperiod with temperature of 25 ±2 °C. The cultures were maintained by regular subcultures at 2 weeks intervals on fresh medium with the same compositions. Well-rooted plantlets were removed from the culture tubes and the roots were washed under running tape water to remove agar trace. Then the plantlets were transferred to small plastic pots containing autoclaved garden soil and compost (1:1) and maintained inside growth chamber set at temperature 28 °C and 70–80% relative humidity. After four weeks they were kept in greenhouse before transferring them to the Botanical Evaluation Garden of Plant Tissue Culture Laboratory finally. Ten to fifteen cultures were used per treatment and each treatment of rooting experiment was repeated thrice. The data on axillary shoot proliferation were recorded after 8 weeks of culture whereas the data on adventitious rooting were recorded after 6 weeks of culture. The data on percentage of shoots rooted and mean number of roots per explant were statistically analyzed by Duncan's multiple Range Test (DMRT) (Gomez, Gomez, 1976).

Results and Discussion

Figure 1 represents the successful results for axillary shoot proliferation from rhizome bud explants. Excised rhizome buds were cultured on MS medium supplemented with different concentrations (0.0 to 9.0 µM) of BA alone or 1.0 and 3.0 µM BA in combination with 0.5 and 1.5 µM NAA. Higher number of multiple shoot induction was observed after 8 weeks of culture and significantly higher number of shoots were found in BA + NAA combinations except for BA alone treatment (Fig. 1). Out of four different BAA + NAA combinations, rhizome buds showed highest performances in 3.0 µM BA + 0.5 µM NAA containing medium (Fig. 2. A & B**)** in terms of three parameters tested- response (%), number of shoots/culture and length of shoots/culture (cm). Bharalee et al. (2005) also found 4.0 mg*l BA + 1.5 mg*l NAA as best growth regulator combination for shoot multiplication of *C. caesia*. In addition, similar results were reported in *C. amada* (Ferdous et al., 2012, Prakash et al., 2004), *C. longa* (Islam et al., 2004, Kambaska et al., 2010) and *Zingiber officinale* (Hoque et al., 1999).

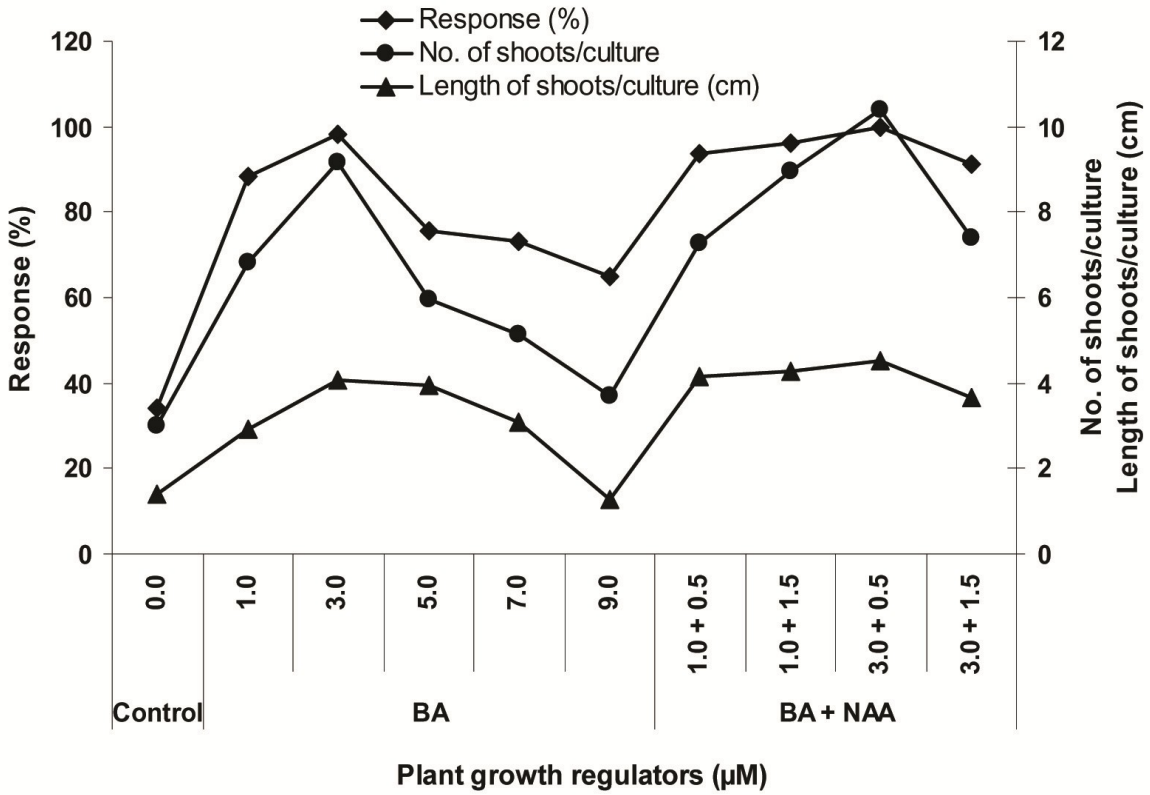

Figure 1. Effect of plant growth regulators on multiple shoot proliferation from rhizome bud explants of *C. caesia* Roxb.

The well developed shoots were excised from shoot clumps and transferred to root induction medium- half strength MS medium containing NAA and IBA alone at different concentrations (0.0, 1.0, 3.0, 3.5 4.0 and 4.5 µM). NAA was found to be best auxin over IBA for *in vitro* rooting (Table 1). Maximum 89.76 ±3.79 % shoots produced 9.73 ±1.07 roots when cultured them onto 3.0 µM IBA containing medium. Higher concentration of IBA than 3.0 µM showed low frequency of root induction. NAA was also reported as best auxin for rooting in *C. longa* (Salvi et al., 2000 & 2001) and in *Z. officinale* (Inden et al., 2003). Well-developed rooted plantlets were transferred to small plastic pots (Figure 2 C) and maintained in controlled growth chamber conditions. Finally, the developed plantlets were kept in greenhouse and transferred to the field condition at Botanical Evaluation Garden of Plant Tissue Culture Laboratory. The survival rate of acclimatized plantlets under field condition was 68%.

Table 1. Effect of two auxins (NAA and IBA) on rooting of *in vitro* regenerated micro-shoots of *Curcuma caesia* Roxb.

	Auxin concentration (μM)	Shoots rooted (%)	Mean no. of roots/explant
Control	0.0	27.13 ±1.09	3.19 ±0.79
	1.0	38.78 ±1.37	3.98 ±0.68
NAA	3.0	58.91 ±2.17	5.29 ±0.63
	3.5	78.54 ±2.23	5.73 ±0.59
	4.0	84.39 ±2.57	7.23 ±0.83
	4.5	69.94 ±1.63	6.49 ±0.34
	1.0	71.28 ±2.37	4.37 ±0.28
	3.0	89.76 ±3.79	9.73 ±1.07
IBA	3.5	73.96 ±2.59	8.34 ±0.47
	4.0	68.24 ±2.87	8.12 ±0.37
	4.5	59.19 ±1.76	5.29 ±0.71

Values represent means ± standard error of 10–15 explants per treatment in three repeated experiments. Values with the same superscripts are not significantly different at 5% probability according to DMRT.

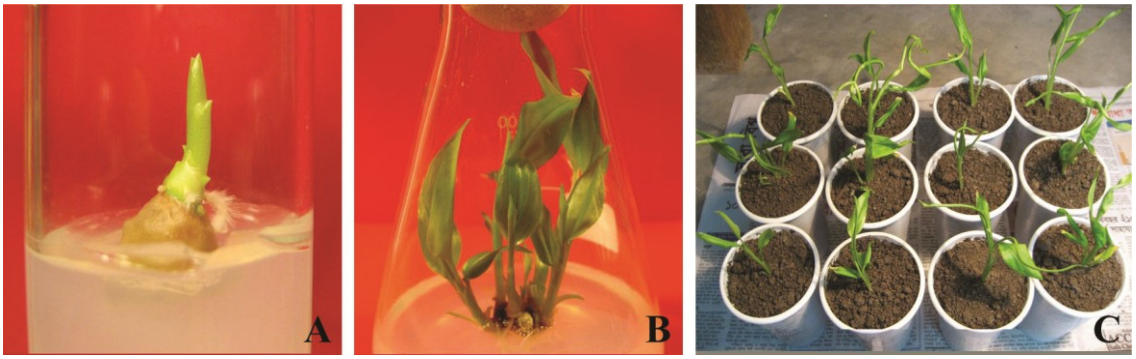

Figure 2.

A. Shoot bud initiation from rhizome explants after 15 days of culture
B. Proliferated shoot clump after 8 weeks of culture
C. Transplanted plantlets onto soil and compost mixture in a small plastic pot

Conclusions

In conclusion, the micropropagation protocol described here was established from rhizome bud explants of *C. caesia*. This efficient micropropagation protocol will be surely useful to produce a higher amount of propagule around the year, thus, this protocol could also be used for large scale multiplication of disease free planting materials and conservation of black turmeric.

Acknowlwdgement

Authors are grateful to Dr Muhammad Nurul Amin and Dr Mustafa Abul Kalam Azad, Plant Tissue Culture Laboratory, Department of Botany, Rajshahi University, Rajshahi-6205, Bangladesh for providing all kinds of laboratory facilities and constant encouragement during the research.

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