In vitro propagation of *Homalomena aromatica* Schott., an endangered aromatic medicinal herb of Northeast India

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Abstract A successful report on the *in vitro* propagation of *Homalomena aromatica* via rhizome axillary bud multiplication is presented. Rhizome bud explants were cultured on Murashige and Skoog medium supplemented with various concentrations of cytokinins to induce multiple shoot formation for micropropagation. The highest number of shoots was achieved in MS medium supplemented with 2.0 mg l^{-1} 6-benzylaminopurine. The regenerated shoots rooted most efficiently on half-strength MS medium supplemented with 0.5 mg l^{-1} α -naphthalene acetic acid. The regenerated plantlets showed no morphological differences from the parent plant. This protocol takes approximately 6 months to reach the acclimatization stage from the initiation stage and facilitates commercial and rapid propagation of *H. aromatica*.

Keywords Cytokinins · *Homalomena aromatica* · *In vitro* propagation · Multiple shoots · Rhizome axillary bud

Introduction

Homalomena aromatica Schott. (Araceae), commonly called ‘Sugandhmantri’ is a rhizomatous aromatic perennial herb commonly found in Assam and adjoining states of the sub-Himalayan regions of India. Its aromatic rhizomes contain an essential oil which is extensively used in perfumery and cosmetic industries. The exhausted material after extraction of essential oil is largely used in *Dhup* manufacturing. Besides having aromatic value, the plant also possesses medicinal properties. In Northeast India, leaves and rhizomes are

commonly use in traditional medicine for treating joint-pain and skin infections (Kar and Borthakur 2008). The plant possesses many pharmacological properties such as analgesic, antidepressant, anti-inflammatory, antiseptic, antispasmodic, sedative, antifungal and insecticidal (Singh et al. 2000; Policegoudra et al. 2012).

At present, production of sugandhmantri oil is completely dependent on the commercial collection of wild and a few cultivated plants. Due to large-scale and indiscriminate collection of plants directly from the naturally growing plants, *H. aromatica* has now become endangered (Ved et al. 2003). As conventional propagation method through rhizome axillary buds is time consuming and provides a limited number of propagules, it is necessary to promote rapid production of *H. aromatica* through tissue culture techniques for its commercial availability and conservation. Tissue culture has been an effective technique to propagate endangered medicinal plants (Pence 2010). Despite the increasing commercial demand for *H. aromatica*, there are no reports for *in vitro* propagation of this plant. The present study was undertaken to develop a suitable protocol using rhizome axillary buds *in vitro* for its rapid multiplication. To our knowledge, this is the first report of *in vitro* propagation of *H. aromatica*.

Materials and methods

H. aromatica plant was collected from Research and Demonstration Farm, Department of Land Resource Development, Government of Nagaland, Ruziephema village, Nagaland and maintained in the glass house of Plant Biotechnology Laboratory, Department of Botany, North-Eastern Hill University, Shillong, India. Sections of the rhizomes containing buds were dissected and washed in detergent for 10 min followed by continuous washing under

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running tap water for 1 h after which the explants were pre-treated with fungicide, bavistin (1 %) for 15 min. After three sterile distilled water rinses, explants were surface-disinfected in 70 % ethanol (v/v) for 1 min, rinsed in sterile distilled water, then soaked in 0.1 % mercuric chloride (w/v) solution for 8 min and finally rinsed thoroughly with sterile distilled water five times. Damaged tissue surrounding the buds was cut off and the rhizome buds were cultured aseptically on nutrient medium.

Rhizome buds were cultured on Murashige and Skoog's (1962) basal medium (MS) supplemented with 0.5 mg l^{-1} 6-benzylaminopurine (BAP) for induction of multiple shoots to raise *in vitro* explants. The *in vitro* plantlets measuring 1.5 to 2 cm were separated individually and subcultured on MS medium supplemented with various cytokinins for shoot induction and multiplication. The medium contained 3 % sucrose (w/v) and after adjusting the pH to 5.8, 0.8 % agar (w/v) was added and sterilized by autoclaving at 105 kPa at 121 °C for 15 min. At least 12 replicates were taken for each treatment. Shoot induction experiment was carried out in culture tubes but further sub-culturing for shoot proliferation was done in 500 ml conical flasks containing 50 ml of medium.

To establish the optimum treatment for multiple shoot induction, the *in vitro*-raised shoots were used as explants and further inoculated onto MS medium containing 0.0–4.0 mg l^{-1} of either of BAP, kinetin (Kn) or thidiazuron (TDZ). For further shoot multiplication, explants with developed shoots were transferred every 4 weeks to fresh medium of same composition. Number of shoots per explant and average shoot length were recorded at every 4 weeks after sub-culturing to fresh medium twice. Cultures were

incubated at 25 ± 2 °C under cool white fluorescent light ($60 \mu\text{mol m}^{-2} \text{ s}^{-1}$) with a photoperiod of 14 h.

Individual regenerated shoots (3–4 cm long) were excised from the shoot clump and transferred to half-strength MS medium fortified with α -naphthalene acetic acid (NAA), indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) at the concentrations of 0.25, 0.5 or 1.0 mg l^{-1} for root initiation. Numbers of roots were counted after 8 weeks of transfer to root induction medium. Well-rooted shoots were washed with water to remove traces of agar and transferred to plastic pots containing a sterilized mixture of soil and compost (2:1). The potted plants were irrigated with 1/4 strength MS basal salt solution devoid of sucrose every 7 d for 4 weeks. The plantlets were kept in a glass house for acclimatization before being transferred to the field.

The data on different parameters was subjected to statistical analysis by ANOVA and the means were compared using student's *t*-test. Each treatment had 12 replications with one explant per culture tube. The experiments were repeated three times, and the values used for statistical analyses were the means obtained from three experiments.

Results and discussion

Sprouting of axillary buds (Fig. 1a) followed by shoot initiation (Fig. 1b) from the basal part of the rhizome buds was observed within 20 d in medium containing 0.5 mg l^{-1} BAP. Due to serious contamination problem (about 70 % contamination), rhizome bud was not used for shoot multiplication. Instead, the induced *in vitro* shoots from the rhizome buds were used in the proceeding experiment in

Fig. 1 *In vitro* multiplication of *H. aromatica*. Bud break from a rhizome bud (a); *in vitro* shoot development (b); multiple shoot formation on MS medium supplemented with 2 mg l^{-1} BAP (c); plantlets rooted on half-strength MS medium supplemented with 0.5 mg l^{-1} NAA (d); plantlets kept in pots for hardening (e) and; *in vitro* raised plantlets in field after 1 year (f). The bar represents 1.0 cm in a–d, and 13.0 cm in e

Table 1 Effect of cytokinins on shoot multiplication of *H. aromatica*, data recorded after 8 weeks of culture

Plant growth regulators (mg l ⁻¹)			Frequency of responding cultures (%)	Number of shoots per explant	Shoot length (cm)
BAP	Kn	TDZ			
0.5			88.83±2.76 ^b	5.4±0.8 ^{de}	2.41±0.2 ^{ab}
1.0			97.20±2.80 ^a	8.1±0.7 ^{cd}	2.23±0.2 ^{abc}
2.0			97.20±2.80 ^a	22.7±0.7 ^a	1.99±0.2 ^{abcd}
4.0			94.40±2.80 ^{ab}	7.8±0.8 ^{cd}	1.57±0.2 ^{bcd}
	0.5		88.83±2.76 ^b	3.1±1.0 ^e	2.48±0.1 ^{ab}
	1.0		97.20±2.80 ^a	3.9±0.7 ^e	2.27±0.2 ^{abc}
	2.0		94.40±2.80 ^{ab}	5.1±0.8 ^{de}	2.64±0.2 ^a
	4.0		88.83±2.76 ^b	3.0±1.0 ^e	2.51±0.2 ^{ab}
		0.5	44.40±2.80 ^c	12.3±0.7 ^b	0.9±0.09 ^{cd}
		1.0	30.53±2.76 ^d	20.2±0.7 ^a	0.6±0.1 ^d
		2.0	30.53±2.76 ^d	22.5±0.7 ^a	0.6±0.1 ^d
		4.0	27.76±2.76 ^d	9.6±0.8 ^{bc}	0.5±0.1 ^d

Values are means ± standard error; mean values followed by different superscript letters within a column are significantly different ($P \leq 0.05$) according to student's *t*-test

order to decrease wastage of *ex vitro* buds and to obtain sufficient number of explants. Bud proliferation initiated at the base of all the explants grown in MS medium supplemented with different concentrations of cytokinins. In the absence of cytokinins, no multiple shoot initiation was observed. The effect of cytokinins on shoot multiplication varied depending upon the kind and concentration of cytokinins used. Of the three cytokinins tested, maximum number of shoots (22.7±0.6) were obtained on medium containing 2 mg l⁻¹ BAP (Table 1 and Fig. 1c). The promotory effect of BAP on multiple shoot induction has been reported earlier in other medicinally important plant species such as *Zingiber officinale* (Balachandran et al. 1990), *Piper* spp. (Bhat et al. 1995), *Houttuynia cordata* (Handique and Bora. 1999), and *Curcuma longa* (Rahman et al. 2004). The number of shoots and length of shoots declined with an increase in BAP concentration beyond the optimal level (Table 1). Similarly, Sivanesan and Jeong (2007) also

observed that multiple shoots decreases beyond 2 mg l⁻¹ BAP in *Sida cordifolia*.

Though both Kn and TDZ effected shoot formation, they were found to be inferior to BAP (Table 1). In Kn containing medium, the number of shoots were greatly reduced compared to BAP. The inferiority of Kn to BAP has been reported in other plants such as *Chlorophytum borivilianum* (Purohit et al. 1994) and *Hibiscus cannabinus* (Herath et al. 2004). Though TDZ had similar effect as BAP with more or less the same number of shoot production, the explants with the initiated shoots in TDZ containing medium turned brown after shoot initiation and died with no further development. The deleterious effect of continued presence of TDZ on growth and multiplication has been reported in *Rauvolfia tetraphylla* (Faisal et al. 2005) and *Capsicum annum* (Ahmad et al. 2006).

Half-strength MS medium was found to be more effective for rooting compared to full-strength MS medium in

Table 2 Effect of different concentrations of auxins on root induction from *H. aromatica* cultured on half-strength MS medium. Data recorded after 8 weeks of culture

Plant growth regulators (mg l ⁻¹)			Frequency of responding cultures (%)	Number of roots per shoot	Root length (cm)
IBA	IAA	NAA			
0.0	0.0	0.0	98.1±1.8 ^b	10.6±0.4 ^{cde}	1.8±0.2 ^c
0.2			99.0±0.9 ^a	8.3±0.6 ^{de}	2.9±0.2 ^{ab}
0.5			100 ^a	10.6±0.6 ^{cde}	2.4±0.2 ^{abc}
1.0			100 ^a	10.6±0.4 ^{cde}	3.4±0.3 ^a
	0.2		100 ^a	7.3±0.6 ^e	1.6±0.2 ^c
	0.5		100 ^a	12.3±0.4 ^{bc}	1.3±0.1 ^c
	1.0		100 ^a	18.1±0.7 ^a	1.5±0.2 ^c
		0.2	100 ^a	10.8±0.6 ^{cd}	1.7±0.2 ^c
		0.5	100 ^a	16.0±0.5 ^a	2.2±0.2 ^{bc}
		1.0	97.2±2.8 ^b	15.0±1.1 ^{ab}	1.8±0.2 ^c

Values are means ± standard error; mean values followed by different superscript letters within a column are significantly different ($P \leq 0.05$) according to student's *t*-test

many of the species (Hu and Wang 1983) and so was employed in this study. The frequency of rooting response was high in all the treatments and also in medium without a growth regulator (Table 2). The ease of root formation on auxin-free medium may be due to the availability of endogenous auxin in the shoots *in vitro* (Minocha 1987). Highest number of roots (18.1 ± 0.7) as well as root length (3.4 ± 0.3 cm) was achieved in half-strength MS medium supplemented with 1.0 mg l^{-1} IAA and 1.0 mg l^{-1} IBA respectively. However, the roots formed in this concentration were thin and unhealthy, which were not suitable for pot transfer. Healthier roots were observed in medium containing 0.5 mg l^{-1} NAA (Table 2 and Fig. 1d). Plantlets obtained in medium supplemented with NAA treatment survived better than IAA and IBA during acclimatization in the green house (data not shown). Approximately 90 % of the regenerated plantlets survived and showed vigorous growth without any apparent morphological variations (Fig. 1e). Through this technique, about 200 plantlets were produced from a single rhizome bud in a year which is not possible through conventional cultivation. To our knowledge, this study reports for the first time, a successful yet simple method for *in vitro* propagation of *H. aromatica*. Using this protocol, it could be possible to propagate this plant species on large scale within a short period of time, thus contributing immensely towards providing raw materials in the perfumery and pharmaceutical industries and its conservation.

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