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In vitro Micropropagation of *Homalomena aromatica* (Roxb.)Schott - A Non Timbre Medicinal and Aromatic Forest Plant of North East India and its Conservation.

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Abstract

Homalomena aromatica (Roxb.)Schott (Araceae), locally called *Sugandhmantri*, is one of the commercially important plants with high demand and exploited from natural habitats in Northeastern region of India. This rhizomatous herb grows naturally in the foot hills as well as in plains of the region. The Montria oilusuallyextracted from its underground parts which contains mostly Linaloolwith traces of Terpineol, Linalool acetate, Limonene, Geraniol and δ-Cadenene. Due to gradual habitat lost for the plant, the availability of raw material for aromatic and perfumery industries during recent years has became a problem .To ensure the availability of this plant for steady supply of raw material for above mentioned industries it becomes imperative to undertake commercial cultivation of the species through Micropropagation.Rhizome bud explants were cultured on Murashige and Skoog medium with cytokinin concentrations to induce multiple shoot formation for micropropagation.The highest number of shoots regenerated under MS medium supplemented with 2.0 mg/l BAP. The regenerated plantlets showed no morphological differences with parent plants. The plantlets were acclimatised with 90% survival rate. The protocol facilitates to constant supply of raw material to both medicinal and aromatic industries and also to conserve the plant in its natural habitat.

Key words: *Homalomena aromatica*,micropropagation, multiple shoots, conservation,

Introduction

The family Araceae is mostly abundantly represented by the genus *Homalomena*which comprises approximately 120 species. Most of the *Homalomena* species are distributed in South and Central Asia, the Mexican lowlands, the Caribbean islands,

Southern Florida and Asian tropical areas (Hay & Herscovitch, 2002; Bown, 2000). Most of the*Homalomena* Species are found in tropical climates. However, many aroids, including *Homalomena*species, are native to temperate climates (Mayo et al, 1997). Species of the genus *Homalomena*have used for traditional medicinal purposes. *H. coerulescens* was used to treat various kinds of skin diseases. *H. graffithi* was found to accelerate child birth and relieve backache. *H. purpurascens* was used to relieve hoarseness, while *H. Sagittifolia* was reported to treat fever and abdominal flatulence. *H. occulta* was commonly used to relieve pain and edema due to traumatic injury (Sulaiman & Mansor, 2002; Bensky& Gamble, 1993). The oil extracted from *H. aromatica* showed significant antifungal activity against *Fusarium graminearium* (Singh *et al,* 2000). A few species, such as *H. philippienensis* and *H. rubescens* are used as ornamental plants.

Among the commercially exploited plants from natural habitats in North-Eastern Region of India, *Homalomena aromatica* (Roxb.)Schott is one of them.. The rich floristic diversity of N.E. India and its uniqueness provide tremendous scope for botanical investigation. During the course of botanical investigation it has been found that among the commercially exploited plants from natural habitats from this region Sugandhmantri is on high demand, which occurs in restricted population in small pockets of its limited habitat. (Khan *etal*,2010).The rhizome of *Homalomena aromatica* is partly subterranean, the aerial portion is clothed with the old withered leaf-sheaths with numerous long, white fibrous cords arising from every parts. The plant is slow growing and attains a height of 30 – 60 cm in wild habitat. Leaves are crowned at the tip of stem andwith sheathing bases, long petiole, lamina sagittate-cordate. The plant flowers during May to August. The essential oil obtained from its rhizome is used for blending of most oriental perfumes. The plants generally grow on forest covered hill slopes (tilla -land) and foothill areas and amongst other types of vegetation. It grows best under 40 – 60 % shade, high humidity (200 to 300 cm annual rainfall), sandy and sandy loam to clay loam soil with organic litter with 4.9 to 5.5 soil pH. Temporary water logging area for 24 – 48 hours is beneficial for quick rhizome elongation.

The dry rhizomes are known in trade as **"**Sugandhmantri".The large rhizomes bearing withered leaf scales and numerous white rootlets are esteemed as an aromatic stimulant.(Anonymous.1959). Its aromatic rhizomes contain an essential oil used for blending of most oriental perfume. The essential oil has a very good demand in perfumery and cosmetic industries. It is mainly used for the extraction of aromatic oil which is used as stimulant and in perfumery. The spent material after extraction of essential oil largely used in Dhup manufacturing (Ahmed, 2005***).*** 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).

Due to increasing exploitation and extraction from the wild habitat, the species is becoming scarce and rapidly depleting from its natural habitat.400 MT of dry rhizomes of Homalomena aromaticaare collected and transported every year to outside the state mainly to Kanauj, Kanpur (U.P.), Delhi, Kolkata, Mumbai etc. from Barak Valley of Assam alone.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). The present study was undertaken to develop a suitable protocol using rhizome axillary buds in vitro for its rapid multiplication.

Materials and methods

H. aromatica plant was collected from Patharia Hills of Karimganj district near Indo-Bangla border and maintained in the germplasm collection centre of the Department of Botany, Srikishan Sarda College, Hailakndi, Assam, India. Sections of the rhizomes (2cm) containing buds were dissected and washed in detergent for 20 minutes followed by continuous washing under running tap water for 1 hour after which the explants were immersed in 90% ethanol for 30 seconds and rinsed with sterile distilled water. They were then surface sterilized with 100 mg L^{-1} (w/v) $Hgcl_2$ solution for 15 minutes, followed by rinsingfive times with sterile distilled water.They were againsurfacesterilizedwith15%Clorox®(containing5.3%sodiumhypochlorite,NaCIO)solution addedwith2-3dropsofTween-20foranother10minutesandagainrinsed fivetimeswithsteriledistilledwater.Thesurfacesterilizedrhizomatousstemsweretrimmedtoremovethedamagedanddeadtissueandcuti ntosmallpieces,eachpiececontainingonebud.

Rhizome buds were cultured on Murashige and Skoog's (1962) basal medium (MS) supplemented with 0.5 mgl^{-1} 6-benzyleaminopurine (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. ThepHofthemediumwasadjustedto5.6-

5.7priortoautoclavingat121^{0}Cat1.06kgcm^{-2}for15 minutes.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 100 ml of medium.

To establish the optimum treatment for multiple shoot induction, the *in vitro*raised shoots were used as explants and further inoculated into MS medium containing 0.0– 6.0 mgl^{-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 explants and average shoot length were recorded at every 4 weeks after sub-culturing to fresh medium twice. Cultures wereincubated at 25±2 °C under cool white fluorescent light (60 μmolm^{-2} s^{-1}) with a photoperiod of 14 hour.

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 mgl^{-1} for root initiation. Numbers of roots were counted after 8 weeks of transfer to root induction medium. Wellrooted 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 day 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.

Result and discussion

Sprouting of axillary buds followed by shoot initiation from the basal part of the rhizome buds was observed within 18 day in medium containing 0.5 mgl^{-1} BAP. Due to serious contamination problem (about 80 % 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 inorder to decrease wastage of axillary 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

Table 1: Effect of cytokinins on shoot multiplication of *H. aromatica*, data recorded after8 weeks of culture

Plant growth regulators (mgl^{-1})			Frequency of responding cultures (%)	Number of shoots per explants	Shoot length (cm)
BAP	Kinetin	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}	20.8±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}
6.0			92.30±2.80^{a}	6.8±0.8^{cd}	1.56±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}
6.0					86.89±2.78^{ab}
3.4±1.3^{e}	2.56±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}
6.0	26.87±2.75^{d}				8.9±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≤0.05) according to student's t-test]

multiplication varied depending upon the kind and concentration of cytokinins used. Of the three cytokinins tested, maximum numbers of shoots (20.8 ± 0.7) were obtained on medium containing 2 mgl^{-1} BAP (Table 1). The promotive effect of BAP on multiple shoot induction has been reported earlier in other medicinally important plant species such as*Homalomena pineodora*(Stanly *et al*, 2012), *Zingiber officinale* (Balachandran *et al*. 1990), *Piper sp*. (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) alsoobserved that multiple shoots decreases beyond 2 mgl^{-1}BAP in *Sida cordifolia*.

Though both Kinetin and TDZ effected shoot formation, they were found to be inferior to BAP (Table 1). In Kinetin containing medium, the number of shoots were greatly reduced com-pared to BAP. The inferiority of Kinetin 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 orless 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 annuum* (Ahmad *et al*. 2006).

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 (mgl^{-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			99.0 ± 0.9^{a}	16.1 ± 0.4^{cde}	3.5 ± 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}	16.1 ± 0.6^{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 ($P\leq0.05$) according to student's t-test]

Half strength MS medium was found to be more effective for rooting compared to full-strength MS medium in 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 auxinfree medium may be due to the availability of endogenous auxin in the shoots *in vitro* (Minocha 1987). Highest number of roots (16.1±0.6) as well as root length (3.5±0.3 cm) was achieved in half-strength MS medium supplemented with 1.0 mgl^{-1} IAA and 1.0 mgl^{-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 mgl^{-1} NAA (Table 2). Plantlets obtained in medium supplemented with NAA treatment survived better than IAA and IBA during acclimatization in the green house. Approximately 90 % of the regenerated plantlets survived and showed vigorous growth without any apparent morphological variations. Through this technique, about 150 plantlets were produced from a single rhizome bud in a year which is not possible through conventional cultivation. This study reports for the first time in the tropical/subtropical environment,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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