The Chittagong Univ. J. B. Sci. 8(1&2): 99-105, 2013

ESTABLISHMENT OF EFFICIENT PROTOCOLS FOR *In vitro* REGENERATION of *Hyptis suaveolens* (L.) Poit. USING APICAL SHOOTS AND NODAL SEGMENTS

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ABSTRACT

The present work was undertaken with a view to standardize an efficient protocol for *in vitro* rapid propagation of *Hyptis suaveolens*. Shoot apices and nodal segments of *H. suaveolens* produced multiple shoot buds when cultured on MS medium supplemented with 1.5-3.0 mg/l BAP or Kn in the combinations with 0.5-1.0 mg/l IAA or NAA. Nodal explants exhibited highest proliferation (90%) and gave maximum number of shoots per culture (8.40 ± 0.18) on MS medium fortified with 1.5 mg/l BAP and 1.0 mg/l IAA. The shoot buds were elongated on MS medium fortified with different concentrations and combinations of auxins and cytokinins. Elongated shoot buds were further cultured in rooting media for root induction. One fourth MS medium without PGR was proved the most effective for induction and proliferation of roots. The rooted plantlets were established in the field through successive phases of acclimatization and the survival rate was 70%.

Key words: *Hyptis suaveolens*, apical shoot and nodal segments, *in vitro* regeneration, aromatic medicinal plant.

INTRODUCTION

Hyptis suaveolens (L.) Poit. is an important aromatic medicinal herb of Lamiaceae family. The plant is distributed in Chittagong and Chittagong Hill Tracts. It is also found to grow in fallow lands of other areas in Bangladesh. Leaves and flowers yield an essential oil containing β-caryophyllens, cineol, terpenol, α-bergamotene, sabinene, menthol, l-sabinene, d-limonene, azulenic sesquiterpens, campesterol and fucosterol, those have curative properties against cancer and tumour (Yusuf *et al.* 2009). Leaf juice is considered to be antispasmodic and antirheumatic, given in cases of colic and stomachaches, infusion is given in fever (Ghani 2003). Seeds contain anti-A haemagglutinin that allay thirst and extract is taken by the Chakma people for the remedy of urinary complications (Yusuf *et al.* 2009). Roots contain β-sitosterol, oleanolic and α-peltoboykinolic acid (Asolkar *et al.* 1992). Juice prepared from root by crushing on stone is taken for treatment of stomach pain by Khumi tribal people (Uddin 2012). Two new diterpènes suaveolic acid and suaveolol have also been isolated from this plant (Rastogi and Mehrotra 1993). The plant is stimulant, carminative, sudorific and lactagogue (Yusuf *et al.* 2009). Now-a-days, the forests and the mountains are already being depleted of medicinal plants as demand increase; and many desirable medicinal plant species are now listed as endangered in their native habitats. Because of over exploitation, due to heavy demand for plant drug resources and pressure of over population, this medicinal plant species is disappearing in our country. Plant tissue culture techniques are

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actively employed biotechnological methods to enhance the multiplication of medicinal plants and to improve the quality of secondary metabolites in a large scale. This article presents an efficient *in vitro* protocol for plant regeneration through apical shoots and nodal segments of *H. suaveolens* and their successful establishment in field conditions.

MATERIALS AND METHODS

Shoot apices and nodal segments of *Hyptis suaveolens* (L.) Poit. were collected from field grown plants. The explants were washed with distilled and double distilled water for three to four times and surface sterilization was done by submerging them in 0.1% (w/v) $HgCl_2$ solution for 1-3 minutes and rinsed with 70% ethanol. Then explants were cultured on MS medium containing 3.0% (w/v) sucrose supplemented with different combinations and concentrations of auxins and cytokinins for multiplication. All the media were made 0.8% (w/v) agar solidified and pH of the media was adjusted to 5.8 prior to autoclaving for 30 min at 121°C under 1.5 kg/cm^2 pressure. Culture vessels with inoculated explants were maintained in a culture room under a regular cycle of 14h light and 10h dark at 25±2°C. The light intensity was 2000-4000 lux. The regenerated complete plantlets were transferred to plastic cups containing sandy-loam soil and humus at 2:1 and then established in outside environment through successive phases of acclimatization. Different growth parameters such as the percentage of response, number and length of shoots and roots were monitored to record data on morphogenetic responses of various explants while under different culture conditions. Five replicates were used in each treatment. All the experiments were repeated thrice. In relevant cases the data were subjected to statistical analysis for computation of the standard error of the mean (SE).

RESULTS AND DISCUSSION

Shoot apices and nodal segments of field grown plants were aseptically cultured on MS medium supplemented with different combinations and concentrations of cytokinins (Kn and BAP) and auxins (IAA and NAA). The obtained results are summarized in Table-1. Shoot apices and nodal segments underwent direct organogenesis producing multiple shoot buds in some of the media combinations. But the responses of shoot apices were not satisfactory, so the data related with shoot apex is not presented. The maximum number (90%) of nodal explants produced multiple shoot buds on MS medium having 1.5 mg/l BAP +1.0 mg/l IAA (Fig. 1A). Similar PGR combination was found to use in *Ficus religiosa* (Hassan *et al.* 2009) for shoot induction. Here, the average number of shoot per culture was 8.40 ± 0.18 (Fig. 1B). Similar direct organogenesis of other medicinal plants were also reported in *Curculigo orchioides* (Wala and Jasrai 2003), *Vitex negundo* (Rahman *et al.* 2004), *Wedelia chinensis* (Sultana and Handique 2004), *Rauvolfia tetraphylla* (Faisal *et al.* 2005), *Curculigo orchioides* (Francis *et al.* 2007) and *Plumbago zeylanica* (Mallikadevi *et al.* 2008).

Shoot buds produced directly from the nodal segments underwent elongation when subcultured on elongation media. The efficiency of the media was assessed in terms of enhancing elongation of shoot buds. The obtained results are presented in Table-2. Maximum elongation took place in MS +1.5 mg/l BAP + 0.5 mg/l IAA (Fig. 1C). Maximum shoot

elongation was reported by Rahman *et al.* (2011) in another medicinal plant *Scoparia dulcis* in the same media combination on MS media with 1.5 mg/l BAP + 1.0 mg/l IAA. The potential of BAP in combination with IAA was also demonstrated in *Plumbago indica* (Hossain *et al.* 2009), *Acorus calamus* (Verma and Singh 2012), *Centella asiatica* (Thangapadian *et al.* 2012), *Ipomoea mauritiana* (Islam and Bari 2013), *Rauvolfia serpentina* (Archana *et al.* 2014).

Full, half and one fourth strength of MS media either individually or fortified with different concentrations of auxins (IAA, IBA and NAA) were used for rooting experiments. *In vitro* grown shoots of 2-3 cm long were separated and transferred to rooting media. Data were recorded after five weeks of inoculation. Responses of shoot to rooting were very much dependent on the concentration of MS basal medium. Results of rooting experiments are presented in Table-3. The increase in length and also the number of roots developed in each plantlet (Fig. 1D) were more on one-fourth MS medium without any PGR. The promotive effect of reducing MS salt concentration without growth regulators have been carried out for *in vitro* rooting of *Adhatoda vasica* (Azad *et al.* 1999), turmeric (Salvi *et al.* 2001), *Anethum graveolens* (Jana *et al.* 2010), *Thalictrum dalzellii* (Sharanappa *et al.* 2011) and *Paeonia lactiflora* (Jeong *et al.* 2013). Well rooted plantlets were transferred to outside environment through a successive phase of acclimatization. The survival rate was 70% (Fig. 1E).

TABLE-1: EFFECT OF GROWTH REGULATORS IN MS BASAL MEDIUM ON SHOOT INDUCTION AND NUMBER OF SHOOTS PER CULTURE ESTABLISHED FROM NODAL SEGMENTS OF *H. suaveolens*.

Supplemented PGRs and their concentrations (mg/l)				% of explants gave response	Time required for induction (d)	Average* no. of multiple shoot buds (mean ± SE)
BAP	Kn	IAA	NAA			
1.0	-	-	-	67	20-25	2.10 ± 0.19
2.0	-	-	-	71	22-28	2.90 ± 0.28
3.0	-	-	-	74	22-30	3.90 ± 0.48
-	1.0	-	-	60	20-28	1.90 ± 0.40
-	2.0	-	-	64	20-28	2.00 ± 0.23
-	3.0	-	-	76	22-25	4.20 ± 0.60
1.5	-	0.5	-	88	22-30	6.80 ± 0.51
1.5	-	1.0	-	90	20-28	8.40 ± 0.18
2.0	-	0.5	-	84	22-28	4.95 ± 0.23
2.0	-	1.0	-	85	22-30	7.10 ± 0.34
-	1.5	0.5	-	72	22-30	5.20 ± 0.17
-	1.5	1.0	-	75	22-30	4.00 ± 0.41
-	2.0	0.5	-	70	24-30	2.30 ± 0.10
-	2.0	1.0	-	68	20-28	2.20 ± 0.18
-	2.0	-	0.5	62	22-30	3.50 ± 0.14
-	2.0	-	1.0	86	22-28	5.30 ± 0.11
-	3.0	-	0.5	65	24-30	2.80 ± 0.31
-	3.0	-	1.0	77	22-28	4.50 ± 0.41
3.0	-	-	0.5	80	20-28	3.10 ± 0.31
3.0	-	-	1.0	82	22-28	5.00 ± 0.19

* Values are the mean of five replicates each with 15 explants.
d = day/s

TABLE-2: ELONGATION OF MULTIPLE SHOOT BUDS GROWN ON 0.8% (W/V) AGAR SOLIDIFIED MS MEDIUM SUPPLEMENTED WITH DIFFERENT PGRS.

PGRs supplement (mg/l)	Average* initial length (cm) of individual shoot bud (mean ± SE)	Average* length (cm) of multiple shoot bud after 30d of culture (mean ± SE)	Average* increase in length (cm) of multiple shoot bud (mean ± SE)
BAP + IAA			
1.5 + 0.5	1.15 ± 0.14	4.10 ± 0.12	3.05 ± 0.18
1.5 + 1.0	1.70 ± 0.34	3.90 ± 0.25	2.25 ± 0.23
2.0 + 0.5	1.10 ± 0.21	2.85 ± 0.27	1.90 ± 0.07
2.0 + 1.0	1.20 ± 0.15	2.50 ± 0.20	1.45 ± 0.11
BAP + NAA			
2.0 + 0.5	2.00 ± 0.17	3.45 ± 0.18	1.70 ± 0.17
2.0 + 1.0	1.89 ± 0.17	3.22 ± 0.18	1.27 ± 0.18
3.0 + 0.5	1.48 ± 0.16	2.90 ± 0.20	1.40 ± 0.13
3.0 + 1.0	1.00 ± 0.08	2.70 ± 0.11	1.55 ± 0.10
Kn + IAA			
1.5 + 0.5	1.50 ± 0.23	3.00 ± 0.16	1.60 ± 0.14
1.5 + 1.0	1.85 ± 0.31	2.95 ± 0.12	1.10 ± 0.08
2.0 + 0.5	2.10 ± 0.24	3.30 ± 0.25	1.30 ± 0.13
2.0 + 1.0	1.90 ± 0.33	3.10 ± 0.28	2.00 ± 0.19
Kn + NAA			
2.0 + 0.5	1.20 ± 0.05	3.50 ± 0.23	2.50 ± 0.18
2.0 + 1.0	1.25 ± 0.18	2.55 ± 0.10	1.35 ± 0.11
3.0 + 0.5	1.78 ± 0.12	2.35 ± 0.20	1.09 ± 0.05
3.0 + 1.0	1.30 ± 0.90	2.50 ± 0.19	1.15 ± 0.06

* Values are the mean of five replicates each with 15 explants.
d = day/s

TABLE-3: EFFECT OF AUXINS IN MS BASAL MEDIUM ON ROOTING OF *H. SUAVEOLENS* AFTER 30 DAYS OF INOCULATION.

Basal medium	Treatments (mg/l)			Mean root no. (± SE)	Length of root (cm) (mean ± SE)
	IAA	IBA	NAA		
MS	-	-	-	2.90 ± 0.19	2.20 ± 0.21
½MS	-	-	-	3.70 ± 0.20	2.95 ± 0.27
¼MS	-	-	-	5.10 ± 0.23	4.70 ± 0.18
,,	0.5	-	-	2.20 ± 0.12	1.56 ± 0.10
,,	1.0	-	-	2.00 ± 0.11	1.90 ± 0.12
,,	-	0.5	-	2.60 ± 0.19	2.25 ± 0.17
,,	-	1.0	-	2.40 ± 0.13	1.85 ± 0.24
,,	-	-	0.5	3.00 ± 0.19	1.77 ± 0.20
,,	-	-	1.0	2.10 ± 0.12	1.65 ± 0.13

Data represent the means ± SE of five replicates each with 15 explants.

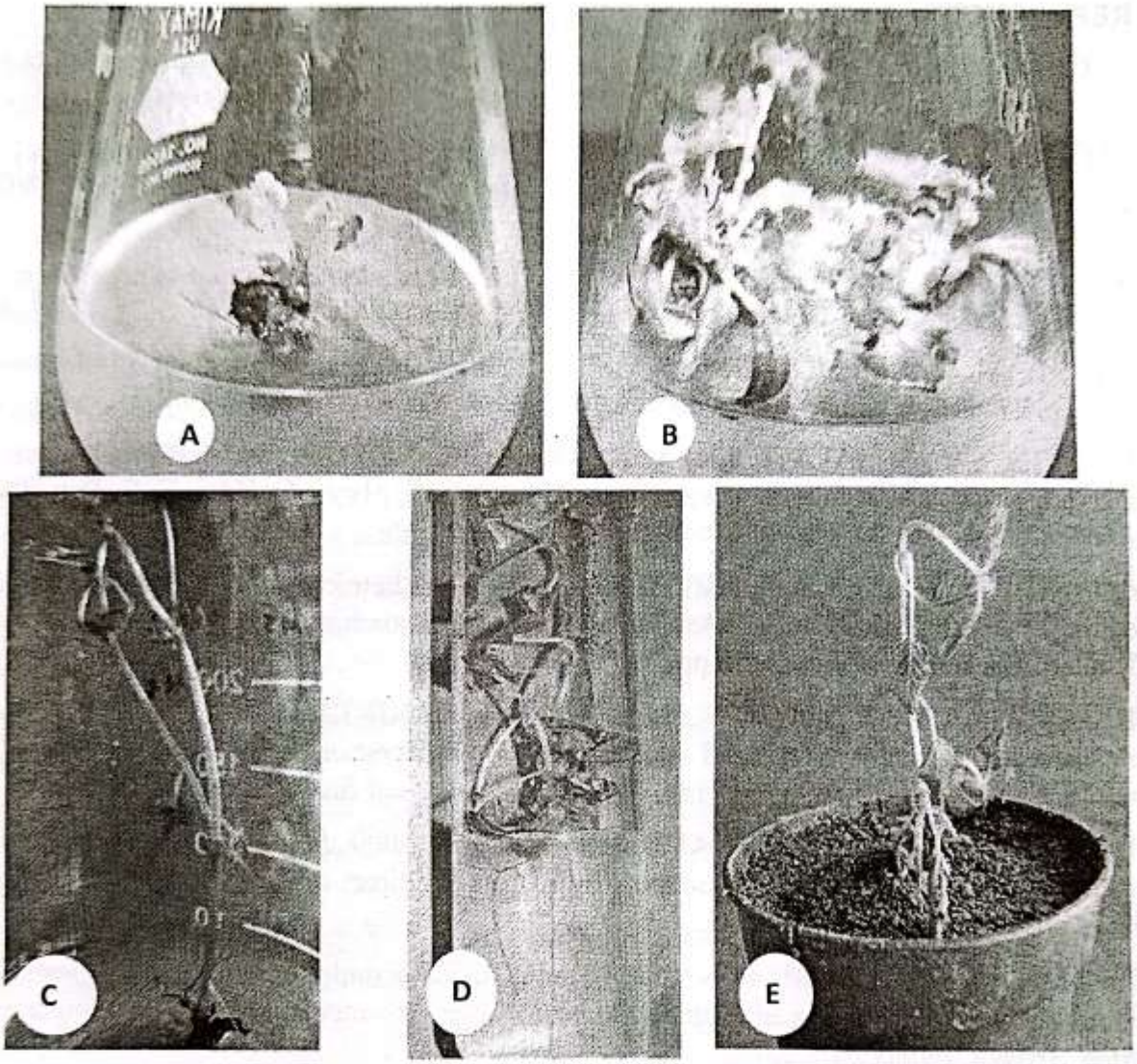

FIGURE 1 : DIFFERENT PHASES OF *IN VITRO* REGENERATION OF *H. SUAVEOLENS* A. DEVELOPMENT OF MULTIPLE SHOOT BUDS FROM NODAL SEGMENTS. B. PROLIFERATION OF MULTIPLE SHOOT BUDS. C. RAPID ELONGATION OF SHOOT BUD IN ELONGATION MEDIUM. D. INDUCTION OF ROOTING. E. ESTABLISHMENT OF *IN VITRO* RAISED PLANTLET IN OUTSIDE.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the valuable guidelines offered by Late Professor Dr. S.K. Bhadra of the Department of Botany, University of Chittagong during the investigation. We also deeply mourn his death.

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Manuscript received on 29.09.2016; Accepted on 17.05.2017

The Chittagong University Journal of Biological Sciences, Vol. 8 (1 & 2). Page No.99-105