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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Full Length Research Paper

Micropropagation of the Indian Birthwort *Arsitolochia indica* L.

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***Aristolochia indica* L. is a medicinal woody perennial climber plant of immense pharmaceutical value. The species is endangered with possible extinction due to its indiscriminate harvesting as raw material for pharmaceutical industry, to manufacture drugs against cholera, inflammation, biliousness, dry cough and snake bite. A rigorous attempt has been made for development of *in vitro* propagation procedure for this species, involving four steps, namely: culture establishment, shoot multiplication, rooting and hardening. Aseptic cultures were established by growing nodal segments (1 to 1.5 cm) as explants on Murashige and Skoog (MS) medium containing 5.0 µM N6-Benzyladenine (BA). Five nutrient media, MS, Woody Plant Medium (WPM), Gamborg Medium (B5), Nitsch and Nitsch Medium (NN), and Schenk and Hildebrandt Medium (SH) supplemented with different cytokinins and auxins at a concentration of 10.0 µM were used in this study. Ads at 10.0 µM proved optimum for *in vitro* shoot multiplication. The treatment resulted in 100% shoot number per explant at 15 days and 61.9% at 30 days on MS medium, 65.2% node number per shoot at 15 days and 196.2% at 30 days on WPM medium and 147.5 and 366.6% node number per explant at 30 days after inoculation on MS medium. The *in vitro* multiplied shoots were used for rooting experiment. Five nutrient media (MS, WPM, B_5, NN and SH) and three auxin sources 10.0 µM each (IBA, IAA and NAA). SH medium with 10.0 µM NAA induced 327.8% rooting at 21days and 654.8% at 28 days and root number per explant 4300% at 21 and 394% at 28 day after inoculation. The *in vitro* propagated hardened plants exhibited excellent growth on transfer to natural condition.**

Key words: *Aristolochia indica* L, *in vitro* propagation, N^6-Benzyladenine.

INTRODUCTION

Aristolochia indica L. (family- Asclepiadaceae.) is a perennial climber with greenish whitish woody stem growing throughout India especially in the tropical and sub-tropical regions. The active constituent “Aristolic acid” is potent drug used in Ayurvedic, Sidda and Homeopathy systems of medicines. Roots are widely used in joint pains and seeds in inflammation, biliousness, dry cough and dyspepsia. The juice of leaves or roots is said to be a specific antidote for cobra poisoning (Kirtikar and Basu, 1987). The species is rare and endangered with extinction due to its indiscriminate collection and over exploitation from natural resources for commercial purpose by pharmaceutical industries (Rahman, 2001). The conventional propagation is hampered due to low seed viability and poor rooting of vegetative cuttings and emphasizes need for the alternative *in vitro* propagation method for large scale multiplication, improvement and conservation of the

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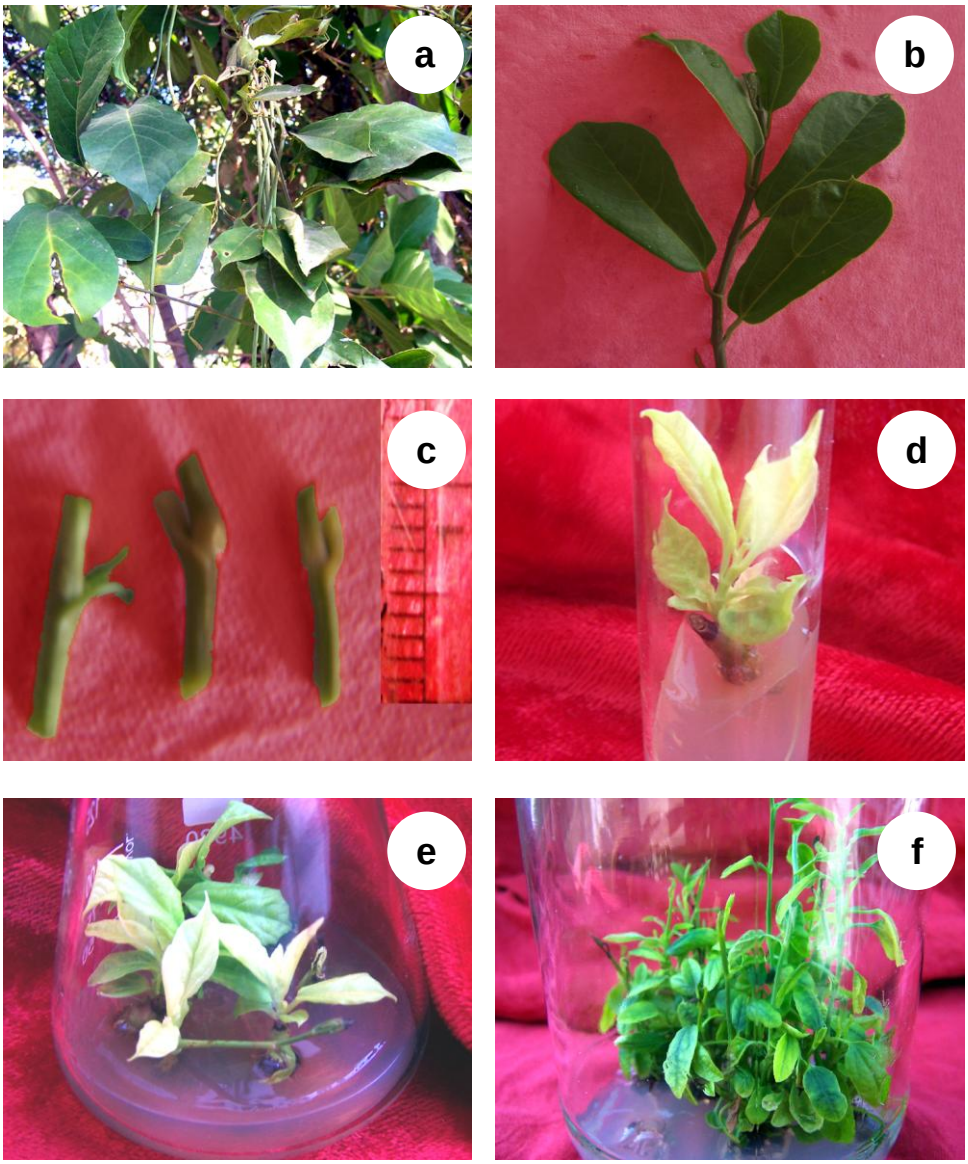

Figure 1. Explant collection, culture establishment and shoot multiplication in *Aristolochia indica* L.; (a) mother plant, (b) a twig, (c) nodal explants, (d) the *in vitro* culture establishment and (e-f) the *in vitro* shoot multiplication.

species. The objective of the study was to develop an efficient protocol for its micropropagation.

MATERIALS AND METHODS

The selected (mother) plant from Jabalpur area of Madhya Pradesh, India (Figure 1a) was used to collect twig (s) (Figure 1b), which were washed thoroughly for 15 min under running water for removing the surface debris. The washed twigs were defoliated and cut into nodal explants (approximately 1 to 1.5 cm long and 0.5 to 0.6 cm diameter) (Figure 1c). These explants were washed with 2% Cetrimide® and kept for 10 min with constant vigorous (shaking 150 rpm) on an orbital shaker incubator followed by rewashing 4 to 5 times with distilled water to remove traces of Cetrimide®. The washed explants were sterilized for 5 min with $HgCl_2$ (0.1%) and Bavistin® (1.0%) in the laminar flow cabinet. Finally, the surface sterilized nodal explants were rinsed 4 to 5 times with sterile distilled water and inoculated on MS medium (Murashige and Skoog, 1962) supplemented with 5.0 µM BA for culture establishment (Figure 1d).

The *in vitro* shoot multiplication (Figure 1e-f) was standardized through a factorial randomized experiment, using single nodal segments from established cultures. In this experiment we screened five nutrient media [MS (Murashige and Skoog, 1962), WPM (Lloyd and McCown, 1980), B_5 (Gamborg et al., 1968), NN (Nitsch and Nitsch, 1969) and SH (Schenk and Hildebrandt, 1972)] along with 10.0 µM each of three cytokinins (BA, TDZ, Ads), and their combinations on shoot number per explant, node number per shoot and node number per explant at 15 and 30 days after inoculation. In second experiment, five nutrient media (MS, WPM, B_5, NN and SH) and three auxins (IBA, IAA and NAA) at

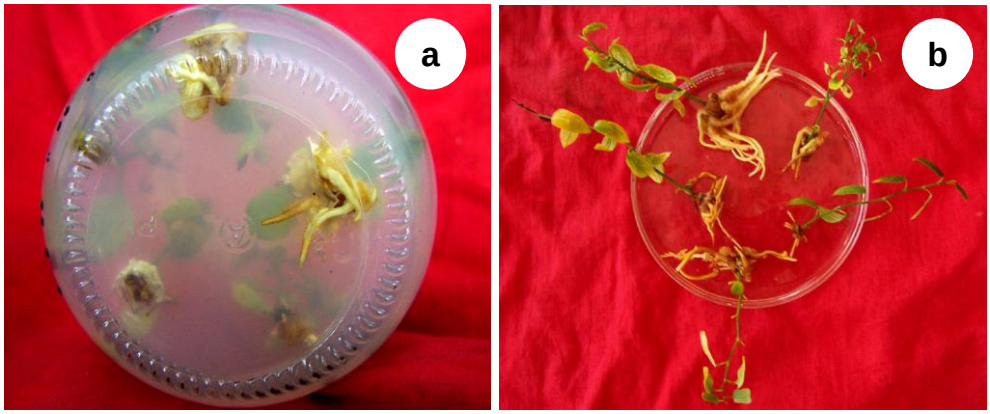

Figure 2. The *in vitro* induction of adventitious root in *Aristolochia indica* L. roots formation on semi-solid medium at (a) 21 and (b) 28 days after inoculation.

concentrations of 10.0 µM and their effect on rooting and root number was recorded at 21 and 28 days after inoculation (Figure 2).

Culture conditions

The inorganic salts used for preparation of culture medium were obtained from Qualigens Pvt. Ltd., India and phytohormones and B vitamins from Sigma Chemicals Pvt. Ltd., India. The medium contained 3% (w/v) sucrose, 0.8% (w/v) agar (Hi-Media chemical Ltd., India). The pH of the medium was adjusted to 6.0 before autoclaving for 15 min at 1.06 kg cm^{-2} (121°C). Explants were cultured in a 150 ml conical Borosil® flasks containing 40 ml semi-solid medium. For *in vitro* shoot multiplication and rooting experiment, the cultures were incubated at 25 ± 2°C under 16 h illuminations with fluorescent light (50 µmol Em^{-2} s^{-1}).

Hardening and transplantation

The *in vitro* raised plantlets were removed from rooting medium washed with distilled water and the plantlets were subsequently transferred to root trainers containing autoclaved soilrite (Figure 3a) and covered with perforated polythene to maintain humidity which were kept under culture room conditions for about 10 days. Subsequently, they were transferred to perforated polythene bags and kept initially in washing room for 5 days and finally transferred to natural condition (Figure 3b-d).

Statisticaly analysis

Each experiment had three replicates for *in vitro* shoot multiplication and rooting. Each replicate had 10 propagules. The data were subjected to two way (factor) analysis of variance for both the experiments with “F” test for ascertaining level of significance. If the data were found significant at $p \leq 0.05$, $LSD_{0.05}$ was computed for comparison of treatment means.

RESULTS

In vitro shoot multiplication

The effect of cultured media, cytokinin sources and their all possible combinations on shoot number per explant, node number per shoot and node number per explant at both the stages of sampling was recorded.

Shoot number per explant

SH medium produced maximum shoot number per explant at 15 days and MS medium at 30 days (Table 1).The enhancement of shoot number per explant in SH medium was 100% in comparison to B_5 medium at 15 days and 61.90% in MS medium at 30 days after inoculation in comparison to that in B_5 medium. Further BA had significantly maximum shoot number $explant^{-1}$ at both stages and was statistically equalled by Ads at 15 days. Shoot number per explant in BA was 1333%, 76% more than that of TDZ at 15 and 30 days, respectively. MS medium with 10.0 µM BA produced maximum shoot numbers per explant which was 189% at 30 days after inoculation.

Node number per shoot

SH medium produced maximum node number per shoot at 15 days and WPM medium at 30 days (Table 2). The enhancement of node number per shoot in SH medium was 65.2% more than that obtained in NN medium at 15 days and 139% at 30 days in comparison to that in B_5 medium which produced the lowest value for the parameter at both stages of sampling . BA induced maximum node number per shoot, which was enhanced by 61% at 15 days and 239% at 30 days in comparison with TDZ. Maximum node number per shoot was observed on SH medium with Ads, at 15 days and WPM 10.0 µM Ads at 30 days. NN medium produced minimum effect on node number per shoot at both the stages of sampling at 15 days and B_5 medium at 30 days.

Figure 3. Hardening and acclimatization of the *in vitro* raised plantlets of *Aristolochia indica* L. Plantlets transferred to root trainers (a) and covered with polythene (b) placed in the culture room, (c) hardened plantlets transferred to polythene bags and (d) growth of the plantlets in the open environment.

Table 1. Effect of culture media and different cytokinins on shoot number per explant in *Aristolochia indica* L. at two stages of sampling.

Cytokinin source (C)	Culture media(M)											
	Inoculation days											
	15 Days						30 Days					
	MS	WPM	B5	NN	SH	Mean	MS	WPM	B5	NN	SH	Mean
BA	1.00	1.00	0.39	0.56	1.11	**0.81**	**2.89**	1.73	1.06	1.67	1.45	**1.76**
TDZ	0.33	0.00	0.00	0.00	0.00	0.06	1.00	1.00	1.00	1.00	1.00	1.00
Ads	0.78	1.00	0.72	0.72	1.11	**0.86**	1.17	1.17	1.11	1.78	1.45	1.33
Mean	**0.70**	**0.66**	0.37	0.42	**0.74**		**1.70**	1.30	1.05	1.48	1.30	

Variable	LSD (0.05) 15Days	30Days
C	0.16	0.13
M	0.20	0.17
C *M	NS	0.29

Node number per explant

SH and MS medium induced maximum node number per explant at 15 days and at 30 days respectively (Table 3). The enhancement of node number per explant in SH medium was 147.6% at 15 days and 366.6% in MS medium at 30 days as compared to B_5 medium. BA and Ads had significantly maximum node number per explants at 15 and 30 days, respectively. BA enhanced node number per explants by 48% at 15 days and Ads by

Table 2. Effect of culture media and different cytokinins on node number per shoot in *Aristolochia indica* L. at two stages of sampling.

Cytokinin sources (C)	Culture media(M)											
	Inoculation days											
	15 Days						30 Days					
	MS	WPM	B5	NN	SH	Mean	MS	WPM	B5	NN	SH	Mean
BA	1.28	1.55	1.17	1.28	2.78	**1.61**	4.04	2.67	1.11	2.28	2.72	2.57
TDZ	1.00	1.00	1.00	1.00	1.00	1.00	1.78	1.06	1.00	1.00	1.06	1.18
Ads	1.56	**2.22**	1.06	1.17	1.83	1.56	3.50	**5.78**	1.11	4.28	5.32	**4.00**
Mean	1.28	1.60	1.80	1.15	**1.90**		3.10	**3.17**	1.07	2.52	3.03	

Variable	LSD (0.05)	
	15 Days	30 Days
C	0.19	0.47
M	0.25	0.61
C *M	0.43	1.06

Table 3. Effect of culture media and different cytokinins on node number per explant in *Aristolochia indica* L. at two stages of sampling.

Cytokinin sources (C)	Culture media(M)											
	Inoculation days											
	15 Days						30 Days					
	MS	WPM	B5	NN	SH	Mean	MS	WPM	B5	NN	SH	Mean
BA	1.28	1.56	0.81	0.67	**3.07**	**1.48**	11.72	4.33	1.36	3.97	4.27	5.13
TDZ	1.00	1.00	1.00	1.00	1.00	1.00	1.06	1.00	1.00	1.00	1.06	1.02
Ads	1.22	2.22	0.76	0.81	2.03	1.41	4.00	6.80	1.27	7.55	**7.73**	**5.47**
Mean	1.16	1.60	0.86	0.82	**2.03**		**5.60**	4.04	1.20	4.18	4.35	

Variable	LSD (0.05)	
	15 Days	30 Days
C	0.21	0.85
M	0.28	1.10
C *M	0.48	1.91

436% at 30 days in comparison to TDZ, which produced the lowest value for the parameter. As for interaction, SH medium with 10.0 µM Ads registered the highest value for the parameter at 30 days after sampling.

In vitro adventitious rooting

Auxin sources and their combinations with different media induced significant rooting and root number per explant at both the stages of sampling.

Percent rooting

SH medium produced significantly high percent of rooting. The enhancement of rooting in SH medium was 327.8% at 21 days and 655% at 28 days in comparison to MS medium. MS, B_5, NN and WPM produced minimum effect on rooting. NAA produced significantly maximum rooting (%), which was 228 at 21 days and 443.7% at 28 days after inoculation in compared to IAA producing minimum value for rooting. SH medium with10.0 µM NAA maximum rooting at both stages of sampling (Table 4).

Root number per explants

SH medium produced maximum root number per explant at both the stages of sampling. The enhancement of root number per explant was 4300% at 21 days and 394% at 28 days after inoculation in comparison with WPM, MS and NN medium. NAA was found to have significant effect on root number per explant at both stages of sampling and resulted in 800% at 21 days and 2900% at 28 days more than that obtained in IAA. SH medium

Table 4. Effect of culture media and different auxins on percent of rooting in *Aristolochia indica* L. at two stages of sampling.

Auxin sources (A)	Culture media (M)											
	Inoculation days											
	Rooting at % 21 Days						Rooting at % 28 Days					
	MS	WPM	B5	NN	SH	Mean	MS	WPM	B5	NN	SH	Mean
IBA	0 (4.16)	0 (4.16)	0 (4.16)	16.66 (24.06)	0 (4.16)	3.33 (8.14)	0 (4.16)	0 (4.16)	5.55 (10.79)	16.66 (24.06)	0 (4.16)	4.44 (9.47)
IAA	0 (4.16)	0 (4.16)	0 (4.16)	0 (4.16)	0 (4.16)	0 (4.16)	0 (4.16)	0 (4.16)	0 (4.16)	5.55 (10.79)	0 (4.16)	1.11 (5.49)
NAA	5.55 (10.79)	0 (4.16)	0 (4.16)	0 (4.16)	50 (45)	11.11 (13.65)	22.22 (24.4)	0 (4.16)	16.66 (24.06)	5.55 (10.79)	100 (85.84)	28.89 (29.85)
Mean	1.9 (6.4)	0 (4.16)	0 (4.16)	5.6 (10.8)	16.7 (17.8)		7.4 (10.9)	0 (4.16)	7.4 (13.00)	9.3 (15.2)	33.3 (31.4)	

Variable	LSD (0.05)	
	21 Days	28 Days
A	2.21	5.49
M	2.85	7.08
A*M	4.94	12.27

Table 5. Effect of culture media and different auxins on root number per explant in *Aristolochia indica* L. at two stages of sampling.

Auxin sources (A)	Culture media(M)											
	Inoculation days											
	Root number 21 Days						Root number 28 Days					
	MS	WPM	B5	NN	SH	Mean	MS	WPM	B5	NN	SH	Mean
IBA	0.00	0.00	0.00	0.16	0.00	0.03	0.00	0.00	0.28	0.39	0.00	0.13
IAA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.03
NAA	0.05	0.00	0.00	0.00	1.33	0.27	0.66	0.00	0.44	0.11	3.28	0.90
Mean	0.01	0.00	0.00	0.05	0.44		0.22	0.00	0.24	0.22	1.01	

Variable	LSD (0.05)	
	21 Days	28 Days
A	0.16	0.27
M	0.20	0.35
A*M	0.36	0.61

along with 10.0 µM NAA was found to have significant effect on root number per explant at 21 and 28 days after inoculation (Table 5).

DISCUSSION

The micro-propagation of *A. indica* comprises four steps, namely: establishment of culture from nodal explants, shoot multiplication, root induction and hardening and acclimatization. The present investigation was intended for the standardization of culture medium and plant growth regulators at second and third steps followed by hardening procedure. For shoot multiplications, the best *in vitro* combination was SH medium supplemented with 10.0 µM Ads. There is no published report on the *in vitro* shoot multiplication of *A. indica* using SH medium. The suitability of SH medium in the present study contrasts with earlier reports of micropropagation for this species wherein MS medium was found to be the most effective

(Siddique et al., 2006a; 2006b; Pattar and Jayraj, 2012). The results indicate that the species requires low amount of nitrogen for growth and differentiation of new shoots. Adenine sulphate was found as the most suitable cytokinin for shoot multiplication. Similar results have been reported in the medicinal plant *Cichorium intybus* also, where multiple shoots proliferation was observed on medium supplemented with BA, IAA and adenine sulphate (Nadagopal and Ranjitha Kumari, 2006).

For *in vitro* rooting also, better performance was obtained on SH medium. High concentration of thiamine (Vitamin B_1) included in SH medium seems to be synergistic with auxins for facilitation of rhizogenesis as reported in teak by Ansari et al. (2002). Of the various auxin treatments, NAA was found to be the best auxin for *A. indica.* Superiority of NAA for *in vitro* rooting may be attributed to its synthetic nature and stability. Further, NAA also eludes the auxin oxidizing/ degrading enzyme systems of the plants (Jacobs, 1972). IAA was found to be inferior to both NAA and IBA. In literature also there are reports of IBA and NAA being more effective than IAA, because of the instability of the latter (Gaspar and Coumans, 1987).

Conclusion

The study demonstrates successful development of *in vitro* propagation procedure for *A. indica*. The procedure offers a potential system for conservation and mass propagation using explants derived from mature plants. SH (medium supplemented with 10.0 µM Ads has been found the best for efficient and rapid multiplication of *in vitro* shoots, while SH medium supplemented with 10.0 µM NAA for optimum induction of *in vitro* adventitious roots. Further, the hardening procedure reported here ensures 70 to 80% field survival of micropropagated plants of *A. indica.*

REFERENCES

Ansari SA, Sharma S, Pant NC, Mandal AK (2002). Synergism between IBA and thiamines for induction and growth of adventitious roots in *Tectona grandis".* J Sustain. For. 15:99-112.

Gamborg OL, Miller RA, Ojima K (1968). Nutrient requirements of suspension cultures of soya bean root cells, Exp. Cell Res. 50:151-58.

Gaspar T, Coumans M (1987). Root formation *In*: J.M. Bonga and D.J. Durzan (eds). Cell and Tissue Culture in Forestry, Specific Principles and Methods: Growth and Developments, Martinus Nihoff Publishers, Dordrecht, 2:202-217.

Jacobs WP (1972). The movement of plant hormones: auxins, gibberellins and cytokinins". In D.J. Carr (ed.), Plant Growth Substances, Springer, New York. pp. 701-709.

Kirtikar KR, Basu LM (1987). Indian Med. Plants. pp. 2117-2118.

Lloyd G, McCown B (1980) Commercially feasible micropropagation of mountain laurel *Kalmia latifolia* by use of shoot tip culture, Comb. Proc. Int. Plant prop. Soc. 30:421-27.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures, Physiol. Plant. 15:473-97.

Nadagopal S, Ranjitha Kumari BD (2006). Adenine sulphate induced high frequency shoot organogenesis in callus and *in vitro* flowering of *Cichoriumintybus* L. cv. Focus - a potent medicinal plant, Acta Agric. Slov. 87(2):415-425.

Nitsch JP, Nitsch C (1969). Haploid plants from pollen grains, Sci. 163:85-87.

Pattar PV, Jayraj M (2012). *In vitro* Regeneration of Plantlets from Leaf and Nodal explants of *Aristolochia indica* L. - An important threatened medicinal plant. Asian Pac. J. Trop. Biomed. 2(2):488-493.

Rahman M (2001). Red data Book of Vascular plants. Bangladesh National Herbarium. Dhaka, Bangladesh.

Schenk RU, Hildebrandt AC (1972). Medium and technique for induction and growth of monocotyledonous and dicotyledonous plant cell culture, Can. J. Bot. 50:199-204.

Siddique NA, Bari MA, Pervin MM, Nahar N, Banu LA, Paul KK, Kabir MH, Huda AKMN, Ferdaus KMKB, Hossin MJ (2006a). Plant Regeneration from Axillary Shoots Derived Callus in *Aristolochia indica* Linn. an Endangered Medicinal Plant in Bangladesh, Pak. J Biol. Sci. 9:1320-323.

Siddique NA, Kabir MH, Bari MA (2006b). Comparative *in vitro* study of plant regeneration from nodal segments derived callus in *Aristolochia indica* Linn. and *Hemidesmus indicus* (L.) R. Br. endangered medicinal plants in Bangladesh, J. Plant Sci. 1(2):106-118.

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Full Length Research Paper

A quick bud breaking response of a surface model for rapid clonal propagation in *Centella asiatica* (L.)

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Present investigation was planned to evaluate time period of bud breaking in *Centella asiatica* with different concentration of plant growth regulators, a medicinal herb distributed throughout the worldwide. For the study, concentrations were designed for response surface model describing bud breaking growth in optimum conditions. A combination of BAP (2 mg/L) + gibberellic acid (GA_3, 0.5 mg/L) was achieved at a best initial bud breaking at 8th hour. Longest time period taken for bud breaking was shown in combination of BAP (0.5 mg/L) + naphthalene acetic acid (NAA, 0.5 g / L) and BAP (0.1 mg/L) + adenine sulphate (0.5 mg/L) which was recorded at 84th hour. Half strength MS media was supplemented with IBA alone (2 mg/L) and in combination with IAA (0.5 mg/L) to attain an early *in vitro* rooting. Their interactions observed were statistically significant (P < 0.05).

Key words: *Centella asiatica*, bud, plant growth regulator, medicinal plant.

INTRODUCTION

Centella asiatica (L.) Urban, synonym *Hydrocotyle asiatica* (Family: Apiaceae) is a small perennial herb, commonly known as Mandukparni. In India, this species is mostly found in the swampy areas up to an altitude of 600 to 1800 m asl (Patra et al., 1998). Medicinally, *C. asiatica* used as memory enhancer and in the treatment of chronic diseases, mental disorders and neuropharmacological disorders like insomnia, insanity, depression, psychosis, epilepsy and stress (Chopra et al., 1980). The major bio-active ingredients in the plant are the triterpenes, asiatic acid, madecassic acid and their glycosides such as asiaticoside and madecassoside (Zheng and Qin, 2007). Due to the presence of these active ingredients, it possesses antileprotic, antifilarial, antibacterial, antifeedant, adaptogenic and antiviral properties (Warrier et al., 1994). The roots contain many polyacetylenic compounds, the major compound being 8-acetoxyfalcarinol (Loc and Tam, 2010).

Over-exploitation of *C. asiatica* from natural habitats for medicinal purposes causes depletion of plant population. There has been an increase interest in *in-vitro* culture techniques for mass multiplication of important species to overcome the pressure of over-exploitation and to restore species diversity (Patra et al., 1998; Tiwari et al., 2000; Bhandari et al., 2010). However, till date *in-vitro* technique has been applied only for < 20% of medicinally important species (Shukla et al., 1999). *In-vitro* propagation of *C. asiatica* was also carried out through leaf explants (Banerjee et al., 1999), axillary buds (George et al., 2004), stolons (Sampath et al., 2001), shoot tips

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Abbreviations: MS, Murashige; **BAP,** 6-benzylaminopurine; **NAA,** naphthaleneacetic acid; **GA3,** gibberellic acid; **IBA,** indole-3-butyric acid, **Kn,** kinetin; **IAA,** indole-3 acetic acid; **PGR,** plant growth regulator; **ANOVA,** analysis of variance.

(Sangeetha et al., 2003), callus cultures (Patra et al., 1998; Rao et al., 1999) and somatic embryogenesis (Martin, 2004). Besides all, there is information available on the methods to initiate early bud breaking, shoot formation and root initiation. Therefore, present study was designed to understand the effect of different PGRs in alone and in combination for bud breaking, shoot formation and root initiation. Present study will be useful in producing quality planting material with in short duration.

MATERIALS AND METHODS

Ex- plant selection

For the Rapid clonal propagation of *C. asiatica,* explants were collected from the Herbal garden (1545 m asl) of Herbal Research and Development Institute, Mandal, Gopeshwar (Chamoli) Uttarakhand, India; It is bounded by North Latitude 30° 27' 13.40" and East Longitude 79° 16' 21.61".

The media for clonal propagation was prepared by following Murashige and Skoog (1962), All Chemicals used for the research purpose were purchased from HiMedia Laboratories (Mumbai, India), and growth regulators were purchased from Sigma Chemical Co. (St Louis, MO) and HiMedia Laboratories, India. Cultures were established for the bud explants on MS medium (Murashige and Skoog, 1962) containing 58 mM sucrose and gelled with 0.7% (w/v) agar. The pH of medium was adjusted between 5.6 to 5.8 using 0.1 N HCL or 0.1 N NaOH solution prior to the autoclaving at 121°C and a pressure of 15 psi for 20 min then allowed to cool at room temperature. The explants thoroughly washed with running tap water for 15 to 20 min, then treated with 1% (v/v), Tween 20 solution and subsequently for 15 min with a sodium hypochlorite solution (0.5% active chlorine) in laminar air flow cabinet and finally the explants were washed thoroughly with autoclaved distilled water for several times to remove the traces of sodium hypochlorite. In support of surface disinfection, bud segments were trimmed from the cut ends in appropriate size, and cultured.

Culture conditions and *in-vitro* establishment of plantlets

For establishment of cultures, the surface disinfected explants were inoculated on full strength MS (Murashige and Skoog, 1962) basal medium having 3% of sucrose, semi-solidified with 0.7% (w/v) agar and supplemented with different concentrations of plant growth regulator viz. BAP (0.1 to 2 mg/L), adenine sulphate (0.1 to 0.5 mg/L) and gibberellic acid (0.5 mg/L). Half strength of MS medium supplemented with growth regulator IBA (0.5 to 2.0 mg/L) and NAA 0.1 to 0.5 mg/L was attempt for rooting. Each hormonal combination was tried in three replicates. 250 ml (Borosil, India) Conical flasks containing 20 ml of medium were used. Cultures vessels were used for incubated at 25 ± 1^{o}C under a 16/8 h light/dark photoperiod with light provided by cool-white fluorescent lamps (Philips India, Mumbai, India) at a light intensity of 1000 lux. The multiplied cultures were taken out; every single shoot was excised and kept in small plastic cup filled with a mixture of soil: sand (1:1) for *ex-vitro* rooting.

Multiple shoots from bud induction

The explants were inoculated in semi-solid MS medium with concentrations (0.1, 0.2, 1.5 and 2 mg/L) of BAP in alone, with combination of BAP (0.5 and 2 mg/L) along with gibberellic acid in 0.5 mg/L, BAP (0.1, 0.5,1 and 2 mg/L) with adenine sulphate 0.5 mg/L and BAP (0.5 to 2 mg/L) with the combination of NAA (0.5 mg/L). Sub culturing was carried out at periodic intervals of three weeks.

Rooting of microshoots

Developed shoots having one or two nodes were excised and transferred to root induction medium comprising of ½ strength MS medium with 3% sucrose and supplemented with different concentrations of IBA (0.5, 1, 1.5 and 2.0 mg/L) in alone and (0.1, 0.2, 0.5, 1.5 and 2.0 mg/L) with IAA (0.5 mg/L) in combination. Number of roots per shoot and root length was score in alternate day.

Hardening and acclimatization

For *in vitro* hardening, rooted shoots were transferred to ¼ MS strength medium having 3% sucrose devoid of PGR for seven days in flasks. Thereafter, they were transferred to polybags containing a mixture of soil: sand: FYM manure (1:1:1) and kept for two weeks in mist-chamber under controlled condition (tamp-25^{o}C ± 2^{o}C), humidity (65% ± 5%). Acclimatized plants were later shifted to soil in pots in agronet-shade house for one week and after that in field.

Statistical analysis

The data collected was subjected to the analysis of variance (ANOVA); using MS Excel 2007 for calculating the significance among different treatments and time of bud breaking and time of root initiation values at $P < 0.05$ were computed to compare means from various treatments.

RESULTS AND DISCUSSION

Initial study on *C. asiatica* was undertaken by Patra et al. (1998), Banerjee et al. (1999) and Tiwari et al. (2000). Tiwari et al. (2000) reported that initiation of nodal culture is better using different combination of plant growth regulators. The results of the present study on bud initiation, bud establishment and root initiation in MS medium supplemented with various combinations of growth regulators are presented in Tables 1 to 3. The earliest bud breaking in this study was achieved in BAP (2 mg/L) + GA_3 (0.5 mg/L). Initiation of bud breaking within 8 h of *in-vitro* culture in *C. asiatica* was reported first time in present study. Achieving early bud breaking is of importance as it produces quality planting material vis a vis reduces time and efforts. Different combinations of PGRs have also been reported to initiate bud breaking (Sen and Sharma, 1991). The longest time period (84 h) taken for bud breaking in present study was noticed in combination of BAP (0.5 mg/L) + NAA (0.5 mg/ L) and BAP (0.1mg/L) + adenine (0.5 mg/L) which are presented in Tables 2 and 3.

BAP alone at higher concentration (2 mg/L) seems to initiate early bud breaking. Similar observations in

Table 1. Morphogenetic response of *C. asiatica* buds cultured on MS medium supplemented with different concentrations of BAP and Gibberellic acid (Bud breaking is found positively significant LSD = 24.25 (P < 0.05).

BAP (mg/L)	Gibberellic acid (mg/L)	Time of bud breaking (h)
Control	-	0
0.1		72
0.1		60
0.1		66
0.2		60
0.2		72
0.2		72
0.5	0.5	36
0.5	0.5	38
0.5	0.5	40
1.5		40
1.5		46
1.5		42
2	0.5	8
2	0.5	12
2	0.5	14
2		24
2		18
2		16

t-value= 2.02, LSD = 24.25 (P < 0.05)

Table 2. Morphogenetic response of *C. asiatica* buds cultured on MS medium supplemented with different concentrations of BAP and Adinine (Bud breaking is found positively significant LSD=19.36; P < 0.05).

BAP (mg/L)	Adinine sulphate (mg/L)	Time of bud breaking (h)
Control	-	0
0.1	0.5	84
0.1	0.5	80
0.1	0.5	78
0.5	0.5	72
0.5	0.5	68
0.5	0.5	66
1	0.5	48
1	0.5	44
1	0.5	40
2	0.5	24
2	0.5	20
2	0.5	18

t-value = 2.13, LSD =19.36; (P < 0.05).

Table 3. Morphogenetic response of *C. asiatica* buds cultured on MS medium supplemented with different concentrations of BAP and NAA (Bud breaking is found positively significant LSD=19.36; P < 0.05).

BAP (mg/L)	NAA (mg/L)	Time of bud breaking (h)
Control	-	0
0.5	0.5	84
0.5	0.5	80
0.5	0.5	76
2	0.5	48
2	0.5	46
2	0.5	42

t-value= 2.03, LSD=19.36; P < 0.05

Ocimum basilicum was found by Pattnaik and Chand (1996). In addition, BAP was found more efficient over kinetine (Kn) in *in-vitro* shoot proliferation in different species (Purohit, 1994; Martin, 2003). In *Swertia chirata*, BAP with higher concentration have optimal response for shoot proliferation (Chaudhuri, 2007; Pant et al., 2010).

In present study, BAP in combination with GA_3 (with different concentrations) significantly (P < 0.05) enhance the rate of bud breaking, shoot proliferation and root initiation (Table 1, Figure 1A-E). Sharma and Sharma (2010) attributed this to the stimulating effects of various hydrolytic enzymes activities thus increasing availability of nutrients for growth. The result of the effects of BAP and GA_3 on shoot proliferation in present study was found comparable to the earlier reports. However, Tiwari et al. (2013) reported improved bud breaking using high concentration of BAP (5 mg/L) and improved root formation in combinations of BAP (4.0 mg/L) and IBA (0.5 mg/L). Karthikeyan et al. (2009) described the rapid clonal propagation through auxiliary shoot proliferation in *C. asiatica*. The shoot elongation with the treatment of BAP and GA_3 might be due to cell enlargement and increase in normal cell division (Karivartharaju and Ramakrishnan, 1985). Earliest root initiation was achieved alone in IBA (2 mg/L) and in combination of IBA (2 mg/L) and IAA (0.5 mg/L) (Tables 4 and 5).

Thus, it is concluded that *in-vitro* micro-propagation offer rapid clonal multiplication of elite clones and further helps in dissemination fulfilling the need of vis a vis to quality planting material. BAP (2 mg/L) in combination with GA_3 (0.5 mg/L) is recommended for effective and earliest bud breaking. Likewise, IBA (2 mg/L) is recommended for earliest rooting in *C. asiatica*.

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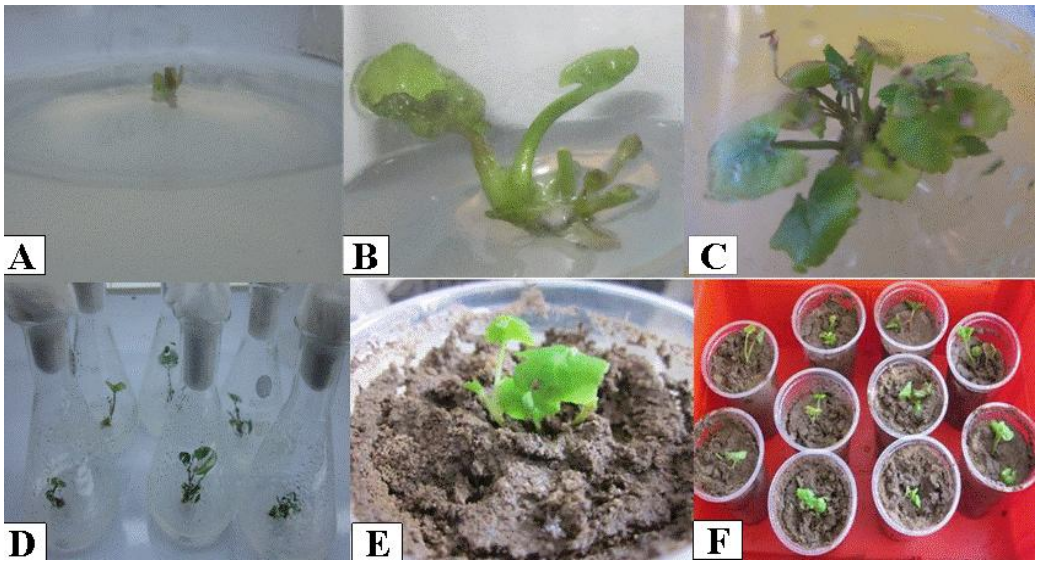

Figure 1. In vitro regeneration of *Centella asiatica,* via bud explants; **(A)** axillary bud induction on nodal segment MS medium+BAP (2 mg/L)+gibberellic acid (0.5 mg/L), **(B)** bud induction on nodal segment, **(C)** culture establishment, **(D)** multiplication of shoots in BAP (0.5 mg/L)+NAA 0.1 mg/L, **(E)** rooted plantlet containing a mixture of soil : sand : manure (1:1:1), **(F)** plantlet in soil for hardening containing a mixture of soil : sand : manure (1:1:1).

Table 4. Morphogenetic response of root initiation explants of *C. asiatica* cultured on half strength MS medium supplemented with different concentrations of IBA (Root initiation is found positively significant LSD=3.16; (P < 0.05).

IBA (mg/L)	Time of root initiation (Days)
Control	0
0.5	16
0.5	14
0.5	15
1	15
1	14
1	13
1.5	12
1.5	11
1.5	11
2	10
2	9
2	8

t-value= 2.07, LSD=3.16; (P < 0.05)

Table 5. Morphogenetic response of root initiation explants of C. asiatica cultured on half strength MS medium supplemented with different concentrations of IBA and IAA (Bud breaking is found positively significant LSD=3.12; (P < 0.05).

IBA (mg/L)	IAA (mg/L)	Time of root initiation (Days)
Control	0	0
0.1	0.5	18
0.1	0.5	16
0.1	0.5	16
0.2	0.5	18
0.2	0.5	16
0.2	0.5	16
0.5	0.5	15
0.5	0.5	14
0.5	0.5	13
1.5	0.5	12
1.5	0.5	11
1.5	0.5	10
2	0.5	10
2	0.5	9
2	0.5	9

t-value= 2.02, LSD=3.12; (P < 0.05)

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REFERENCES

Banerjee S, Zehra M, Kumar S (1999). *In vitro* multiplication of *Centella asiatica*, a medicinal herb from leaf explants. Curr. Sci. 76:147-148.

Bhandari AK, Negi JS, Bisht VK, Bharti MK (2010). *In vitro* culture of *Aloe vera* - A plant with medicinal property. Nat. and Sci. 8(8):174-176.

Chaudhuri RK (2007). Production of genetically uniform plants from nodal explants of *Swertia chirata* Buch.-Ham.ex Wall.: an endangered medicinal herb. *In-vitro* Cell. Div. Biol. Plant 43:467-472.

Chopra RN, Nayar SL, Chopra IC (1980). Glossary of Indian Medicinal Plants (Including the Supplement). CSIR, New Delhi.

George S, Remashree AB, Sebastian D, Hariharan M (2004). Micropropagation of *Centella asiatica* L. through axillary bud multiplication. Phytomorphology 54:31-34.

Karivartharaju TV, Ramakrishnan V (1985) Seed hardening studiesin two varieties of ragi. Indian J. Pl. Physiol. 28:243-248.

Karthikeyan K, Chandran C, Kulothungan S (2009). Rapid clonal multiplication through *in- vitro* axillary shoot proliferation of *Centella asiatica* L. Ind. J Biotechnol. 8:232-235.

Loc NH, Tam NT (2010). An Asiaticoside production from Centella (*Centella asiatica* L. Urban) cell culture. Biotech. Bioproc. Eng. 15:1065-1070.

Martin KP (2003). Rapid *in-vitro* multiplication and ex-vitro rooting of *Rotula aquatic* Lour., a rare rhoeophytic woody medicinal plant. Pl. Cell Rep. 21:415-420.

Martin KP (2004). Plant regeneration through somatic embryogenesis in medicinally important *Centella asiatica* L. *In vitro* Cell. Dev. Biol. Plant. 40:586-591.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays for tobacco tissue cultures. Physiol. Plant. 15:473-97.

Pant M, Bisht P, Gusain PM (2010). *In vitro* propagation through axillary bud culture of *Swertia chirata* Buch.-Ham ex Wall: an endangered medicinal herb. Int. J. Int. Bio.10:48-53.

Patra A, Rai B, Rout GR, Das P (1998). Successful plant regeneration from callus cultures of *Centella asiatica*. Pl. Growth Regul. 24:13-16.

Pattnaik SK, Chand PK (1996). *In vitro* propagation of the medicinal herbs *Ocimum americanus* Pl. Cell Reports 15:846-850.

Purohit SD (1994). Micropropagation of safed musli (*Chlorophytum borivilianum*), a rare medicinal herb. Plant Cell Tiss. Org. Cult. 39:93-96.

Rao KP, Rao SS, Sadanandam M (1999). Tissue culture studies of *Centella asiatica*. Indian J. Pharm. Sci. 61:392-394.

Sampath P, Muthuraman G, Jayaraman P (2001). Tissue culture studies in *Bacopa monnieri* and *Centella asiatica*.National Research Seminar on Herbal Conservation, Cultivation, Marketing and Utilization with Special Emphasis on Chattisgarh, The Herbal State, Raipur, Chattisgarh, India. 12:18-21.

Sangeetha N, Buragohain AK (2003). *In vitro* method for propagation of *Centella asiatica* (L.) urban by shoot tip culture. J. Plant Biochem. Biotechnol. 12:167-169.

Sen J, Sharma AK (1991). Micropropagation of *Withania somnifera* from germinating seeds and shoot tips. Plant Cell Tiss. Org. Cult. 26:71-73.

Sharma S, Sharma RK (2010). Seed physiological aspects of pushkarmool (*Inula racemosa*), a threatened medicinal herb: response to storage, cold stratification, light and gibberellic acid. Curr. Sci. 99(12):1801-1806.

Shukla A, Rasik AM, Jain GK, Shankar R, Kulshrestha DK, Dhawan BN (1999). *In vitro* and *in vivo* wound healing activity of asiaticoside isolated from *Centella asiatica*. J. Ethnopharmacol. 65:1-11.

Tiwari C, Bakshi M, Vichitra A (2013). A rapid two step protocol of *in vitro* propagation of an important medicinal herb *Centella asiatica* Linn. Afr. J. Biot.12:1084-1090.

Tiwari KN, Sharma NC, Tiwari V, Singh BD (2000). Micropropagation of *Centella asiatica* (L.) a valuable medicinal herb. Plant Cell Tiss. Organ Cult. 63:179-185.

Warrier PK, Nambiar VP, Ramankutty C (1994). Indian medicinal plants. A compendium of 500 species.Vol-I, Orient Lonhman Pvt Ltd, Chennai, India: pp.52-55.

Zheng CJ, Qin LP (2007). Chemical components of *Centella asiatica* and their bioactivities. J. Chin. Integ. Med. 5:348-351.

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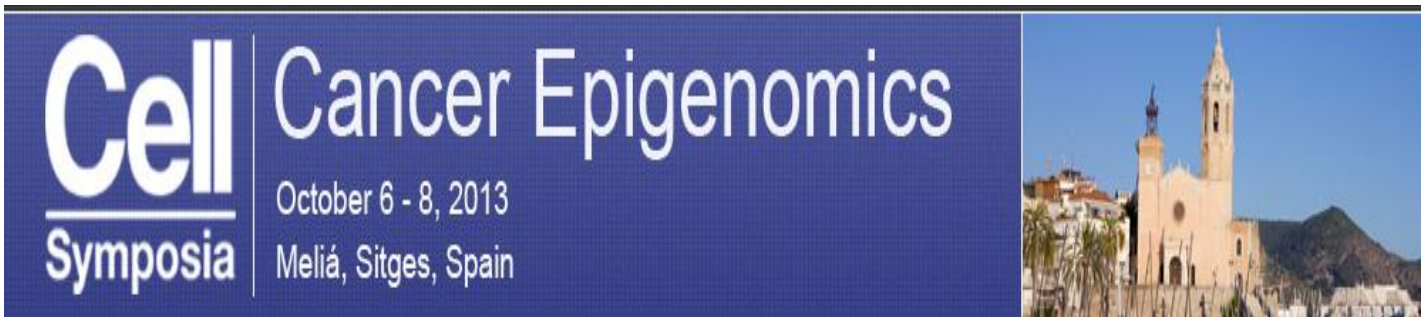

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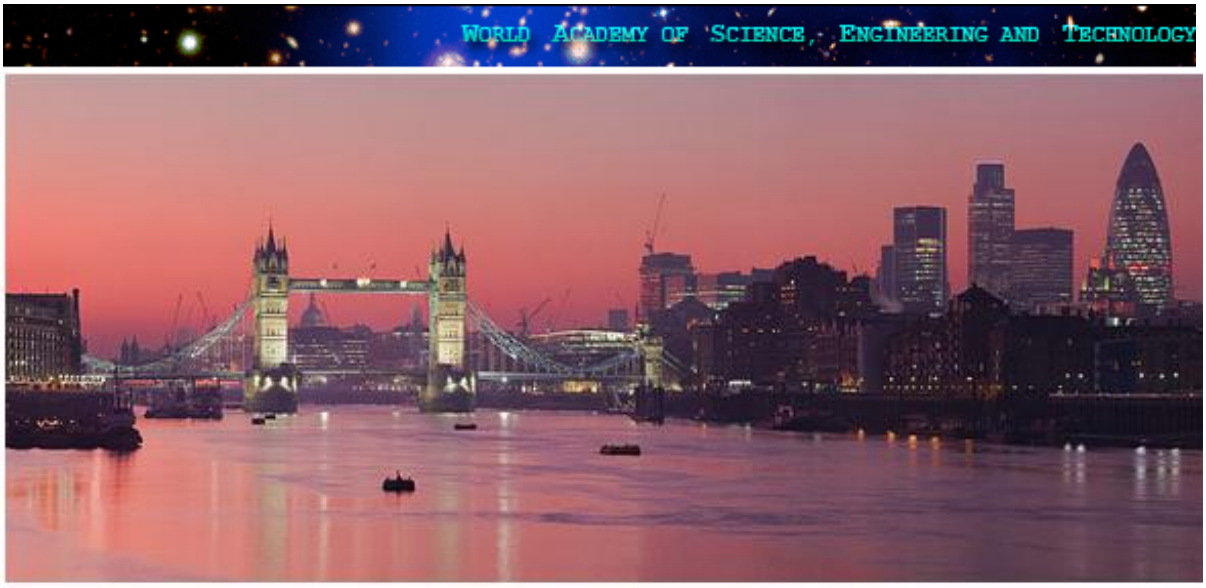

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