Pogostemon cablin (Blanco) Benth. (*Lamiaceae*): It's Ethnobotany & *in vitro* regeneration

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ABSTRACT

Objectives: Since the beginning of human civilization various herbal medicines are employed for healing human and animal. *Pogostemon cablin* (Blanco) Benth., locally known as *Patchouli* (Assamese) is a very important medicinal plants belongs to mint family i.e. *Lamiaceae*. The main aim of this study was to collect ethnobotanical information's and to study its *in vitro* regeneration results. All possible ethnobotanical literatures have been cited here. **Methods:** *In vitro* propagation was achieved from leaf and nodal explants of *Pogostemon cablin* on MS medium. **Results:** Callus development and *in vitro* axillary shoot formation was successfully made in MS basal medium containing BA (4.0 mg/L), NAA (2.0 mg/L) + IAA (1.0 mg/L) and BA (3 mg/L) + IAA (1 mg/L). MS basal medium containing IBA (0.1/L) and Kn (2.5 mg/L) was best for induction of multiple shoots within 4 weeks of culture. Combination of NAA (0.1 mg/L). Kn (0.1 mg/L) and CH (100 mg/L) was best for callus induction which later on formed multiple shoots and caused elongation of roots. Micro shoots of varied length were produced on MS medium. Rooted plantlets were successfully acclimatized in green house for 1 month and then were transferred to the field. **Conclusion:** It can be concluded that *pogostemon cablin* has immense ethno botanical importance. For its rapid multiplication, *in vitro* technique was found very successful. In MS medium supplemented with Kn 2.5 mg/L and IBA 0.1mg/L found maximum multiplication rate. In this proportion rates of shoot generation, leaf, rooting, callus formation was maximum.

Key words: Ethnobotany, *in vitro* study, MS medium, *Pogostemon cablin.*

Highlights of the paper :

- *Pogostemon cablin* (Blanco) Benth., locally known as Patchouli (Assamese)is a very important medicinal plants belongs to mint family i.e. Lamiaceae.
- A plant of immense ethno-botanical importance.
- After several trials, it was found that MS medium with Kn 2.5mg/L and IBA 0.1 mg/L gave a maximum multiplication rateof shoot, root and callus initiation. Hence, recommended.

INTRODUCTION

Medicinal plants have provided modern medicine with numerous plant derived therapeutic agents.[1] In aromatherapy, lots of medicinal plants are used, in which volatile plant materials are used, known as essential oils, and other aromatic compounds for the purpose of altering a person's mind, mood, cognitive function or health.[2] The anti-microbial effects has demonstrated from tea tree, but there is still a lack of clinical evidenced monstrating efficacy against bacterial, fungal, or viral infections.[3] Human culture has been augmented by plants and plant products since time immemorial. Perhaps ethnobiology is the first science that originated with the evolution of men in this planet.[4] The plant patchouli belongs to the family *Lamiaceae* under the genus *Pogostemon*, which comprises about 130 species and native to tropical Asia and is widely grown in India, Malaysia, Philippines, Indonesia and Singapore.[5-7]

The patchouli oil, obtained by steam distillation of shade-dried leaves is commercially used in perfumes and cosmetics.[8,9] Patchouli has insecticidal activities, anti-fungal and bacteriostatic properties.[10-12] Patchouli plants regeneration from stem tip, leaf and nodal callus, protoplasts

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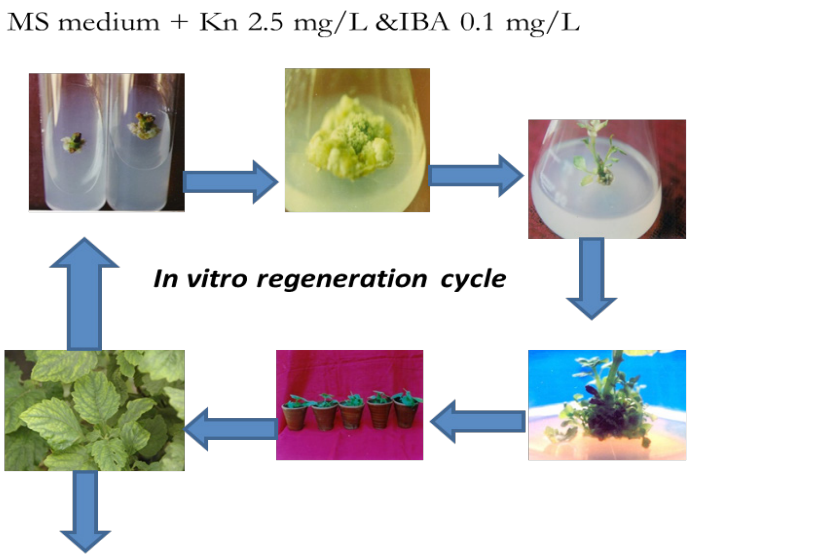

Graphical Abstract

encapsulated in alginate beads, somatic embryogenesis have been reported earlier.[13-17]

CLASSIFICATION[18,19]

- Domain : Eukaryota
- Kingdom : Plantae-Plants
- Subkingdom: Tracheobionta- Vascular plants
- Super division: Spermatophyta – Seed plants
- Division: Magnoliophyta – Flowering plants
- Class: Magnoliopsida – Dicotyledons
- Subclass: Asteridae
- Order: Lamiales
- Family: Lamiaceae– Mint family
- Genus: Pogostemon Desf. – pogostemon
- Species: Pogostemon cablin (Blanco)Benth. – Patchouli (Common name)

VERNECULAR NAMES[20-22]

Malaysia: Dhalum Wangi, Tilam Wangi, Nilam

English: Patchouli

Indonesia: Nilam Wangi (General), Nilam (Acheh), Singalon (Batak)

Thailand: Phimsen (Bangkok)

Vietnam: (Ho (aws) ch (uw) (ow) ng)

Philippines: Kabling (Tagalog); Katluen (Bisaya) Kadlum (Bikol, Bisaya, Sulu)

China: GuangHuo Xiang

Korea: Hyangdulkkaephul

India: Pachi (Sanskrit); Pachauli (Hindi); Pachapat,Patchouli (Bengali); Pachila, Kattam (Malayalam);Pacha, Sugandhipandi (Gujarati);Panch (Marathi)

French: Patchouli

Spanish: Pachuli

ETHNOBOTANICAL REPORTS

Cooling and tonic, is used in asthma cough and debilitating diseases.[23] Whole plant ash used to kill worms in animal wounds.[24] Because of its primary antiseptic properties, it is used to treat athlete's foot, dandruff, wounds and scars. It gives relief from constipation and acts as an antidote against insect bites temporarily. Patchouli alcohol is a fragrance ingredient used in decorative cosmetics, fine fragrances, shampoos, toilet soaps, non-cosmetic products such as household cleaners and detergents.[25] It also used as daily dosage along with other herbs for treatment of asthma.[26]

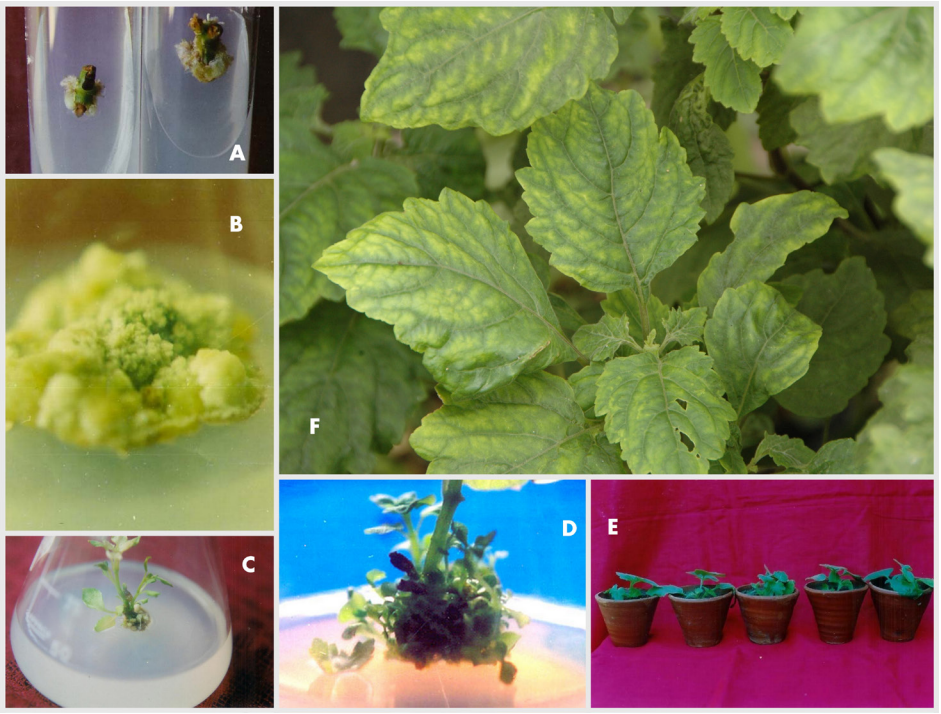

Figure 1: A. Callus initiation; B. Callus development; C. Root initiation from callus D. Multiple shoot formation; E. transferred to pots; F. A *Pogostemon cablin* twig.

MATERIALS AND METHODS

Plant Material

Nodal stem with axillary buds were used as explants were surface sterilized with 70% alcohol and then with 0.01% $HgCl_2$ solution. The explants were immersed in 100 ml of tap water containing 1-2 drops of extra for 5 minutes and later washed with sterile distilled water. The stem segments were further cut into 1 cm pieces having one node and were used as explants.

Culture media and condition[27]

Medium supplemented with PGRs–BA, 2, 4-D, IAA, IBA, Kn, and NAA was used under aseptic condition. Myo-inositol 100 mg/1 (wt/vol), sugar (3%), casein hydrolysate (100 mg/1) were used in the media for shoot bud proliferation. The p^H of the media was adjusted to 5.7 and solidified with agar (0.8%). The medium was sterilized in an autoclave at 121°C for 15 minutes. Aseptic condition was maintained throughout the whole operation. Cultures were maintained at 25 ± 2°C under fluorescent light of about 2500–3000 lux with 16 hr photoperiod/day.

For further multiplication

For acclimatization and transfer of plants to soil rooted shoots were removed from culture flasks and washed dipped in 0.2% bavistin fungicide for 5 minutes and plantlets were potted in a sterilized mixture of garden soil and sand. They were irrigated with half strength MS solution for 1 week and subsequently with sterile distilled water. The plantlets were acclimatized under laboratory condition before transferring to Green House and then to natural condition.

RESULTS AND DISCUSSION

In the present study, different combination of BA, IBA, IAA, Kn, NAA and 2, 4-D were tried. Among the combination tried, MS medium supplemented with Kn 2.5 mg/L and IBA 0.1 mg/L gave a maximum multiplication rate with 72.33 ± 0.80 shoot number (Figure 1, D), 15.64 ± 0.44 cm shoot length (Figure 1, C), 5.7 ± 3.10 leave no and 3.3 ± 1.28 no's of roots (Table 1).Callus formation was also maximum in this concentration (Figure 1, A & B). It was followed by Kn (0.5 mg/L)+IBA (0.5 mg/L) concentration which gave multiplication rate 30.0 ± 3.08 shoot number, shoot length 2.2 ± 0.57cm, 5.25 ± 2.70 leave no and 20.0 ± 0.58 no's of roots with well-developed callus.

When BA was added in MS medium at concentration ranging from 0.1 to 4.0 mg/L with NAA and IAA not much development of shoots observed on lower concentration. But in BA (4.0 mg/L) with NAA (2.0 mg/L) and IAA (1.0 mg/L) resulted higher rate of shoot multiplication with 58.0

Table 1: Effect of Plant Growth regulators on MS medium

MS Medium+PGRS (mg/L)							Intensity of Development			Observation after 30 days			
BA	NAA	IAA	IBA	Kn	2,4-D	CH	C	S	R	No.of shoots	Shoot length	No.Of leaf	No.of roots
0.1	05	-	-	-	-	-	++	++	-	2.3±0.57	2.53±0.68	2.5±0.70	1.5±0.57
0.5	1.0	-	-	-	-	-	++	++	-	1.5±0.57	2.63±0.89	5.68±1.49	0
1.0	0.5	-	-	-	-	-	++	+++	++	14.3±0.66	06.5±0.31	4.75±1.705	2.0±0
3.0	-	1.0	-	-	-	-	+++	+++	++	17.00±.44	05.2±0.17	1.60±0.06	18.0±0.58
4.0	2.0	1.0	-	-	-	-	+++	+++	+	58.00±1.0	09.17±0.37	1.37±0.03	25.3±0.88
-	0.1	-	-	0.1	-	50	+++	+++	+++	27.67±2.02	7.80±0.15	1.23±0.03	5.0±0.40
-	0.5	-	-	0.1	-	50	+	+++	++	16.00±01.0	6.13±0.20	1.17±0.06	4.6±1.53
-	1.0	-	-	0.1	-	50	+	++	+	12.33±0.33	6.03±0.13	1.27±0.03	3.3±0.57
-	2.0	-	-	0.1	-	50	+	+	+	10.00±0.58	9.97±0.23	1.47±.03	1.5±0.57
-	3.0	-	-	0.1	-	50	+	+	+	1.5±0.57	1.17±0	1.5±0.29	1.25±0.50
-	-	-	-	0.1	0.1	100	+	++	++	1.5±0.57	1.62±0.85	3.5±1.29	1.50.57
-	-	-	-	-	1.0	100	+	+++	-	1.75±0.50	1.62±0.63	5.0±0.82	-
-	-	-	-	1.0	0.5	100	+	++	++	1.25±0.50	1.17±0.69	3.5±1.29	2.00.82
-	-	-	-	1.0	1.5	100	+	+++	-	2.5±0.57	2.25±0.95	2.5±0.57	-
-	-	-	-	1.0	2.5	100	+	+++	-	1.5±0.57	1.12±0.57	1.5±0.57	-
-	-	-	0.5	0.1	-	-	+	+++	+	2.3±0.57	2.53±0.68	5.68±1.49	1.5±0.57
-	-	-	1	0.1	-	-	+	++	-	1.5±0.57	2.63±0.89	4.75±1.70	0
-	-	-	0.5	0.5	-	-	+	+++	+	30.0±3.08	2.2±0.57	5.25±2.70	20.0±0.58
-	-	-	0.5	1.5	-	-	+	+++	-	2.0±0.0	2.65±0.96	4.42±1.65	0
-	-	-	0.1	2.5	-	-	+	+++	+	72.33±0.88	15.64±0.44	5.7±3.10	3.3±1.28

C- Callus, S- Shoot, R- Root ; +++ →Excellent, ++ → Good, + → Positive

± 1.0 shoot number, 0.9 ± 0.37 cm shoot length, 1.37 ± 0.03 leaf number and 25.3 ± 0.88 nos. of root. Also callus formation was excellent in this combination. Even lowering the kinetin concentration to 0.1 mg/L with NAA 0.1 mg/L and CH 50 mg/L a good number of shoots 27.67 ± 2.02, 7.80 ± 0.15 cm shoot length, 1.23 ± 0.03 leave no and 5.0 ± 0.40 no of roots were noticed at laboratory stage (Table 1).

The callus induction was found to be good with combination of Kn (1.0 mg/L)+2,4-D (2.5 mg/L)+CH (100 mg/L). The combined effect of NAA (0.1 mg/L)+Kn (0.1 mg/L)+CH (100 mg/L) produced best callus which later developed both shoot and root in MS medium. With increase in concentration of auxin the two axillary buds developed only a few shoots. Development of callus was also found in combination of BA (3.0 mg/L) + IAA (1.0 mg/L), Kn (1.0 mg/L)+2, 4-D (1.5 mg/L, 2.5 mg/L)+CH (100 mg/L) in MS medium. Multiple shoot formation also resulted in combinations of BA (3.0 mg/L)+IAA (1.0 mg/L) and in BA (1.0 mg/l)+NAA (0.5 mg/L) After 40 days plants are transferred to pots for better growth (Figure 1, E & F).

CONCLUSION

From this study it can be concluded that *pogostemon cablin* has immense ethno botanical importance. Due to its enormous importance and demand, the mass propagation through *in vitro* technique was found very successful. After successful experiments with lots of combinations, it has been found that MS medium supplemented with Kn 2.5 mg/L and IBA 0.1 mg/L gave a maximum multiplication rate. In this proportion rates of shoot generation, leaf, rooting, callus formation was maximum.

ABBREBIATION

MS : Murashige and Skoog (1962) medium,
BA : N^6 Benzyladenine,
IBA : Indole-3-Butyric Acid,
IAA : Indole-3-Acetic Acid,
NAA : Naphthelene Acetic Acid,
Kn : Kinetin,
2,4-D : 2,4 Dichloro Acetic Acid,
CH : Casein Hydrolysate

CONFLICTS OF INTEREST

The authors are declared that there is no conflict of interest.

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