Indian J. Genet., **67**(2): 187-189 (2007)

Short Communication

Organogenic regeneration from different explants of patchouli (*Pogostemon cablin*)

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(Received: June 2006; Revised: October 2006; Accepted: November 2006)

Patchouli [*Pogostemon cablin* Benth. (syn. *P. patchouli* Pellet)] a native of South East Asia, is a hardy perennial herb adapted to hot and humid climatic conditions. Oil from patchouli blends with other essential oils and is a basic ingredient of high value perfumes, cosmetics and toiletries because of its strong fixative properties. However, the feasibility of mass production of patchouli has been limited due to the recurrence of mosaic virus, root knot nematode and insect pests. This plant never or very rarely flowers and hence needs to be propagated vegetatively. Feasibility of mass propagation of high yielding and disease/pathogen resistant patchouli through tissue culture approaches has been envisaged. *In vitro* studies on clonal propagation [1-3] and regeneration from callus [4, 5] have been reported. Present study reports high frequency organogenic regeneration from leaf and nodal explants of an elite strain that can be suitably employed for large-scale propagation.

An exotic superior strain of patchouli (Indonesian) was obtained from WIMCO seedlings [Rudrapur, U.S. Nagar, Uttaranchal]. The leaf and nodal bud explants from field grown plants were used for *in vitro* studies. The explants were washed thoroughly under running tap water, surface sterilized with 0.1% $HgCl_2$ for 5 min followed by treatment with 20% sodium hypochlorite for 10 min. The explants were then washed 5x with sterile distilled water. Basal MS medium of Murashige and Skoog supplemented with 3% sucrose and gelled with 0.8% agar was used. The medium was supplemented with different concentrations of BAP (2-3 mg/l) and auxins of either NAA (0.2 mg/l) or IAA (1 mg/l) for organogenic shoot induction. The cultures were incubated at 25±2°C under 16h light with 2000-3000 lux provided by cool white fluorescent tubes. After four weeks, data was recorded for either callus induction or callus with shoots. These calluses were then transferred to MS medium supplemented with 0.5 mg/l BAP and 0.1 mg/l NAA. Data was recorded after four weeks on this media with a total period of eight weeks of culture for number of shoots induced. The proliferated and elongated shoots were transferred to rooting medium which is MS supplemented with 0.5 mg/l NAA. These plantlets were then transferred to plastic pots containing vermiculite, sand, soil and manure in 1:1:1:1 ratio for hardening in a chamber with 80% relative humidity. These plants after establishment in soil were shifted to bigger pots and kept in the natural environment for further growth.

Leaf and nodal explants were cultured on MS medium supplemented with different concentrations of BAP alone or in combination with auxin (NAA or IAA). After 8 weeks of culture nodal explants showed a single shoot only when medium was not supplemented with any of the growth regulators (Table 1). The media containing BAP alone or in combination with auxins showed induction of either callus or callus with shoots after 4 weeks of culture (Fig. 1a&b). These calluses with or without shoots were then transferred to MS medium containing 0.5 mg/l BAP and 0.2 mg/l NAA. After 4 weeks of culture on this medium it was observed that the explants initially cultured on media containing 2 and 3 mg/l of BAP alone showed 4.2 and 3.3 average number of shoots per nodal explant, respectively. While the explants cultured on medium containing 2 mg/l BAP when supplemented with 0.2 mg/l NAA showed 9.2 shoots per explant and when BAP containing media was supplemented with 1 mg/l IAA then 16.3 shoots per nodal explant were obtained (Fig. 1c). Thus, in comparison to medium containing 2 mg/l BAP the medium containing 2 mg/l BAP and 1 mg/l IAA showed a four fold increase in number of shoots per nodal explant. Nodal explants cultured initially on a medium containing 3 mg/l BAP showed 3.3 shoots per explant. However when the same 3 mg/l BAP containing medium was supplemented with either 0.2 mg/l NAA or 1 mg/l IAA auxins then on an average 13.5 and 15.3 shoots per explant were observed. Thus it seems that BAP (2 or 3 mg/l) when combined with 1 mg/l IAA showed a synergistic effect in enhancing the shoot formation

capacity from nodal explants via morphogenic callus induction. Therefore, it can be said that IAA acts as a better supplement with BAP for multiple shoot induction from the nodal explants.

Leaf explants when cultured on media containing 2 and 3 mg/l BAP cytokinin followed by transfer to medium containing 0.5 mg/l BAP and 0.2 mg/l NAA showed 6.5 and 10.7 average number of shoots per explant, respectively after 8 weeks of culture. Leaf explants showed 16 shoots per explant on a medium containing 2 mg/l BAP and 0.2 mg/l NAA as compared to 12.4 shoots per explant when medium contained 2 mg/l BAP and 1 mg/l IAA (Fig. 1d). Thus, there was 2.5 fold increase in number of shoots when medium contained 2 mg/l BAP and 0.2 mg/l NAA over medium containing 2 mg/l BAP alone. Likewise when medium contained 3 mg/l BAP alone 10.7 shoots per explant were obtained and there was increase in number of shoots to 13.8 per explant when BAP medium was further supplemented with 0.2 mg/l NAA. However, number of shoots declined to 12.4 per explant when 3 mg/l BAP containing medium was supplemented with 1 mg/l IAA. Thus with leaf explants medium containing 2 mg/l BAP and 0.2 mg/l NAA was found to be the best for multiple shoot induction followed by medium with 3 mg/l BAP and 0.2 mg/l NAA. The role of cytokinin alone in shoot bud proliferation [3] and BAP cytokinin with auxin [2] has been reported. However, the present study clearly demonstrates the synergistic effect of auxin and cytokinin in enhancing shoot induction. The different explants showed better response under different growth regulators regime. Higher number of shoots were obtained on MS medium supplemented with 2 mg/l BAP and 1 mg/l IAA from nodal explants and on MS medium with 2 mg/l BAP and 0.2 mg/l NAA from leaf explants. The proliferated and elongated shoots when transferred to medium containing 0.5 mg/l NAA showed good rooting within 2-3 weeks of culture (Fig. 1e). The plants with vigorously growing root system

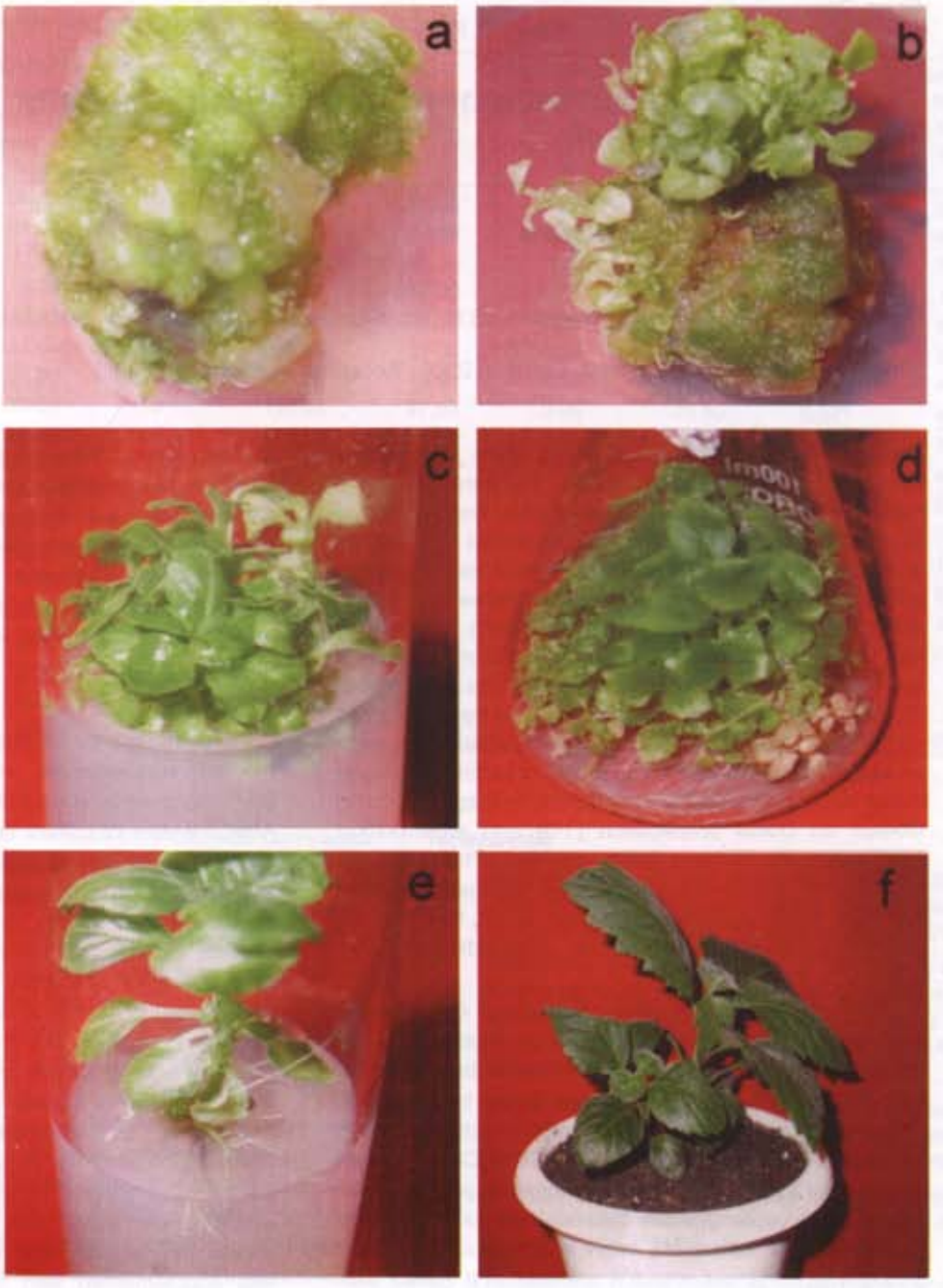

Fig. 1. Different stages of regeneration: a - callus; b - callus with shoots; c - multiple shoot induction; d - multiple shoots with roots; e - root induction from shoot; f - potted plant

Table 1. Response of shoot induction from different explants of patchouli on MS medium with different concentrations of growth regulators

Media	Response after four weeks			Response after eight weeks		
	No. of explants	Cal	Cal with sh	Av. No of sh from cal	Av. No of sh from cal with sh	Overall Av. No. of sh/ explant
Nodal explant						
MS_0	20	-	20	-	1.0	1.0
MS_{B2}	20	4	16	3.5	4.3	4.2
$MS_{B2N0.2}$	22	4	18	2.0	11.0	9.2
MS_{B2I1}	16	4	12	8.5	18.8	16.3
MS_{B3}	22	14	8	2.1	5.2	3.3
$MS_{B3N0.2}$	44	14	30	14.4	13.0	13.5
MS_{B3I1}	20	4	16	7.5	17.2	15.3
Leaf explant						
MS_0	26	22	4	2.1	3.0	2.3
MS_{B2}	20	16	4	9.3	5.0	6.5
$MS_{B2N0.2}$	16	6	10	13.3	17.6	16.0
MS_{B2I1}	14	-	14	-	12.4	12.4
MS_{B3}	26	-	26	-	10.7	10.7
$MS_{B3N0.2}$	18	8	10	11.2	16.0	13.8
MS_{B3I1}	16	-	16	-	12.4	12.4

[Suffix as B - BAP; I - IAA; N - NAA and numbers indicate concentration of growth regulators in mg/l; Cal - Callus, Sh - Shoots]

were transferred to pots for hardening and acclimatization (Fig. 1f). This protocol is being extensively used for large scale production of plants.

Acknowledgements

Financial support from DBT, India is thankfully acknowledged.

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