Environment & Ecology 29 (3) : 1152—1155, 2011
 ISSN 0970-0420

Somaclonal Variation in Patchouli (*Pogostemon patchouli* Pellet.)

K. M. RAMACHANDRA, V. B. NARAYANPUR AND A. A. FAROOQI

Division of Horticulture, University of Agricultural Sciences, GKVK Bangalore 560065, India

Abstract

A procedure for high frequency plant regeneration from leaf explant culture of patchouli was standardized. Murashige and Skoog medium supplemented with 2, 4-dichlorophenoxyacetic acid (2 mg/liter) along with 6-benzyl aminopurine (0.1 mg) supported the callus production and regenerated callus were repeatedly subcultured at 15 days interval. After eighth subculture, callus were transferred to media containing 6-benzyl amino purine (2 mg/liter) along with α–naphthalene acetic acid (0.1 mg/liter) for shoot production and *in vitro* derived shoots readily developed roots when cultured on half strength MS medium. Regenerated plantlets were successfully transferred to field with high rate of establishment. A wide range of variation was observed in morphological characters, especially in leaf pigmentation. The leaves of one variant (Sc-1) had variegation with whitish yellow pigments which looks quite ornamental. However, quantitative and qualitative traits failed to show any significant difference to the parental plant.
Key words : Patchouli, Somaclonal variation, Plant regeneration.

Patchouli (*Pogostemon patchouli* Pellet. Syn. *Pogostemon cablin* Benth.) belonging to the family Lamiaceae, is an important source of patchouli oil used in soaps, perfumery, cosmetic and flavoring industries. It is a vegetatively propagated crop and virtually there is no scope for generating variability by conventional breeding methods based on mating system, whereas in spite of flowering the seed setting either does not occur or is poor. Non-fertile patchouli exhibit narrow genetic variations. Therefore, somaclonal breeding has been quite useful and recommended for enhancing desirable variation in patchouli. Under a genetic improvement program aimed at developing high oil yielding strains possessing desirable oil composition, the present study focuses the role of tissue culture in generating variability, standardization of an efficient procedure for long term high frequency plant regeneration by *in vitro* method, screening of somaclones for various economic traits and evaluation of performance of selected somaclones in field condition.

Methods

Cuttings of commercial cultivar 'Johore' was planted in earthen pots and plants sprouted there from were used as parent source for obtaining explants in tissue culture experiments. Leaf explants from 5-month old parent plant maintained under controlled conditions in glasshouse, were washed with distilled water along with tween-20 and surface sterilized with 0.1% mercuric chloride solution for 1 minute before implanting on the nutrium medium of Murashige and Skoog medium supplemented with sucrose (3%) and agar (0.8%). Auxins like 2, 4-D (0.5 to 2.0 mg/liter) and NAA (0.5 to 2.0 mg/liter) along with BAP (0.1 mg/liter) were supplemented to basal medium in different con-

Table 1. Performance of selected somaclones in relation to plant height and number of branches in Patchouli (*Pogostemon patchouli* Pellet.)

Treatments	Plant height (cm)	Number of branches/ plant
Control	82.00	34.00
Sc—1	81.00	33.00
Sc—2	79.00	36.00
Sc—3	80.00	32.00
Sc—4	78.00	34.00
Sc—5	83.00	35.00
Sc—6	80.00	33.00
Sc—7	81.00	34.00
Sc—8	79.00	33.00
Sc—9	80.00	34.00
Sc—10	82.00	35.00
Mean	80.45	33.90
F-test	NS	NS
SE ±	2.33	2.00

Table 2. Performance of selected somaclones in relation to herb yield, oil content and oil yield in patchouli (*Pogostemon patchouli* Pellet.)

Somaclones	Herb yield/harvest/ha (t)	Herb yield ha/yr (t)	Oil content (%)	Oil yield/harvest/ha (l)	Oil yield ha/yr (l)
Control	0.93	2.79	2.4	22.68	68.04
Sc—1	0.91	2.73	2.5	22.81	68.43
Sc—2	0.95	2.85	2.3	21.77	65.31
Sc—3	0.93	2.79	2.4	22.68	68.04
Sc—4	0.95	2.85	2.3	21.77	65.31
Sc—5	0.95	2.85	2.3	22.14	66.42
Sc—6	0.93	2.79	2.4	22.30	66.90
Sc—7	0.91	2.73	2.5	22.81	68.43
Sc—8	0.95	2.85	2.3	22.47	67.41
Sc—9	0.91	2.73	2.5	22.81	68.43
Sc—10	0.95	2.85	2.3	22.77	68.31
Mean	0.94	–	2.3	22.36	–
F-test	NS	–	NS	NS	–
SE ±	0.04	–	0.24	2.18	–

centration and combination. The pH of the media combination was adjusted to 5.8±1 before autoclaving at 1.04 kg cm^2 at 121 C for 15 minutes. Cultures were incubated at 25±3C temperature under 1500 to 2000 Lux light intensity. The regenerated callus were repeatedly subcultured at 15 days intervals. After 8th subculture, healthy callus were transferred to shoot regeneration medium consisting of BAP (0.5 to 2.5 mg/liter) alone and in combination with NAA (0.1 mg/liter) and *in vitro* derived shoot readily developed the roots when cultured on half strength MS medium. Healthy rooted 200 plants were transferred to thumb pots in hardening trays and hardened in glass house under high humidity conditions and were subsequently transferred to field along with the parents plant to serve as control. All these plant were maintained in the field following standard agronomic practices.

Observation on five agronomic traits namely plant height, number of branches per plant, herbage yield, oil content and oil yield were recorded indivudally after 5 months of planting in the field. The ten plants which performed better among the 200 plants with respect to quantitative and qualitative traits were selected. Following initial screening, cutting from each somaclones were taken for further evaluation. These somaclones were planted in the field in there replications along with control plant and observations were recorded at the harvest.

After five months of planting, plants were readily for harvesting. The harvesting was done with the help of secreature at 0.30 m above the ground and herb yield was recorded under each treatment, separately. The oil estimation was done by using clavenger's apparatus and qualitative assessment of oil sample was performed by gas chromatograph analysis.

Results and Discussion

Leaf explants cultured on MS medium supplemented with Auxins (2, 4-D and NAA) in the range of 0.5 to 2.0 mg/liter and cytokinin (BAP) in 0.1 mg/liter induced the callus. The treatment, 2 4-D (2 mg/liter) and BAP (0.1 mg/liter) was optimum for this response, while NAA was least effective as compared to 2, 4-D. Shoot development from healthy callus was enhanced by supplementing BAP (0.5 to 2.5 mg/liter) along with NAA (0.1 mg/liter) containing MS medium. Addition of NAA along with BAP resulted in multiple adventitious shoots from the hard compact green callus as compared to BAP alone. BAP (2 mg/liter) along with NAA (0.1 mg/liter) was optimal and produced the maximum shoots. Occasionally, a few small and thick roots also emerged from the basal nodes of regenerated shots. However, subsequent growth of these roots was not satisfactory. Half strength MS medium proved to be optimal for rhizogenesis among different Auxins (IAA and NAA) tested and 80—90% of cultures exhibited root induction with 2—3 weeks of incubation . Among the treatments tried with respect to cost of production, half strength MS medium was optimal to get better rooting. Results revealed that hormonal requirements for various morphogenetic response in patchouli is largely confirmed with results obtained by Bharati (1) and Shashikala (2) in patchouli. Following the above procedure for tissue culture, *in vitro* raised plantlets were transferred to thumb pots in hardening trays and maintained in the glass house. A high survived rate was evident when the plant were transferred in the field where they grew into normal plant.

Analysis of data recorded on each somaclones revealed little variation in their qualitative and quantitative traits. Considerable variation was observed in the color of the leaves, but there is no significant

difference in growth and yield parameters. Even in the composition of oil also, we observed only a slight variation with respect to patchouli alcohol content, which is of major economic significance in patchouli oil. From the results it was observed that somaclone - 1 (Sc-1) recorded 33.82% of patchouli alcohol as against the control.

Among the somaclones tried, somaclone-1 (Sc-1) showed variegation in the leaf color. Five successive generation were tried by taking the cuttings from variegated parent plant to confirm the variegation. Even after the five generations also the plant showed similar variation in their leaf colour. Such variegation were also observed by Mathure et al. (3) in citronella, where he detected red pigment leaves. Similar observations were also reported by Yadav et al. (4) in wild *Cymbopogan* sp. where he encountered a wide range of marpho-physiological variations amongst the regenerates with reference to leaf pigmentation. He also reported that about 4—6% of regeneration were completely albinos and no incidence of reversal was observed. These mutations are generally called as somaclonal variations. Bajaj (5) and Philips et al. (6) also studied the application of this approach in plant breeding in various species. The mutants are the criteria to know the extent of variation and provide the indication for quantitative and qualitative variation in crop improvement program.

The most important question with respect to somaclonal variation is related to the origin and the understaying mechanism of such variations. Evidence have accumulated that most of the variation have a genetic or at least epigentic basis. The nature, extent and possible origin of somaclonal variation have been reviewed from various points. These variation might be a long term culture cycle (7), callus culture Skirvin and Janick (8), use of explants from specified tissues (9), growth medium (10, 11) and hormone / growth regulators effect (12).

Recently, molecular studies have indicated that the plant genome is remarkably unstable, capable of undergoing changes, thus, generating variability. During vegetative or asexual propagation, these variations might be enhanced by cryptic chromosomal changes such as small additions and transition or transversion. Besides, *in vitro* genomic instability may be caused largely by changes in the repetitive DNA sequence, which is more common in plants (13)

The summaries, evidence has been presented that genetic variability results from the regeneration of patchouli from leaf explant by passing callus interphase. From the present work an approach for developing improved clones *Pogostemon patchouli* for herb and oil yield can be adopted, where in probably large populations are necessary to isolate specific somaclones statistically superior than control under field condition. The albinos variant had three colored patches on the leaves it looks like ornamental rather than aromatic. Since it is a herbaceous perennial plant, it can be grown in pots for decorative purpose, apart from this ornamental value, the plant produces, the characteristic patchouli odor during its maturity, which is sensually evocative and deeply releasing fragrance and insect repellent nature of the oil, make the patchouli premises fragranceable and free from insects. This findings suggests that, *in vitro* conditions do induce somaclonal variation but the analysis of stability behavior of these *in vitro* raised genotypes is extremely important in the genetic improvement programs of patchouli using somaclonal breeding as one of the alternative approach.

References

1. Bharati N. 2002. Biotechnology in commercial production of patchouli in NE Region. Pp. 46—51. *In Patchouli.* Nat. Works. on commercialization of patchouli in NE region. N.E.D.F.C. & NHB 9—11 Apr. 2002. Guwahati, Assam, India.
2. Shashikala R. 1997. *Studies on micro propagation and production of metabolites in patchouli.* M. Sc. thesis. Uni., Agri. Sci., Bangalore, India.
3. Mathure A. K., P. S. Ahuja, B. Pandey, A. K. Kukreja, S. Mandal. 1998. Screening and evaluation of somaclonal variations for quantitative and qualitative traits in a aromatic grass (*C. winterianus*).*Pl. Breed.* 101 : 321—334.
4. Yadav U., A. K. Mathur and N. S. Sangwan. 2000. *In vitro* calli clone regeneration to enhance qualitative variability in essential oil of a wild *Cymbopogon* sp. *J. Med. Arom. Pl. Sci.* 22 : 485—487.
5. Bajaj Y. P. S. 1990. Somaclonal variations—origin, induction, cryopreservation and implication in plant breeding. *In Biotechnology in agriculture and forestry—II.* Publ. Springer—Veralg, Berlin Heideberg, New York, USA. 3—48 pp.
6. Philips R. L., S. M. Kaeples and P. Olhoft. 1994. Genetic instability of plant tissue cultures : break down of normal controls. *Proc. Nat. Acad. Sci. US* 91 : 5522—5526.
7. Bush S., E. Earle and R. W. Langhans. 1976. Plantlets from petal segments, petal epidermis and shoot

tip of the periclinal chimera ; *Chrysanthemum morifolium. Am. J. Bot.* 63 : 729-737.
8. Skirvin R. M. and S. Janick. 1978. Natural and induced variation in tissue culture. *Int. J. Trop. Agric.* 27 : 241—266.
9. Van Harten A. M., H. Bouter and C. Broerties. 1981. *In vitro* adventitious bud techniques for vegetative propagation and mutation breeding of potato (*Solanum tuberosum*) ; significant for mutation breeding. *Euphytica* 30 : 1—8.
10. Bayliss M. W. 1980. Chromasomal variation in plant tissue culture. Pp 113—144. *In* I. K. Vasil, *(ed). Perspective in plant cell and tissue culture.* Publ. Academic Press, New York, USA.
11. Furner I. J., J. King and O. L. Gamborg. 1978. Genetic resources in plants, their exploration and conservation. *Pl. Sci. Lett.* 11 : 169—176.
12. Damato F. 1975. The problem of genetic stability in plant tissue and cell culture. Pp. 343—358. *In* Frankel O. H. and J. G. Howkes. (eds). *Crop genetic resources for today and tomarrow. Cambridge Univ. Press, Cambridge,* USA.
13. Patnaik J., B. K. Debata and S. Shahoo. 2000. Somaclonal variation in cell suspension culture—desired regenerates of *Cymbopogon martinii* (Roxb.) Wats Var. Motia. *Pl. Breed. (In Press).*