Environment & Ecology 29 (3) : 1143—1145, 2011
 ISSN 0970-0420

Mutagenic Effect of Ethyl Methane Sulphonate (EMS) on Growth, Yield and Composition of Oil in Patchouli (*Pogostemon patchuli* Fellet.)

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Abstract

Mutagenic effect of different concentrations of EMS was studied to develop high yielding mutant in patchouli. The *in vitro* developed callus were treated with 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5% EMS solution for 1 hour and the treated callus were cultured on regeneration media. The regenerated plants were screened in the field for their quantitative and qualitative traits. The study results in isolation of several economically useful mutant showing variation in agronomic and essential oil characteristic. The vegetative growth shows the inverse relationship with concentration of EMS solution. Whereas, high frequency of alteration in oil content and oil yield was noticed in several treatments, especially in the plants obtained from the callus treated with 0.3% EMS solution which exhibited the pronounced effect in enhancing oil content from 2.4 to 3.6% and oil yield from 67.56 to 86.31 liters per hectare per year.

Key words : Mutagenic effect, Ethyl methane sulphonate, Growth, Yield, Patchouli.

Patchouli (*Pogostemon patchouli* Pellet. Syn. P. cablin Benth.) is an important aromatic crop and source of commercial Patchouli Oil available in domestic and international market. A native of Philippines it grows wild in many South Asian countries and is presently cultivated on commercial scale in Indonesia, Malaysia, China and Brazil. The commercial oil used in the perfumery industry is obtained by steam distillation of shade dried leaves. Though it is not a dominant source of fragrance, its blending with other essential oils and attars provides a strong base for lasting character, fixative properties and aids prevention of evaporation thus, promoting tenacity. The oil is widely used in manufacture of soaps, scents, body lotions, detergents etc. It is also used in flavouring tobacco, alcohol, beverage. As it is a vegetatively propagated crop the available genetic variation in this crop is narrow. Traditional breeding methods were also not successful in breaking the yield barriers as the plant rarely flower and does not set seeds. Therefore, considerding an alternative to conventional breeding technique, present study was designed for induction of mutation using ethyl methane sulphonate (EMS) for genetic variability, so as to improve the quantitative and qualitative traits.

Methods

In vitro developed callus were treated with different concentrations (0.05, 0.1, 0.2, 0.3, 0.4 and 0.5%) of EMS solution. The stock solution of EMS of different concentrations was prepared by dissolving the required quantity of EMS in known quantity of double distilled water. The stock solution was prepared under laminar air flow chamber at 27 ± 2^0C with 75% relative humidity. The well developed callus were agitated in different concentrations of EMS solution for 1 hours and treated callus were transferred to culture room. The pre-requisite EMS response curve was first established and LD_{50} was determined. The treated callus were subcultured for 5 cycle at 12 days intervals and cultured in regeneration media. Healthy plant-

Table 1. Plant height, number of branches and leaf area as influenced by EMS treatment in the plants obtained from the callus of patchouli (*Pogostemon patchouli* Pellet.).

	Treatments	Plant height (cm)	Number of branches/ plant	Leaf area/ plant (cm^2)
T_1	Control	82.5	35.75	4555.75
T_2	0.05	78.5	31.50	4344.00
T_3	0.1	68.5	26.75	3836.50
T_4	0.2	64.5	21.00	3247.00
T_5	0.3	62.5	19.75	3082.25
	Mean	71.30	26.95	3825.1
	F-test	*	*	*
	SE ±	1.04	0.98	31.37
	CD 5%	3.21	3.02	96.67

Table 2. Herb yield, oil content and oil yield as influenced by EMS treatment in the plants obtained from the callus of patchouli (*Pogostemon patchouli* Pellet).

	Treatments	Herb yield/harvest/ha (t)	Herb yield/ha/yr (t)	Oil content (%)	Oil yield/harvest/ha (I)	Oil yield ha/yr (I)
T_1	Control	0.93	2.79	2.4	22.52	64.25
T_2	0.05	0.91	2.73	2.5	22.99	68.97
T_3	0.1	0.86	2.58	2.9	25.15	75.45
T_4	0.2	0.82	2.46	3.3	27.52	82.56
T_5	0.3	0.79	2.37	3.6	28.77	86.31
	Mean	0.86	–	2.94	25.39	–
	F-test	*	–	*	*	–
	SE ±	0.12	–	0.07	0.97	–
	CD 5%	0.03	–	0.24	3.00	–

lets were hardened and the treated plants along with control plants were transplanted in the field at 60 × 60 cm spacing under uniform field condition. Cultural operations were carried out at timely interval and after five months of planting, plants were harvested observation on five agronomic traits viz., plant height, number of branches, leaf area, herb yield, oil content and oil yield were recorded at the time of harvesting.

Oil estimation of dry herb was done by hydrodistillation using Clavanger's apparatus. The percentage of oil was calculated on moisture free basis and quality was assessed with the help of gas chromatographs. The mean value for all the economic traits were subjected to statistical analysis (randomized complete block design) for different characters pairs at different treatments.

Results and Discussion

The treatments of plant cells and tissues in *in vitro* with chemical mutagen were found to enhance the spectrum and frequency of variations in many plants. In the present investigation also the callus treated with different concentration of EMS solution showed a wide variability in both quantitative and qualitative traits.

Growth Parameters

The vegetative growth showed significant difference among the treatments (Table 1). The callus treated 0.3% EMS solution recorded the minimum plant height (62.50 cm), number of branches (19.75/plant) and leaf area (3082.25 cm^2), it is the maximum concentration of EMS solution where we observed 50% of survivality (LD_{50}), whereas the untreated callus recorded the maximum plant height (82.50 cm), number of branches (35.75/plant) and leaf area (4555.75 cm^2). From the results it can be seen that as the concentration of EMS solution increased the growth parameters decreased which may be due to the fact that, the enzymatic activity associated with the biosynthesis of primary metabolic traits might have recorded with increases concentration of EMS solution. There results are in conformity with the results observed by Mishra et al. (1) in muskdana and Meelpathak and David (2) in garlic.

Yield Parameters

Chemical mutagen (EMS) also influenced the herb yield (Table 2). The maximum dry herb yield (2.79 t/ha per year) was recorded in untreated callus and the minimum (2.37 t/ha)in callus treated with 0.3% EMS solution. The results showed that as the concentration of EMS solution increased the dry herb yield decreased. This is due to the reason that a higher concentration there was a decrease in the vegetative growth which in turn has given the lower herb yield. With regard oil content, the callus treated with 0.3% EMS solution recorded the maximum oil content (3.6%) whereas, untreated callus recorded the minimum (2.4%) oil content. The results show that as the concentration of EMS increased, the oil content also increased. This may be due to the reason that the 0.3% concentration of EMS solutions might favorably activate the enzyme which is associated with biosynthesis of essential oil and could cause the enhancing the rate of biosynthesis leading to higher oil content. Similar observations were reported by Patnaik et al. (3) in *Cymbopogon* and Malpathak and David (2) in garlic, where the oil yield was found to be influenced by EMS treatment. Similarly, the maximum oil yield (86.31 l/ha per year) was recorded in the callus treated with 0.3% EMS solution and the minimum (64.25 l/ha per year) in the untreated callus. The oil content and oil yield also increased with the increase in the concentration of EMS. This may be because, the oil yield mainly depends upon herb yield and oil content and when the 0.3% EMS concentration re-

corded the maximum oil content, it resulted in more oil yield.

Regarding the composition of oil, chemical mutagen (EMS) had a greater influence in the callus of patchouli. The callus treated with 0.3% concentration of EMS solution recorded the maximum patchouli alcohol (49.94%) which is of major economic significant in patchouli oil as against the untreated callus which recorded 32.02% of patchouli aocohol.

In conclusion it can be said that a considerable progress has been made in the application of induced mutation in vegetatively propagated plants. In seeds or vegetative propagated aromatic plants, chemical mutagens have been successfully used to enhance their industrial utility. In the present study, plant treated with 0.3% EMS solution may be selected as mutant clone which has higher oil content and oil yield. However, further, evaluation of these clones are needed to known their consistency.

Thus, mutation breeding offers considerable scope for altering both quantitative and qualitative characters in patchouli in a desirable direction. Only through careful screening and selection, the range of variability induced by gamma rays can be widened which can be further exploited by recombinant breeding.

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