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Radiation induced methyl-eugenol deficient mutant of *Cymbopogon flexuosus* (Nees ex Steud) Wats

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Abstract. The presence of methyl eugenol in the oil and the difficulty experienced in its separation was considered to be a negative attribute in the *Cymbopogon flexuosus* (var. RRL–59). A mutation breeding programme was initiated to rectify this inherent defect. A massive screening of irradiated vegetative slips of this essential oil-bearing plant resulted in the isolation of a methyl-eugenol deficient mutant. Our results show that once the methyl eugenol is absent from the oil, the oil as such closely resembles the oil of citronella (Java type) and can be a good substitute for the same. This investigation supports the view that beneficial mutations at specific gene loci once isolated in a vegetatively propagated species can be easily stabilised and propagated.

Keywords. *Cymbopogon flexuosus*; Java citronella; methyl eugenol; geraniol; geranyl acetate.

1. Introduction

The essential oils obtained from a number of *Cymbopogon* species are used in perfumery as such or yield pure isolates used in high grade perfumery. As a continuing programme of screening of *Cymbopogon* species, Thapa *et al* (1971) identified a new cultivar of *C. flexuosus* (var : RRL–59) which gives an oil yield of about 400 kg per hectare. The major constituents of the oil were geraniol and methyl eugenol. The presence of methyl eugenol in the oil and difficulty experienced in its separation made this, otherwise high yielding cultivar, rather less attractive. Hence, the presence of methyl eugenol was considered to be a negative attribute in this oil. It was pointed out by Bradu and Atal (1974) that in the absence of methyl eugenol the oil would resemble the oil of citronella (Java type) which is highly valued by perfumers and gets a premium in the market. A mutation breeding programme was thus initiated with a view to isolating a methyl-eugenol free mutant. Results achieved are reported in this paper.

2. Materials and methods

Dormant vegetative slips (1500) of *Cymbopogon flexuosus* (RRL–59) were exposed to 10 kR x-rays at a dose rate of 770 R/min in air. The vegetative slips were

arranged in a single layer in a circle with roots pointing towards the centre. The roots were covered by a circular lead sheet to protect them from radiation damage. The x-ray machine was operated at 110 kV, 11 mA without any filter. Preliminary experiments had established 10 kR to be LD–50 for this species (Choudhary *et al* 1976). Immediately after irradiation, they were planted in the field along with the control (mother strain) and allowed to grow under normal field conditions. Nearly half of them survived and when they attained full growth after about 5 months, a massive screening programme was initiated. The foliage harvested from each plant was hydro-distilled in a cleavenger type apparatus. The qualitative examination of oil was done by thin layer chromatography (TLC). During this process a single methyl-eugenol deficient muant isolated in 1975 was multiplied vegetatively during 1976. Data on various quantitative characters were recorded before flowering on 10 randomly selected plants of this mutant and mother strain. Qualitative differences in the oil components were ascertained with TLC using ethyl-acetate benzene (1 : 9) as a solvent system and 2% vanillin-sulphuric acid as the spraying reagent. Quantitative differences were estimated with the help of Perkin Elmer gas chromatograph model No. 881, with flame ionisation detector. The separation was done in column of SE–30 (10%) on chromosorb W (acid-washed). The flow rate of nitrogen during analysis was maintained at 25 ml/min. The column temperature was kept at 135° C, injector temperature at 180° C and detector was maintained at 220° C. The constituents were assigned by comparing their retention times with those of the authentic samples under identical conditions.

3. Results and discussion

A comparative study of various quantitative characters revealed (table 1) that the mutant exhibited a decrease in the plant height, reduced leaf length and width and number of vegetative slips per plant. The oil percentage was also reduced

Table 1. Comparative study of various quantitative characters between mutant and normal plant of *Cymbopogon flexuosus* (RRL–59)

Character	Plant type	
	Normal	Mutant
	Mean ± SE (cm)	Mean ± SE (cm)
Plant height	88·40*±2·281	83·11±2·24
Leaf length	67·53*±1·966	57·33±1·673
Leaf width	1·70*±0·094	1·32±0·037
No. of vegetative slips/plant	57·58*±5·19	53·93±5·12
Oil percentage	0·98*±0·030	0·87±0·026

* Significant at 5% level.

in comparison to control. However, fresh herbage yield on an average was not significantly different from the control. As the growth proceeds both the normal and mutant plants show similar morphological characters and are apparently indistinguishable. The composition of oils based on GLC analysis is presented in table 2 which shows that important constituents like citronellal, citronellol and geraniol remained undisturbed while the percentage of geranyl acetate was more than doubled. Methyl eugenol was totally absent in the oil of the mutant. Olfactory odour evaluation confirmed the belief that once the methyl eugenol is absent from the oil, it resembles the oil of citronella (Java type) and can be a good substitute of the same.

A comparative study of the major constituents of the oil of *C. flexuosus* (RRL–59), the mother strain as well as the methyl-eugenol free mutant and that of *C. winterianus* (Java citronella) was undertaken. The data are given in table 3. It

Table 2. Composition of oil in the mutant and normal plant of *Cymbopogon flexuosus* (RRL–59)

Peak No.	Component	Plant type	
		Normal %	Mutant %
1.	..	7·10	9·7
2.	..	9·40	12·0
3.	..	1·10	1·10
4.	Citronellal	7·0	6·70
5.	..	7·5	8·60
6.	Citronellol	5·6	5·30
7.	Geraniol	31·6	31·30
8.	Geranyl Acetate	2·60	6·70
9.	Methyl eugenol	23·30	..
10.		0·50	0·70
11.		1·8	2·50
12.		1·8	2·50

Table 3. Comparative study of the composition of four major components in the oils of *Cymbopogon flexuosus* (RRL–59) and *Cymbopogon winterianus*

Component	*C. flexuosus* (Var. RRL–59)		*C. winterianus* (Java type)
	Normal %	Mutant %	Normal %
Citronellal	7·00	6·70	25·39
Citronellol	5·60	5·30	16·83
Geraniol	31·60	31·30	20·08
Geranyl acetate	2·60	6·70	14·46
Methyl eugenol	23·30	0	0

is apparent that in comparison to 20·08% of geraniol present in Java type, the mutant shows 31·30% of geraniol. The absence of methyl eugenol in the oil of the mutant brings it at par with Java citronella with respect to the quality of oil. Hence the mutant which also has a greater yielding potential than the Java type, can become a good substitute for the same.

These experiments establish the basis for induced mutation in vegetatively propagated *Cymbopogon* species. Beneficial mutations at specific genetic loci once isolated can be easily stabilised and propagated. The desired trait in this particular case was maintained through two successive propagation. The marginal reduction in the herb yield can be made up through greater inputs and better field management.

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