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Mutation studies in *Mentha spicata* L.

S N KAK and B L KAUL
Regional Research Laboratory, Jammu Tawi 180 001, India

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Abstract. Dormant rhizomes of *Mentha spicata* were subjected to various x-ray doses to induce mutations for herb yield and/or improvement in the quality characters of the essential oil. The investigation has led to the isolation of fourteen mutant clones including one with double the quantity of carvone.

Keywords. *Mentha spicata* ; rhizomes ; spearmint oil ; carvone ; carveol.

1. Introduction

Mentha spicata also called spearmint, is an important polymorphic essential oil bearing species of genus *Mentha*. Fresh leaves and flowering tops on steam distillation yield a pale yellow oil. The principal constituent of this is carvone, for which it is mainly cultivated in Europe and the United States (Shimzu and Ikeda 1962). The oil is also used in flavouring, confectionary and pharmaceutical preparations.

Due to its low herb and oil yield, this crop has not gained enough commercial importance in India (Anon 1962) although spearmint oil worth Rs. 15 lakhs is imported annually (Anon 1978). It was therefore considered worthwhile to breed suitable plant types giving better herb yield and/or containing higher percentage of carvone in the oil. Experiments were initiated to develop clones capable of giving higher yield of herb and oil through induced mutations and the results are presented in this paper.

2. Materials and methods

Dormant rhizomes of *Mentha spicata* L. containing single bud per piece were exposed to various doses of x-rays (2–9 kR) at a dose rate of 550 R/min. The x-ray machine was operated at 110 kV, 11 mA without any filter. Irradiated rhizomes were immediately planted in the experimental fields along with non-irradiated control. Germination of rhizomes was noted two weeks after planting and three weeks later the surviving rhizomes were determined. During harvest, each clone was individually weighed and hydrodistilled in a Cleavanger type apparatus. The essential oil thus obtained was assayed for quality characters by thin layer chromatography (TLC) using ethyl acetate-benzene (2 : 8) as solvent system and 2% vainillin-sulphuric acid as the spraying agent. Quantitative determination was done by gas liquid chromatography (GLC) using a Perkin-Elmer gas chromatograph model No. 881 with flame ionisation detector. The column used for separation was SE–30 (10%) on chromosorb W (acid washed). The nitrogen flow rate during analysis was maintained at 25 ml/min. The column temperature was kept at 130° C, the injector temperature at 200° C and the detector was maintained at 200° C. The constituents were discerned by comparing their retention times with those of the authentic samples under identical conditions.

3. Results and discussion

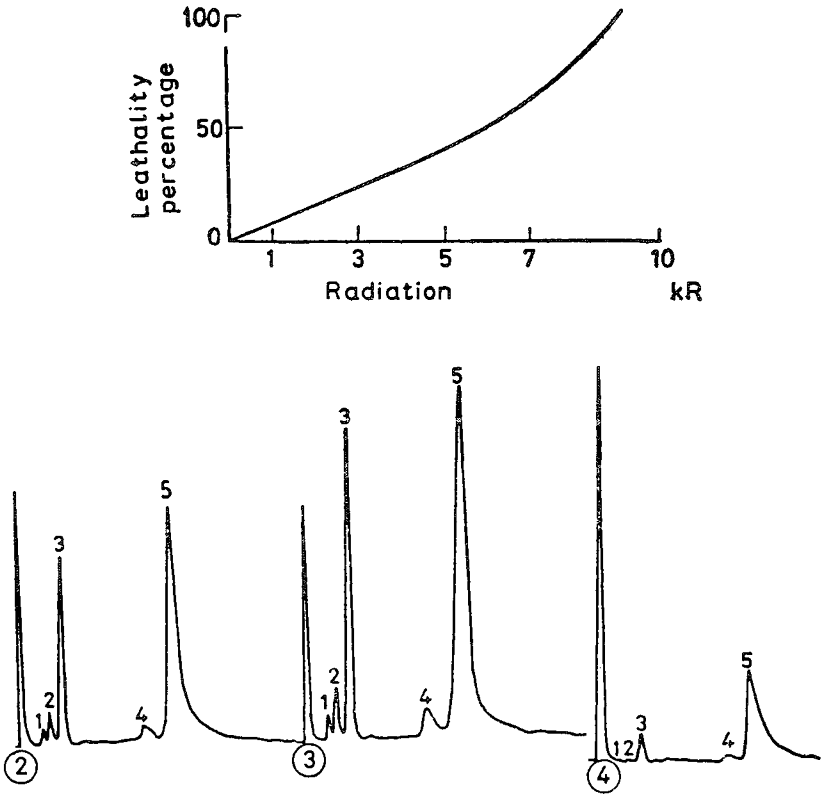
Germination and survival of rhizomes were adversely affected following irradiation. Almost 50% lethality was obtained following 6 kR treatment which went upto 70 and 90% at 7·5 and 9 kR respectively (table 1). The latter two doses seem to be drastic. Most of the germinated rhizomes survived to maturity. The data on survival (table 1) and the dose-response curve (figure 1) suggest that LD_{50} against x-rays for this species is around 5 kR.

At maturity each treated clone was individually harvested and analytically assayed for its oil content. The data are given in table 2. The yield was drastically low in the 4 to 9 kR treated material. However, the range of variation was greater in 4 kR treated population, where some clones out-yielded the parental clone (table 2). Nevertheless, the oil content was not appreciably affected.

Considering the peculiarity of the growth habit, morphological traits and variation in the quality of oil, 14 different mutant clones were isolated. Data on their yield and the quality of oil are given in table 3. Three clones, viz R-sp/40, R-sp/59 and R-sp/87 appear to be promising ones on the basis of the herb yield and the carvone content in the oil. While R-sp/40 exhibits higher herb yield potential retaining the parental quality of oil, clones R-sp/59 and R-sp/87 are characterised by a higher content of carvone without any effect on their herb yield. These two mutant clones had carvone content of 61 and 97% respectively compared

Table 1. Effect of various doses of x-rays on germination and survival of *Mentha spicata* rhizomes.

Treatment (kR)	Germination (%)	Survival (%)
Control	100	100
2·0	78	78
4·0	65	61
6·0	53	47
7·5	39	30
9·0	10	10



Figures 1–4. **1.** Dose-response curve of *Mentha spicata*. **2.** GLC curve of parental clone of *Mentha spicata*. **3.** GLC curve of mutant clone R-sp/59. **4.** GLC curve of mutant clone R-sp/87. (In figures 2–4, peaks 1–3 show hydrocarbons and unidentified compounds ; peaks 4 and 5 show carveol and carvone respectively).

Table 2. Effect of various doses of x-rays on herb yield and oil content of *Mentha spicata*.

Sl. No.	Treatment (kR)	Herb yield per plant (g) Range	Mean	Mean oil content (%)
1.	Control	434–517	458	0·19
2.	2·0	394–505	426	0·18
3.	4·0	312–538	340	0·19
4.	6·0	364–476	315	0·19
5.	7·5	209–446	228	0·19
6.	9·0	228–320	235	0·18

Table 3. Qualitative and quantitative characters of some of the mutant clones of *Mentha spicata* isolated as a result of x-ray treatment.

Mutant	Herb yield/ plant (g)	Oil content (%)	Oil composition		Others	Source
			Carvone	Carveol		
Control	465	0·19	42	2	56	—
R-Sp/7	473	0·19	39	2	59	2 kR
R-Sp/24	488	0·18	41	5	55	2 kR
R-Sp/13	367	0·19	35	3	62	4 kR
R-Sp/40	505	0·18	40	2	58	4 kR
R-Sp/56	470	0·19	49	2	49	4 kR
R-Sp/59	440	0·19	61	6	33	4 kR
R-Sp/87	456	0·19	79	8	13	4 kR
R-Sp/149	384	0·19	30	5	65	6 kR
R-Sp/107	410	0·18	36	3	61	6 kR
R-Sp/94	225	0·19	42	4	54	7·5 kR
R-Sp/163	360	0·18	38	7	55	7·5 kR
R-Sp/178	434	0·19	41	4	55	7·5 kR
R-Sp/83	228	0·18	32	3	65	9 kR
R-Sp/119	270	0·17	39	6	55	9 kR

to 42% in the parental clone (figures 2, 3 and 4). Higher carvone content makes them more useful and fetches an increased price. The oil content of herb has not undergone any change.

Artificially induced mutations have been used very effectively in the improvement of different crop plants (Swaminathan 1969; Sigurbjornsson and Micke 1974). This method of breeding has been particularly useful in the improvement of vegetatively propagated plants, where it is perhaps the only method of generating new variability (Broertjes 1968). In the genus *Mentha* this technique has already been used quite successfully to develop new high yielding and improved cultivars (Murray 1969; Kak and Kaul 1977a, b). The present investigation clearly shows the usefulness of this method of breeding in yet another species of the genus *Mentha*. The unique feature of breeding by induced mutagenesis is the ability to rectify one of a few minor characters of an otherwise good species of cultivar (Broertjes *et al* 1976). Mutation breeding, therefore, offers considerable scope for improving vegetatively propagated species, when the characters involved are governed by one or a few genes.

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