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NOTES ON NEGLECTED AND UNDERUTILIZED CROPS

Induction and assessment of morpho-biochemical mutants in *Artemisia pallens* Bess.

Kanti Rekha · Anima Langer

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Abstract *Artemisia pallens* Bess. is a low volume and high value essential oil plant used in perfumery, cosmetic and flavouring industries. On account of the failure of conventional procedures to induce variability in species, mutation techniques have been tried in our experiments. Dry and viable seeds (moisture content 8%) of homozygous pure breeding lines were subjected to 150–500 Gy doses of gamma rays and 0.01–0.1% ethyl methane sulphonate (EMS) for 8 h. Desirable qualitative mutants were recovered from segregating M_2 generation (4,283 plants scored) raised as single plant progenies. The spectrum of morphological mutants included late and early flowering types; bushy and high yielding types; tall and more capitula-producing types and high oil and high davanone yielding types. These were raised through M_3 families to evaluate stability and transmission of mutant characters. As such out of 15 different types selected in M_2, only 11 types bred true to their characteristic variability. Based on their performance, the mutants were characterised depending upon their distinguishing features. Davanone, the main component of oil showed the maximum increase (64.22% against 54.64% in control) in mutant 'S–5' recovered from exposure with 250 Gy γ-rays. Mutant 'E-6' was economically most viable having increased oil biosynthesis (0.36% against 0.22% in control) and hence yields higher oil per unit area than the parental control (isolated from 0.05% EMS treatment).

Keywords *Artemisia pallens* · Characterisation · Davanone · EMS · γ-rays · Mutants

K. Rekha (✉)
Plant Breeding and Crop Husbandry Division,
Regional Research Laboratory, Canal Road, Jammu
Tawi 180 001, India
e-mail: kantirekha2000@yahoo.co.in

A. Langer
Department of Botany, University of Jammu, Jammu
180 004, India

Introduction

Artemisia pallens Bess. belonging to family Asteraceae is native of India and has gained considerable industrial importance. Popularly known as davana, it is a traditional underexploited Indian herb prized for its fruity fragrance. The oil has a delicate aroma and is used in high grade perfumes, cosmetic and flavouring industries (Thakur and Misra 1989; Jeffrey 2001). At present, the production of davana oil is estimated around 2.5 tonnes per annum and sold at the high price of Rs. 7,500/kg (Jhunjhunwalla 2006). There is huge demand for the oil in the foreign market. But the oil produced in India is not keeping pace with the increasing demand for

the oil. As such necessity has been felt for the development of the crop. There is no significant natural genetic variability in the crop (Farooqi et al. 1990). Davana produces tiny flowers due to which it is tedious to produce variability through recombination breeding. Therefore, mutation breeding is more desirable to create variation in davana. The present author has induced enormous variability in the crop through physical and chemical mutagenesis (Rekha 1999; Rekha and Kak 1997; Rekha et al. 2000). The present investigation was undertaken to characterise the various morphological and biochemical mutants isolated from M_2 and M_3 generations raised from γ-rays and EMS treated seeds.

Material and methods

Dry and viable seeds with 8% moisture of homozygous pure breeding lines of *A. pallens* maintained at the Herbal garden of Regional Research Laboratory (RRL), Jammu were used for the study. The seeds were irradiated with gamma rays (Cobalt ^{60} as radiation source). The doses applied were 150, 200, 250, 300, 350, 400 and 500 Gy at the dose rate of 5 Gy/min. As a chemical mutagen, ethyl methane sulphonate (EMS) was used in three concentrations viz. 0.01, 0.05 and 0.1% for 8 h. After chemical treatment, the seeds were washed under running tap water for 30 min. Three replications, each of 200 seeds were used for various treatments. Untreated seeds were soaked in distilled water for the same period to serve as control. All the treated seeds along with control were immediately sown in pots to raise in the nursery. The seedlings about 5–8 cm tall were transplanted to experiment field following randomized block design to raise in the M_1 generation. The seeds of the M_1 plants were collected plant wise and again sown in the field in the next season to raise M_2 generation on plant to a row basis and similarly, those of the M_2 generation were used to raise the M_3 generation.

Plant height was measured at the flowering stage. The height represented the size from soil surface to the tip of tallest branch. All capitula borne on an individual were counted. The average was calculated from 25 plants selected at random in each treatment and the control. Fully grown 100 capitula were picked at random from each plant and weight over the electronic balance. The data were recorded and computed. Pollen fertility was estimated through aceto-carmine staining test. For this purpose the anthers, about to dehisce, were squashed in 1% aceto-carmine. The plump and deeply stained pollen grains were treated as fertile, whereas, the unstained, empty and denatured ones were considered as non-fertile. The crop at bloom was cut 5–6 cm above ground by sickles, weighed over the balance and expressed as herbage yield/plant. All the floral heads borne on individual plant were harvested, weighed and expressed as floral yield/plant. The oil extraction was carried out by hydro-distillation in a Clevenger apparatus at 60–70°C for 3 h. The oil percentage was calculated on fresh weight basis per plant. The oil obtained from each sample was subjected to gas liquid chromatography (GLC) for determination of davanone content in it. Davanone is a sesquiterpene ketone and is the main constituent of the oil. Analysis was performed on Nucan gas chromatograph, model 5765 with FFAP fused silica capillary column (30 m × 0.25 mm i.d.) with helium as the carrier gas (30 ml/min). The column temperature was programmed from 90°C to 230°C at 2°/min. The injector and detector temperatures were maintained at 250 and 270°C, respectively. The identification of the main component was done by comparing the retention time of the peak with reference sample run under similar conditions.

The M_2 population was screened for the presence of morphological mutations. The M_3 plants were mainly evaluated to determine the stability and transmission of mutant characters recorded in M_2 generation. Most of M_2 mutants were found to breed true in the M_3 generation. The morphological and biochemical mutants were characterised on the basis of growth, qualitative and quantitative performance. Statistical analysis of the data obtained in the present study was carried out after Mungikar (1997).

Observations

Treatment with γ-rays

Selections at the upper boundaries of the plant characteristics were made from the M_2 and M_3

generations under various gamma ray treatments. Besides these, selections in the other parameters such as earliness, late flowering, synchronous flowering etc. were also made. A total of six different morphological and biochemical mutants were isolated which breed true to their characteristic variability. The data on the individual performance of these mutants in M_3 generation along with the control are given in Table 1. The salient features of these mutants are described as below:

(a) Early flowering mutant (S-1): In this mutant, flower initiation took place two weeks earlier than control which led to an early seed setting. It grew to a height of 63.50 cm. This selection exhibited increase in capitulum number (400), floral head diameter (0.65 cm) and 100 floral head weight (10.50 g). It is also superior to control in herbage and flower yield/plant, oil and davanone content. Meiosis was normal with 72.04% pollen fertility. This mutant was isolated from seeds exposed to 150 Gy gamma rays.

(b) Tall mutant (S-2): A vigorous tall, high yielding type grew 65.40 cm in height as against 60.29 cm in the mother plant. This also showed better performance in all other parameters studied particularly herbage (39.50 g) and flower yield (16 g)/plant. Meiosis was normal with 72.69% pollen fertility. This mutant was isolated following 150 Gy gamma ray treatment.

(c) More capitula producing mutant (S-3): This mutant possessing more capitula was characterised by increase in number and yield of capitula. It exhibited capitulum count of 422 against 390 in the control and capitulum yield of 22.08 g against 13.50 g in control. However, it exhibited reduced floral head diameter of 0.52 cm against 0.55 cm in control plants. With respect to other parameters like herbage yield, oil and davanone content it was better than control. Meiosis was normal with high level of pollen fertility (80.0%). This mutant was isolated from among 250 Gy irradiated seeds.

(d) High yielding mutant (S-4): This mutant recorded higher yield of 52.72 g of fresh

Table 1 Growth and yield characteristics of the mutants selected after gamma ray irradiation in M_3 generation

Selection	Plant height (cm)	No. of capitula per plant	Flower head diameter (cm)	100 flower head wt. (g)	Pollen fertility (%)	Herbage yield per plant (g)	Floral yield per plant (g)	Oil (%)	Davanone (%)	Remarks
Control	60.29 ± 0.55	390 ± 8.66	0.55 ± 0.13	9.64 ± 0.88	80.92 ± 1.91	33.00 ± 1.09	13.50 ± 0.64	0.22	54.64	-
S-1	63.50 ± 0.63	400 ± 6.66	0.65 ± 0.03	10.50 ± 0.91	72.04 ± 1.62	35.00 ± 1.00	16.69 ± 0.92	0.24	59.09	Early maturing type
S-2	65.40 ± 0.48	412 ± 9.32	0.62 ± 0.05	10.00 ± 0.94	72.69 ± 2.00	39.50 ± 1.34	16.00 ± 0.72	0.23	58.33	Tall type
S-3	62.64 ± 0.48	422 ± 6.52	0.52 ± 0.02	12.50 ± 0.55	80.00 ± 2.20	34.84 ± 1.04	22.08 ± 0.55	0.24	60.20	More capitula producing type
S-4	64.36 ± 0.60	414 ± 6.04	0.56 ± 0.04	9.32 ± 0.59	71.93 ± 1.96	52.72 ± 1.26	25.00 ± 0.55	0.23	59.39	High yielding type
S-5	58.85 ± 0.72	400 ± 5.63	0.66 ± 0.02	12.93 ± 0.80	70.52 ± 1.59	40.00 ± 0.94	16.62 ± 0.42	0.26	64.22	High davanone type
S-6	60.88 ± 0.49	420 ± 7.92	0.60 ± 0.04	10.66 ± 0.74	68.65 ± 1.77	39.00 ± 1.06	20.10 ± 0.52	0.28	58.26	Early maturing, high oil yielding type

herbage as against 33 g in the control and an average of 25 g flowers as against 13.50 g/control plant. It exhibited an increase in all other agronomic parameters studied. Meiosis was normal with 71.93% pollen fertility. This mutant was isolated following 200 Gy gamma ray irradiation.

(e) High davanone yielding mutant (S-5): This mutant was characterised by possessing high davanone content of 64.22% in its oil as compared to 54.64% in control. It is also rich in oil concentration (0.26%). The mutant also excelled in other agronomic and yield parameters. Meiosis was normal with 70–52% pollen fertility. It was isolated from treatment with 250 Gy gamma rays.

(f) High yielding mutant (S-6): This mutant contained oil as high as 0.28% in its leaves and flowers as against 0.22% in the parental line accompanied by increased number of flower/plant (420). Apart from being high oil yielding type, it also exhibited early maturity. Therefore, it matured 15 days earlier than the control. Its performance was better than control in general growth and other yield parameters. Meiosis was normal with 68.59% pollen fertility. It was isolated following 300 Gy gamma ray treatment.

Treatment with EMS

Observations were recorded from M_2 and M_3 generations and five mutants were selected which excelled over the parent in terms of growth and yield potential. These also transmitted the mutated traits from M_2 to M_3 generation. Data on agronomic and yield parameters of the five mutant genotypes along with their controls are presented in Table 2. The mutants are characterised as below:

(a) Late flowering mutant (E-1): From 0.01% EMS treatment, a late flowering plant type was isolated in which flowering was delayed by three weeks. This mutant exhibited sufficient increase in height and vegetative foliage. It exhibited increase in herbage yield, oil, and davanone content. Meiosis

Table 2 Growth and yield characteristics of the mutant genotypes originated from EMS treatment in M_3 generation

Mutant Genotype	Plant height (cm)	No. of floral heads (cm)	Floral head diameter (cm)	100 floral head wt. (g)	Pollen fertility (%)	Herbage yield per plant (g)	Floral yield per plant	Oil (%)	Davanone (%)	Remarks
Control	60.29 ± 0.55	390 ± 8.66	0.55 ± 0.13	9.64 ± 0.88	80.92 ± 1.91	33.0 ± 1.09	13.50 ± 0.64	0.22	54.64	-
E-1	66.64 ± 0.42	320 ± 9.32	0.53 ± 0.12	9.10 ± 0.64	72.93 ± 1.84	47.34 ± 0.99	11.69 ± 0.56	0.25	55.23	Late flowering type
E-2	57.63 ± 0.63	432 ± 8.34	1.12 ± 0.09	10.63 ± 0.74	81.00 ± 2.00	30.66 ± 0.89	18.83 ± 0.72	0.26	58.94	Early flowering type
E-3	58.29 ± 0.39	396 ± 6.45	0.61 ± 0.11	10.68 ± 0.53	77.69 ± 1.76	61.84 ± 1.10	16.32 ± 0.66	0.23	51.56	Bushy and high yielding type
E-5	58.74 ± 0.58	483 ± 10.04	1.15 ± 0.12	11.84 ± 0.89	78.55 ± 1.99	35.49 ± 1.12	21.00 ± 0.59	0.22	59.64	More capitula producing type
E-6	58.93 ± 0.64	410 ± 7.93	0.92 ± 0.13	10.54 ± 0.90	70.67 ± 1.92	35.20 ± 0.77	14.00 ± 0.74	0.36	60.32	High oil yielding type

was normal with 72.93% pollen fertility. However, there was decrease in other growth parameters like capitulum count/plant, capitulum diameter and 100 capitulum weight as compared to the control.

(b) Early flowering mutant (E-2): This mutant flowered and matured earlier than the control by 18 days. Meiosis was normal with very high level of pollen fertility (81%). This mutant also possessed more number of capitula (432) with average diameter of 1.12 cm. It showed increase in 100 flower weight, floral yield, oil and davanone content but was poor in height and herbage yield. This mutant was isolated from 0.01% EMS treatment.

(c) Bushy mutant (E-3): This high herbage yielding mutant manifested bushy appearance by having increased number of branches, delayed germination and flowering accompanied by remarkably high herbage yield (61.84 g)/plant. Meiosis was found normal with 77.69% pollen fertility. It showed increase in capitulum diameter (0.61 cm) and 100 flower weight (10.68 g). This mutant was obtained from 0.05% EMS treatment.

(d) High capitulum yielding mutant (E-5): This mutant was characterised by capitulum number as high as 483 against 390 in the control plants. It also exhibited increase in floral head diameter, 100 floral head weight, floral yield etc. Oil concentration was comparable to control. However, davanone content was as high as 59.64% against 54.64% in the control. Meiosis was normal with 78.55% pollen fertility. This mutant originated from 0.01%EMS treatment. It also exhibited synchronous flowering.

(e) High oil yielding mutant (E-6): This mutant possessed high oil concentration of 0.36% in fresh herbage as compared to 0.22% in mother plant. It also showed higher davanone content of 60.32% as against 54.64% in control. It excelled in all other agronomic and yield parameters except the plant height. Meiosis was normal with 70.67% pollen fertility. However, this mutant exhibited loose branching as compared to compact nature of mother plant. It was isolated from 0.05% EMS treatment.

Discussion

In the present investigation, the two mutagens were found to be effective in inducing a broad spectrum of productive mutants (Table 3). Prominent among them are mutants like tall, early maturing, high yielding and bushy mutants where the morphology of the vegetative parts as well as the maturity and yield were affected. These have been recorded in the M_2 and M_3 generations of the physical and chemical mutagen treated populations. In this study a tall mutant (S-2) was obtained. The tallness is fundamentally due to an initial increase in internode length, sometimes accompanied by an increase in internode number (Jana 1963). Increased length of cells and their number per unit area also contribute to tallness (Ehrenberg et al. 1961; Miura et al. 1974). Tall mutants have been observed by Gaikwad and Kothekar (2003) in *Lens culinaris* Medik.

Early maturing mutants (S-1 and S-6) obtained in the present study showed rapid growth and enhanced productivity. Jana (1963) and Basu

Table 3 Different desirable plant types obtained in *A. pallens* following mutagenesis

S. No.	Plant type	No. of mutants	Treatment
1.	Early maturing type	2 (S-1 and E-2)	150 Gy, 0.01% EMS
2.	Tall type	1 (S–2)	150 Gy
3.	More capitula producing type	2 (E-5 and S-3)	0.01% EMS, 250 Gy
4.	High herbage yielding type	2 (E-3 and S-4)	0.05% EMS, 200 Gy
5	High oil yielding type	2 (E-6 and S-6)	0.05% EMS, 300 Gy
6.	High davanone type	1 (S–5)	250 Gy
7.	Late maturing type	1 (E-1)	0.01% EMS

(1966) explained that early maturity may be due to physiological changes caused by irradiation and increased production of flowering hormone.

High yielding mutants (S-3, S-4, E-3 and E-5) were isolated during the course of present study. Yield comprises one of the most important characters for judging the agronomic value of the mutant as related to that of mother variety. In these mutants the yield attributes like herbage and capitulum yield have increased. High yielding mutants have been reported by Pawar and Wanjari (1994) in different crops using induced mutations.

Apart from causing morphological alterations, the mutagenic treatments resulted in producing earliness or delay in mean time of flowering and seed setting in *A. pallens.* The genetic nature and stability of these characters were evident from their re-appearance in the M_3 progenies. Variation in the flowering period obtained, through mutagens in the present investigation is of great practical utility as its exploitation through selection could provide ample scope for getting desired genotypes capable of maturing in different durations. Variation in this character (earliness or lateness) can be attributed to enhanced or suppressed activity of the genes controlling or involved in the development of flowering and seed setting (Gunckel et al. 1953). Variation in maturity time following treatment with mutagenic agents has been reported in various other crops by Basu (1966), Conger et al. (1976), Pawar et al. (1979), Kak and Koul (1980), Sengupta and Datta (2004) etc.

High oil and high davanone yielding mutants (S-6, S-5 and E-6) showed appreciable increase in mean oil content. These results are in accord with these of Sadowska (1975) who reported a positive increase in peppermint oil following mutagenic treatments. The results obtained are also in conformity with earlier reports whereby induced mutations have resulted in significant changes in secondary metabolite formation in plants (Hegnauer 1975; Levy 1982). There is evidence that mutagens (radiations) stimulate the metabolic activity of plants such as respiration (Romani 1966), glycolysis and oxidative phosphorylation (Mergen and Johnson 1964) and cytochrome oxidase and catalase activity (Goldon 1957) which may ultimately influence and enhance synthesis of plant products (Kaul et al. 1973). The enhancement in the oil concentration noted in the present study can be also due to such influence. Change in the main essential oil constituent in *A. pallens* may be attributed to the changes produced in the enzyme systems which control the biosynthesis of essential oil (Hefendehl and Murray 1976).

Based on these results, it has been concluded that both the mutagens succeeded in induction of viable mutants in *Artemisia pallens*. All these 11 mutants have great potential to be incorporated in further breeding programmes for developing new productive cultivars.

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