Indian J. Pl. Genet. Resources 13(1): 37-41, 2000

# EMS INDUCED VARIABILITY IN *Artemisia pallens* Wall.*

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**Davana (*Artemisia pallens*) is native to India and has gained considerable importance in industry for its fragrance. Because the crop is harvested at pre-flowering stage, there is threat to its extinction, and thus variability is also reduced. This paper revealed that a wide range of variability could be generated through the induced chemical mutagenesis in *A. pallens*.**

**Key words:** Davana, Artimisia pallens, induced mutagenesis, variability

The genus *Artemisia* comprises a large number of economically important species, some of which yield essential oil while others have medicinal properties (Anonymous, 1985). Among theses, *Artemisia pallens* Wall. popularly known as davana is a native of India and has gained considerable industrial importance. The davana oil, prized for its fruity fragrance, is mainly used in perfurmery, flavouring and cosmetic industries. There is a huge demand of the oil in the foreign market. But the oil produced in India is not keeping pace with the increasing demand of the oil. As such necessity has been felt for the development of the crop. In view of the fact that cultivation of the crop is confined to a limited area and crop is harvested at pre-flowering stage, there is hardly any variability in the crop (Farooqi *et al.*, 1990), which is a pre-requisite for any improvement programme. Thus one of the best options available is to generate variability through induced mutations. The present study on induced mutagenesis in *Artemisia pallens* was carried out with a view to assess the amount of variability, which can be induced by Ethyl methane sulphonate (EMS).

## MATERIAL AND METHODS

A set of selfed, dry and viable seeds with 8-10 per cent moisture were treated with aqueous solution of EMS prepared in distilled water. Concentrations of EMS used ranged from 0.01-0.1 per cent. Another set of same concentrations was prepared by dissolving the mutagen in 2 per cent Dimethyl Sulphoxide (DMSO). Three replicates, each of 200 seeds, were used for each treatment along with the controls. All the treatments lasted for 8 hrs at a temperature of 25 ± 1°C with intermittent shaking. At the end of treatments, seeds were thoroughly washed. Following each treatment, 50 treated seeds along with respective controls were separately placed in petridishes for germination and cytological studies. The remaining seeds were sown in pots to raise the nursery. The seedlings about 5-8 cm tall were transplanted to experimental beds to raise $M_1$ generation. The biological effects of different treatments were evaluated with respect to the germination, seedling growth, survival percentage and other agronomic characteristics of the plants and compared to the control. All the observations in the control were

*Part of PhD Thesis of the first author, sumbitted to University of Jammu, Jammu (J&K)

taken as 100%. $M_1$ abnormalities scored from seedling stage to maturity were determined from the percentage of the $M_1$ plants survived. Pollen fertility was estimated by Aceto-carmine staining test over 2,000 pollen grains per treatment. Impact of EMS treatments has been assessed on mitotic and meiotic systems. The cytological studies were carried out as described by Sharma and Sharma (1980). Data on plant height, capitulum number/plant, herbage and flower yield per plant were recorded on 25 plants per replicate from each treatment. Oil percentage of the herb was determined by hydro-distillation in Clevenger apparatus.

Selfed seeds from $M_1$ plants were harvested individually and grown to raise $M_2$ generation as progeny rows the following year. These progenies were studied for various parameters like $M_1$ plants. The data collected for quantitative traits were put to statistical analysis. The statistical significance of the difference in mean values between the control and treated plants was calculated by applying student's t-test. The variance of the control and treated plants was compared by applying variance ratio (F) test.

## RESULTS AND DISCUSSION

Germination and survivality were adversely affected under all the treatments of EMS. These physiological processes revealed a parallel relationship and showed a decreasing trend with the increasing doses. Application of EMS dissolved in DMSO induced more reduction than the corresponding concentration in water. The concentration of EMS used and seedling height bear inverse relation. Maximum reduction to 48.87% was observed at 0.1% EMS dissolved in DMSO (Table 1). The observed decrease in survival and germination is attributed to disturbed metabolic activity in the treated seeds and also to the damage caused to the meristimatic cells (Konzak *et al.*, 1965).

**Table 1. Seed germination, seedling growth and seedling survival following various EMS doses in *Artemisia pallens* seeds in $M_1$ generation**

| Treatment (EMS) | Germination (%) | | Seedling height (cm) | | Survival % of control |
|---|---|---|---|---|---|
| | Mean | % of control | Mean | % of control | |
| Control | 92.00 | 100.00 | 2.73 ± 0.09 | 100.00 | 100.00 |
| 0.01%/$H_2O$ | 85.00 | 92.39 | 2.37 ± 0.11** | 86.18 | 90.64 |
| 0.05%/$H_2O$ | 71.50 | 77.50 | 2.24 ± 0.15** | 81.45 | 74.65 |
| 0.10%/$H_2O$ | 56.34 | 51.74 | 1.86 ± 0.14** | 67.63 | 52.58 |
| DMSO (2%) | 88.36 | 100.00 | 2.66 ± 0.08 | 100.00 | 100.00 |
| 0.01%/DMSO | 74.74 | 84.59 | 1.66 ± 0.13** | 81.20 | 81.50 |
| 0.05%/DMSO | 63.28 | 71.62 | 1.84 ± 0.12** | 69.17 | 64.04 |
| 0.10%/DMSO | 48.35 | 42.73 | 1.30 ± 0.11** | 48.87 | 54.63 |

EMS induced a number of morphological aberrations which were mostly confined to leaves. Leaf curling was the most predominant effect. The frequency of these abnormalities were dose related (Table 2). These abnormalities fail to re-appear in the $M_2$ generation. This class of variation represent the outcome of physiological imbalance or biochemical disturbances caused by mutagen (Gunckel and Sparrow, 1961).

All the EMS treatments induced significant reduction in plant height in $M_1$ generation. Mean values for this character skewed towards positive direction following 0.01 and 0.05% EMS treatments in water during $M_2$ generation and all the treatments exhibited a significant increase in variance. A dose related increase in pollen fertility was observed in the treated plants and was found enhanced in case of treatments applied in presence of DMSO. All the concentrations

**Table 2. Morphological abnormalities induced by various doses of EMS in $M_1$ generation of *Artemisia pallens***

| Treatment (EMS) | Abnormal plants (%) | Types of morphological abnormalities (%) | | | | |
|---|---|---|---|---|---|---|
| | | Curled leaves | Fused leaflets | Albino seedlings | Colour changes | Other types |
| Control | - | - | - | - | - | - |
| 0.01%/$H_2O$ | 9.39 | 3.21 | 2.22 | - | 2.00 | 1.96 |
| 0.05%/$H_2O$ | 16.45 | 5.05 | 4.13 | 0.55 | 3.08 | 3.64 |
| 0.10%/$H_2O$ | 18.52 | 12.22 | 2.86 | - | 1.44 | 2.00 |
| DMSO (2%) | - | - | - | - | - | - |
| 0.01%/DMSO | 19.94 | 8.36 | 5.94 | - | 3.33 | 2.28 |
| 0.05%/DMSO | 26.74 | 14.84 | 4.31 | 0.84 | 2.12 | 4.63 |
| 0.10%/DMSO | 29.54 | 13.09 | 9.99 | - | 3.46 | 3.00 |

widened the range of pollen fertility in $M_2$ and more variability has been induced by EMS treatments dissolved in DMSO (Tables 3 and 4).

Reduced fertility has been ascribed to the physiological damage produced by the hydrolic products of the alkylating chemicals (Gaul *et al.*, 1966). Stimulatory effect on capitulum count per plant was observed as a result of EMS (0.01 and 0.05%) treatments in $M_1$ generation. All other treatments showed reduction. Herbage and flower yield per plant also suffered reduction except at the treatment 0.05 per cent EMS/$H_2O$ An appreciable increase in oil percent was noticed at all the treatments. This increase was not linear to the concentration of EMS applied (Table 5). Variance was increased significantly for most of the treatments for all the parameters under study during $M_2$ generation. Range got further widened than within the control (Table 6 & 7). Increased variability in these traits is much desired aspect of mutagenic treatments.

The stimulatory effect of low EMS doses observed on some yield attributing characters has been attributed to the rapid turn over of auxin

**Table 3. Plant height and pollen fertility following EMS treatment in *Artemisia pallens* in $M_1$ generation**

| Treatment (EMS) | Plant height (cm) | | Pollen fertility (%) | |
|---|---|---|---|---|
| | Average | % of control | Range | Average |
| Control | 59.32 ± 0.69 | 100.00 | 54.22 - 93.81 | 77.63 |
| 0.01%/$H_2O$ | 56.72 ± 0.97* | 95.62 | 56.64 - 77.03 | 68.91 |
| 0.05%/$H_2O$ | 52.00 ± 0.70* | 87.66 | 48.19 - 85.93 | 69.74 |
| 0.10%/$H_2O$ | 47.80 ± 0.91** | 50.58 | 52.00 - 70.81 | 61.75 |
| DMSO (2%) | 57.64 ± 0.52 | 100.00 | 69.74 - 89.99 | 78.32 |
| 0.01%/DMSO | 52.61 ± 0.62** | 91.27 | 50.96 - 90.02 | 70.98 |
| 0.05%/DMSO | 47.52 ± 1.03** | 82.44 | 33.63 - 90.02 | 53.32 |
| 0.10%/DMSO | 37.69 ± 0.82** | 65.39 | 37.93 - 60.64 | 48.20 |

**Table 4. Variation in plant height and pollen fertility in $M_2$ generation raised after treatment with EMS in *Artemisia pallens***

| Treatment (EMS) | Plant height (cm) | | | Pollen fertility (%) | |
|---|---|---|---|---|---|
| | Range | Average | % of control | Range | Average |
| Control | 47.20 - 70.50 | 85.84 ± 1.41 | 100.00 | 76.82 - 90.50 | 82.23 |
| 0.01%/$H_2O$ | 46.30 - 72.40 | 61.76 ± 1.59 | 104.96 | 68.88 - 87.21 | 79.07 |
| 0.05%/$H_2O$ | 50.50 - 77.00 | 66.56 ± 1.44** | 113.12 | 64.52 - 83.68 | 75.91 |
| 0.10%/$H_2O$ | 39.40 - 68.20 | 54.40 ± 1.85* | 92.45 | 55.85 - 81.87 | 69.23 |
| DMSO (2%) | 53.10 - 70.40 | 60.36 ± 0.94 | 100.00 | 69.14 - 86.99 | 78.44 |
| 0.01%/DMSO | 39.40 - 70.00 | 55.92 ± 1.82** | 92.64 | 46.86 - 89.91 | 69.36 |
| 0.05%/DMSO | 36.70 - 66.50 | 51.44 ± 1.75** | 85.22 | 49.99 - 73.63 | 62.51 |
| 0.10%/DMSO | 32.60 - 60.00 | 47.28 ± 1.57** | 37.33 | 38.85 - 74.22 | 57.39 |

**Table 5. Effect of various doses of EMS on different yield attributing characters in *Artemisia pallens* in $M_1$ generation**

| Treatment (EMS) | Capitula count/plant | | Herbage yield/plant (g) | | Flower yield/plant (g) | | Oil (%) |
|---|---|---|---|---|---|---|---|
| | Mean | % of control | Mean | % of control | Mean | % of control | |
| Control | 370.70 ± 9.52 | 100.00 | 31.08 ± 0.89 | 100.00 | 13.63 ± 0.45 | 100.00 | 0.20 |
| 0.01% / $H_2O$ | 424.40 ± 3.24** | 114.49 | 28.04 ± 1.36* | 90.22 | 14.12 ± 0.79 | 103.22 | 0.36 |
| 0.05% / $H_2O$ | 397.95 ± 15.91 | 107.35 | 33.28 ± 1.40 | 107.07 | 15.12 ± 0.72* | 110.52 | 0.32 |
| 0.10% / $H_2O$ | 313.95 ± 15.99** | 84.60 | 26.48 ± 123** | 85.20 | 12.36 ± 0.65 | 90.35 | 0.30 |
| DMSO (2%) | 374.00 ± 11.59 | 100.00 | 32.56 ± 0.78 | 100.00 | 13.55 ± 0.47 | 100.00 | 0.24 |
| 0.01% / DMSO | 355.43 ± 13.64 | 94.50 | 29.04 ± 1.07** | 89.18 | 12.53 ± 1.32 | 93.57 | 0.37 |
| 0.05% / DMSO | 321.94 ± 14.08** | 86.06 | 26.07 ± 1.35** | 81.69 | 11.60 ± 0.98 | 84.61 | 0.37 |
| 0.10% / DMSO | 301.37 ± 16.31 | 80.58 | 24.00 ± 1.15** | 73.71 | 10.08 ± 0.67* | 74.39 | 0.39 |

**Table 6. Variation in capitulum count and herbage yield/$M_2$ plants raised following treatments with EMS**

| Treatment (EMS) | Capitula count/plant | | | Herbage yield/plant (g) | | |
|---|---|---|---|---|---|---|
| | Range | Mean | % of Control | Range | Mean | % of control |
| Control | 280-450 | 373.04 ± 10.00 | 100.00 | 25-38 | 30.92 ± 0.86 | 100.00 |
| 0.01% / $H_2O$ | 290-510 | 401.24 ± 11.47* | 107.56 | 22-48 | 33.92 ± 1.43* | 109.13 |
| 0.05% / $H_2O$ | 190-525 | 359.20 ± 15.15 | 96.29 | 28-48 | 38.08 ± 1.22** | 122.52 |
| 0.10% / $H_2O$ | 260-445 | 334.92 ± 10.53** | 89.78 | 21-38 | 29.44 ±1.26 | 94.72 |
| DMSO (2%) | 291-420 | 355.04 ± 7.72 | 100.00 | 28-37 | 32.66 ± 0.79 | 100.00 |
| 0.01% / DMSO | 280-512 | 373-28 ± 11.00 | 105.14 | 22-40 | 34.10 ± 1.00 | 104.40 |
| 0.05% / DMSO | 245-425 | 340.76 ± 9.63 | 95.97 | 18-36 | 30.14 ± 1.24* | 92.28 |
| 0.10% / DMSO | 240-410 | 329.20 ± 9.22* | 92.72 | 15-36 | 28.54 ± 1.23** | 87.38 |

**Table 7. Variation in flower yield and oil yield/$M_2$ plant raised following treatments with EMS**

| Treatment (EMS) | Flower yield/plant (g) | | | Oil yield (%) | |
|---|---|---|---|---|---|
| | Range | Average | % of control | Range | Average |
| Control | 9.5-17.4 | 12.64 ± 0.57 | 100.00 | 0.16-0.32 | 0.23 |
| 0.01%/$H_2O$ | 7.7-22.3 | 14.18 ± 0.64* | 112.21 | 0.26-0.38 | 0.32 |
| 0.05%/$H_2O$ | 4.5-20.0 | 12.17 ± 0.96 | 96.32 | 0.26-0.35 | 0.30 |
| 0.10%/$H_2O$ | 4.6-19.4 | 11.71 ± 0.99 | 92.66 | 0.22-0.33 | 0.28 |
| DMSO (2%) | 8.9-17.1 | 12.00 ± 0.48 | 100.00 | 0.18-0.28 | 0.22 |
| 0.01%/DMSO | 7.5-15.6 | 11.38 ± 0.93 | 94.84 | 0.21-0.34 | 0.28 |
| 0.05%/DMSO | 5.8-16.3 | 10.80 ± 0.52* | 90.04 | 0.26-0.36 | 0.32 |
| 0.10%/DMSO | 4.5-162 | 10.28 ± 0.80* | 85.72 | 0.23-0.36 | 0.29 |

± Standard error; *Significant at 5% probability level; **Significant at 5% probability level

in metabolically active tissue and increase in cell division or size of cells (Sax, 1962). Similar results have been recorded in *Sorghum* (Wanjari and Kutarekar, 1977). Treatment with EMS induced no change in the cytological behaviour of treated plants. Therefore no alteration was observed in the chromosome complement during mitosis as well as meiosis. Chromosome synapsis was perfect and 8 bivalents were observed at prophase I and metaphase I. They disjuct and 8 chromosomes segregate to each pole at anaphase I.

The use of DMSO as solvent modified the mutagenic effectiveness of EMS. All the biological parameters were reduced as compared to corresponding EMS treatments in water. However, the mean value for oil content was increased. The role of DMSO in increasing mutagenecity of EMS has been reported in many other plants also and it has been attributed to its property of enhancing penetration and absorption of the mutagen (EMS) by the treated tissues (Bhatia, 1967; Singh and Chaturvedi, 1982).

The mutagenic effects of EMS in *A. pallens* are in conformity with the results obtained in a large number of plants under similar treatments (Ram and Kaul, 1991; Padmavathi *et al.*, 1992; Mary and Jayabalan, 1995; Kumar and Mani, 1997; Singh *et al.*, 2000). Moreover, variability observed in $M_1$ generation was manifested through $M_2$ generation suggesting the genetic origin of these variations. This variability seems to be of great practical utility as its exploitation through selection could provide ample scope for selection of desired genotypes. The present investigation shows that a wide range of variability could be generated through the induced chemical mutagenesis in *A. pallens*.

## ACKNOWLEDGEMENTS

The authors are grateful to Director and Chairman, Botanical Sciences Division, RRL, Jammu (J&K) for encouragement and facilities.

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