Mutagenic Effects of EMS on Pollen Mother Cells of *Catharanthus roseus* (L.) G. Don

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**ABSTRACT:** Mutation can be beneficially utilized for tailoring crop plants. Chemical mutagen become one of important tools in crop improvement. Each mutagen has its important role as positive or negative effects on crops. Cytological abnormalities during meiosis has also been regarded one of the dependable parameters for estimating mutagenic sensitivity of a species. Studies undertaken in $M_1$ and $M_2$ generations on pink flower and white flower strains of *Catharanthus roseus* showed EMS elicit various chromosomal aberration in meiosis. Different types of meiotic abnormalities such as fragments, univalents, stickiness and unorientation of bivalents could be observed at metaphase I and bridges, laggards, unequal separation, unorientation of bivalents and stickiness could be seen at anaphase I. Meiotic abnormalities increased along with the increase in concentration in mutagens. The percentage of meiotic abnormalities was recorded maximum in $M_1$ generation as compared to $M_2$ generation. Contrary to pink, white flower strain exhibited maximum meiotic abnormalities and pollen sterility and hence, more sensitive to EMS. The highest pollen fertility was recorded in 0.2% concentration of EMS in pink flower strain of *C. roseus*.

**KEYWORDS:** *Catharanthus roseus*, EMS, mutagenic aberration, pollen fertility

*Catharanthus roseus* (L.) G. Don is an economically important medicinal plant. Among various types of medicinal plants *C. roseus* occupies a unique place in that its alkaloid possesses potent anticancerous activity apart from having other role in treating diseases like diabetes, high blood pressure, rheumatism, asthma, malaria and wasp sting (Chopra et al.1956). It is popularly known as “Flowers That Cure Cancer” and it was aptly so by the virtue of its unique medicinal properties to cure blood cancer due to presence of anticancerous alkaloids, vinristine and vinblastin. Ethyl Methane Sulphonate (EMS) is a chemical mutagen capable of inducing alteration in genotype as well as cytological aberrations which are used as a tool for estimating the efficacy and potency of the mutagen. EMS affects a wide range of chromosomal aberrations resulting into abnormal behavior. Mutagens have clastogenic effects on plant (Abbasi and Anis 2002). Induced mutation has great potential and serve as complementary approach in genetic improvement of crops.

The present work was intended to investigate the effect of EMS on the pollen mother cell (PMC) and pollen fertility of both the strains of *C. roseus*.

**MATERIALS AND METHODS**

Two strains of *Catharanthus roseus* i.e. pink flower and white flower were taken for the present investigation. Both the strains of *C. roseus* were treated with different concentrations of EMS i.e. 0.1%, 0.2%, 0.3%, 0.4% and 0.5% for 4 hours. After maturity, young and juvenile flower buds of both the strains were fixed in 1:3 acetolcohol solution with pinch of ferric chloride for 24 hours and there after transferred to 70% alcohol for preservation. Staining was done in 2% propionic carmine solution. Micro photographs were taken in ORWO photofilm. The data were collected from 100 PMCs per plant and aberrations were recorded.

Pollen grains from mature buds were taken and stained with 2% acetocarmine. Deeply stained pollen grains were taken as fertile and unstained pollen grains were taken as sterile. Percent pollen fertility was calculated by the following formula :-

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Pollen\; fertility\; (%) = \frac{\text{Total number of fertile pollen grains}}{\text{Total number of pollen grains}} \times 100
\]

**RESULTS AND DISCUSSION**

The induced mutagenic aberrations in PMC revealed univalents, fragments stickiness and unorien- tations at metaphase. I while bridges, laggards, unequal separation and stickiness at anaphase I (Figs.1 - 12).

The data depicted in Table 1 and 2 revealed that the percentage of abnormalities at meiotic metaphase in white strain following treatment with EMS increased gradually with the increase in level of concentration of EMS both in $M_1$ and $M_2$. $M_2$ generation showed a lesser degree of aberration as contrast to $M_1$ generation. The maximum (40.57%) and minimum (20.79%) meiotic aberrations during metaphase I could be observed at 0.5% and 0.1% concentration of EMS, respectively in $M_1$ generation. In $M_2$, the maximum (25.47%) and minimum (6.60%) percentage of abnormalities at metaphase I could be noticed at 0.5% and 0.1% concentration, respectively.
Figs. 1-8

1. PMC showing sticky metaphase with precocious movement of bivalent, 2. PMC at diakinetic metaphase showing univalent and trivalent, 3. PMC at metaphase I showing precocious movement, 4. PMC showing univalent, 5. PMC showing stickiness among bivalent, bridge at anaphase I and Translocation ring., 6. PMC showing lagging bivalent at Anaphase I, 7. PMC showing unequal separation, 8. PMC showing unequal separation. Scale bar = 10µm.
As compared to metaphase I, the anaphase I showed lower percentage of meiotic abnormalities both in M₁ and M₂ generation. During anaphase I, maximum (20.75%) aberrations were recorded at 0.4% and minimum (5.94%) at 0.1% concentration in M₁ generation. In M₂ generation, percentage of meiotic abnormalities were recorded maximum (8.82%) at 0.3% and minimum (1.88%) at 0.1% concentration of EMS.

In pink flower, the observation revealed that the percentage abnormality in metaphase I increased in ascending order with the increase in level of concentration of EMS both in M₁ and M₂ generation. However, the M₂ generation showed a lesser degree of aberrations as compared to M₁ generation. The maximum (35.78%) and minimum (12.74%) meiotic aberration during metaphase I could be observed at 0.5% and 0.1% level of concentration respectively in M₁ generation. In M₂ generation, the maximum (4.76%) percentage of abnormalities during metaphase I could be noticed at 0.5% and 0.1% concentration respectively. Anaphase I showed, maximum (17.64%) and minimum (4.90%) aberrations percentage at 0.2% and 0.1% concentration, respectively. Rest of the values lay between these two values but pollen fertility was less as compared to control. Contrary to white flower, pink flower showed gradual increase in pollen fertility with the increase of EMS concentration except at 0.5% concentration. The highest pollen fertility was recorded at 0.2% concentration of EMS, which is being reported for the first time. The minimum pollen fertility i.e. 90.96% was exhibited by 0.5% concentration. Rest of the values lay between them.

The data related to pollen fertility of both the strains of *C. roseus* have been depicted in Table 3. Manifestation of the results indicated a sequential decrease in the pollen fertility rate with increase in EMS concentration. Pollen fertility in white flower was maximum at 0.1% (98.11%) while it was observed to be minimum (80.05%) at 0.5% concentration. Rest percentage of pollen fertility lay between these two values but pollen fertility was less as compared to control. Contrary to white flower, pink flower showed gradual increase in pollen fertility with the increase of EMS concentration except at 0.5% concentration. The highest pollen fertility was recorded at 0.2% concentration of EMS, which is being reported for the first time. The minimum pollen fertility i.e. 90.96% was exhibited by 0.5% concentration. Rest of the values lay between them. Highest concentration of EMS manifested low pollen fertility.

The perusal of the results indicated that the aberrations induced by EMS were transferred from one generation to another finally reaching the stage of sporogenesis. The abnormalities, observed during the study are accordance with the findings of Sudhakaran (1971). The chemical mutagen EMS has influenced the synaptic behaviour of the bivalents thus univalents have been reported. Rees (1961) indicated that the pairing and recombination of
Table 1. Percentage of meiotic abnormalities in white and pink flower of *Catharanthus roseus* after treatment with EMS in M₁ Generation

| Concentration of EMS | Strains | Total No. of PMC Observed | No. of PMC with Abnormalities | % of PMC with abnormalities | % of Abnormalities |
|----------------------|---------|---------------------------|-----------------------------|-----------------------------|-------------------|
| Control              | WF      | 100                       | —                           | —                           | —                 |
|                      | PF      | 100                       | —                           | —                           | —                 |
| 0.1%                 | WF      | 101                       | 27                          | 26.73                       | 20.79             |
|                      | PF      | 102                       | 18                          | 17.64                       | 12.74             |
| 0.2%                 | WF      | 107                       | 41                          | 38.31                       | 25.23             |
|                      | PF      | 102                       | 39                          | 38.23                       | 20.59             |
| 0.3%                 | WF      | 104                       | 55                          | 52.88                       | 39.42             |
|                      | PF      | 102                       | 39                          | 38.23                       | 25.49             |
| 0.4%                 | WF      | 106                       | 65                          | 61.32                       | 40.57             |
|                      | PF      | 103                       | 44                          | 42.71                       | 33.98             |
| 0.5%                 | WF      | 106                       | 56                          | 52.83                       | 40.57             |
|                      | PF      | 109                       | 55                          | 50.45                       | 35.78             |

Table 2. Percentage of meiotic abnormalities in white and pink flower of *Catharanthus roseus* after treatment with EMS in M₂ Generation

| Concentration of EMS | Strains | Total No. of PMC Observed | No. of PMC with Abnormalities | % of PMC with abnormalities | % of Abnormalities |
|----------------------|---------|---------------------------|-----------------------------|-----------------------------|-------------------|
| Control              | WF      | 100                       | —                           | —                           | —                 |
|                      | PF      | 100                       | —                           | —                           | —                 |
| 0.1%                 | WF      | 106                       | 9                           | 8.49                        | 6.60              |
|                      | PF      | 105                       | 7                           | 6.66                        | 4.76              |
| 0.2%                 | WF      | 103                       | 20                          | 19.41                       | 11.65             |
|                      | PF      | 104                       | 18                          | 17.30                       | 10.57             |
| 0.3%                 | WF      | 102                       | 26                          | 25.42                       | 16.66             |
|                      | PF      | 102                       | 20                          | 19.60                       | 13.72             |
| 0.4%                 | WF      | 105                       | 34                          | 32.38                       | 24.76             |
|                      | PF      | 106                       | 27                          | 25.47                       | 20.75             |
| 0.5%                 | WF      | 106                       | 35                          | 33.01                       | 25.47             |
|                      | PF      | 103                       | 31                          | 30.09                       | 25.24             |
chromosome in prophase I is under genetic control and governed by a single pair of genes. Stickiness could be a sequela to depolymerisation of nucleic acid caused by EMS or partial dissociation of the nucleoproteins and alterations in their pattern of organization (Evans 1962). Lagging chromosomes may be explained on the basis of abnormal spindle formation and chromosomal breakage (Tarar and Dnyansagar 1978). Bridges noticed in the present study seemed to be more a result of paracentric inversion as reported by Swanson (1965). According to Sax (1960) and Saylor and Smith (1966) formation of bridge can be due to the failure of chiasmata in a bivalent to terminalize and chromosome get stretched between the poles. Unorientation of chromosome at metaphase I sometimes lead to unequal separation at anaphase I. Khan (1996) also held the similar view. Incorrect movement of laggards may also lead to unequal separation.

The increase in pollen sterility observed in the present study could be attributed to increase in cytological abnormalities. Similar observations have also been reported by Gaul et al. (1966). The gibberellic acid present in the plant might have reduced the aberration in EMS treated plant which in turn could have reduced the pollen sterility and therefore, cent per cent pollen fertility could be observed at low concentration (Narsinghani and Kumar 1971).

From the present findings it is inferred that the EMS is capable of inducing meiotic aberration and enhancing the pollen fertility. It is expected that increase in pollen fertility will in turn increase the population of plant and there by simultaneous increase in production of alkaloid which is medicinally and economically important.

**Table 3. Pollen fertility in two strains of *Catharanthus roseus* after treatment with EMS**

| Concentration of EMS | White flower | Pink flower |
|----------------------|-------------|-------------|
| Control              | 98.30       | 97.93       |
| 0.10%                | 98.11       | 99.61       |
| 0.20%                | 97.63       | 100.00      |
| 0.30%                | 95.48       | 99.42       |
| 0.40%                | 92.46       | 98.85       |
| 0.50%                | 80.05       | 90.96       |

**LITERATURE CITED**

Abassi, N. and Anis, M. 2002. Clastogenic effect of chemical mutagens in *Trigonella foenum-graceum* L. J. Cytol. Genet. 3 : 109-114.

Chopra, R. N., Nayar, S. L. and Chopra, I. C. 1956. Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research, New Delhi.

Evans, H. J. 1962. Chromosome aberration induced by ionizing radiation. Int. Rev. Cytol. 13 : 221–232.

Gaul, H., Bendrer, K., Vlonska, E. and Sato. M. 1966. EMS induced genetic variability in barley. In The problem of EMS induced sterility and a method of increase the efficiency of EMS treatment mutation in plant breeding IAEA Vienna. pp. 12-13.

Khan, I. A. 1996. Meiotic irregularities induced by DES in chilli peper (*Capsicum annum*) var. NP46A. Prog. Hort. 28 : 36-40.

Narsinghani, V. G. and Kumar S., 1971. The effect of gibberellic acid on chromosomal aberration in EMS and MMS treated *Pisum sativum* Linn. Theoretical and Applied Genetics 41 : 18-20.

Rees, H. 1961. Genotype control of chromosome form and behaviour. Bot. Rev. 27 : 288-318.

Sax, K. 1960. Meiosis in inter specific pine hybrids. Forest Sci. 6 : 135-138.

Saylor, L. G. and Smith, B. N. 1966. Meiotic irregularities in species of inter specific hybrids in *Pisum*. Am. J. Bot. 53 : 453–468.

Sudhakaran, I. V. 1971. Meiotic abnormalities induced by gamma rays in *Vinca rosea* Linn. Cytologia 36 : 67-69.

Swanson, C. P. 1965. Cytology and Cytogenetics. Prentice Hall Inc. USA. Upper Saddle River, New Jersey.

Tarar, J. L. and Dnyansagar, V. R. 1978. Composition of ethyl methane sulphonate and radiation induced meiotic abnormalities in *Turnera ulmifolia* Linn. var. *augustifolia* Willd. Cytologia 45 : 221-231.