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Cytogenetic effect of EMS on root meristem cells of *Catharanthus roseus* (L.) G. Don var. Nirmal

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ABSTRACT

Cytological effects of the ethyle methane sulphonate (EMS) were investigated in root tip cells of Catharanthus roseus (L.) G. Don var. Nirmal. The root tip of C. roseus treated with various concentrations (0.50%, 0.75% and1%) of ethyle methane sulphonate. The results showed dose dependent increase in mitotic indices. The chromosomal anomalies observed include condensation, persistent nucleolous, fragmentation, C- metaphase, bridge, laggard, cleft and binucleolated cells.

KEYWORDS: *Catharanthus roseus, ethyle methane sulphonate, root tip, mitosis*

INTRODUCTION

Cytogenetic studies are necessary to obtain information regarding the role and effect of mutagen and in elucidating the response of genotypes to a particular mutagen. Mutations can be beneficially utilized for tailoring better varieties of crop plant. Among chemical mutagen alkylating agents effects a wide range of chromosomal aberrations causing meiotic and mitotic disturbances which leads to various chromosomal abnormalities. EMS is more efficient in inducing chromosomal aberrations keeping in view the economic importance of *Catharanthus roseus* (L.) G. Don (2n=16) being an important medicinal plant source of active principles vincristine, vinblastine, and ajmalcine the present study was undertaken to assess the mutagenic effect of EMS on a promising variety of *C. roseus* "Nirmal" by studying mitotic behavior of chromosomes after treatment.

MATERIALS AND METHODS

Pure and healthy seeds of *Catharanthus roseus* (L.) G. Don (var. Nirmal accession no-CIMAP-0865) was presoaked in distilled water for 18 hours; to facilitate permeability to the mutagen and divided into four lots of 50 seeds each. Out of four, three lots were treated with different concentrations (0.50%, 0.75% and1%) of EMS (CH_3 $SO_2OC_2H_5$) solution prepared in phosphate buffer pH 7.0. at 30±1^{0}C temperature for about six hours and remaining lot was maintained in distilled water for the same time duration as control . After treatment, seeds were thoroughly washed than kept in petridishes on moist blotting paper for cytological investigations. On germination, radicle of appropriate length were fixed in 1: 3 (acetic acid: alcohol) in between 1:15 -1:35 pm. After 24 hours, fixative was removed and tips were preserved in 90% alcohol. Chromosomal preparations were made by following haematoxylin squash procedure[1]. Random scoring were made from the 15 different microscopic fields in 10 root tips for determination of the frequency of mitotic index (MI) and chromosomal aberrations (CA). Pooled data were statically analyzed using on STATISTICA (version 6.0) software.

RESULTS

The effects of chemical mutagen (EMS) have been studied on mitotic activity of the root meristems. The percentage of dividing cells (mitotic indices) increased with increasing doses of mutagen. The value of mitotic index was maximum for 1.0% EMS and minimum at control. Mean values for mitotic indices at 0.50% EMS, 0.75% EMS and 1.00% EMS were significantly higher (P>0.01) than that of control (**Table-1**).

The percentage of various mitotic anomalies increased with the increasing doses of mutagen. Percentage of dividing cells with abnormalities were found 4.17 ± 0.81 for control, 53.43 ± 0.45 for 0.50% EMS, 62.35 ± 4.66 for 0.75% EMS and 68.13 ± 1.61 for 1% EMS(**Table-1**).

Table 1: Effect of EMS treatments on cell division of root meristem of *C. roseus* (L.) G. Don (var. Nirmal)

Treatments	Mitotic index	Abnormality %	Total cells in prophase	Total cells in metaphase	Total cells in in anaphase	Total cells in telophase
Control	4.71 ± 0.43	4.17 ± 0.81	38.27 ± 2.38	26.31 ± 1.69	26.13 ± 1.67	10.10 ± 2.97
0.50 % EMS	7.19±0.45**	53.43 ± 9.25**	30.81±4.44ns	16.24 ±4.29ns	18.65 ± 2.49ns	35.95 ± 7.12*
0.75 % EMS	8.31±0.15**	62.35 ± 4.66**	25.13±4.43ns	17.03 ±2.43ns	21.97 ± 3.86ns	34.35 ± 2.96*
1.00 % EMS	9.44±0.27**	68.13 ± 1.61**	25.05±3.84ns	18.39 ±1.53ns	25.65 ± 3.06ns	33.75 ± 5.84**

ns- p>0.05, *- p<0.05, **- p<0.01- in comparison with "control"

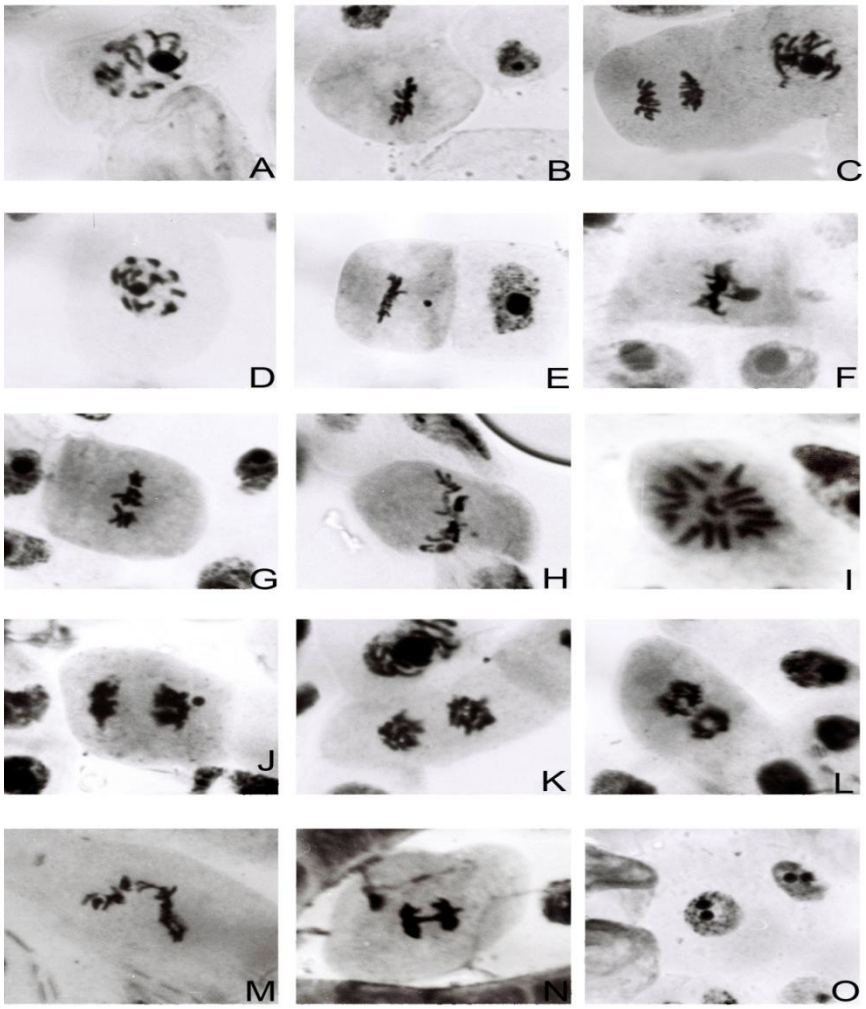

Figure: A-C: Control prophase, metaphase and anaphase, D: Condensed prophase, E: Persistent nucleoli at metaphase, F: Balloonous metaphase, G: Cleft at metaphase ,H: Fragmented metaphase, I: C-metaphase, J: Anaphase with persistent nucleolous, K: Condensed anaphase, L: Cleft at anaphase, M: Disturbed Anaphase, N: Anaphase with bridge,O: Binucleolated cell.

The various types of cytological anomalies observed (**Fig- E-O**)) were condensation, metaphase with persistent nucleolous, anaphasic bridges, and C-metaphase, metaphasic cleft, stickiness /clumping, strays, fragmentation, laggards and disturbed anaphase (**Table-2**). Besides these anomalies, vacuolated anaphase, binucleated condition, micronuclei, were also found.

Table 2: Percentage of different abnormality type of *C. roseus* (L.) G. Don (var. Nirmal) roots treated with different concentrations of EMS.

Treatments	Con-pro	Clu-meta	Meta-pn	C-meta	M-cleft	F-meta	Ana-pn	Co-ana	F-ana	Lag	Bri	Binu	Other
Control	-	-	-	-	-	-	-	-	-	-	-	-	-
0.50 %EMS	4.74	-	12.11	2.11	5.26	11.57	3.16	-	-	-	-	47.89	13.16
0.75 %EMS	15.14	2.17	6.49	7.57	3.24	10.62	18.92	2.16	1.62	1.08	0.52	20.46	10.01
1.0 % EMS	16.77	9.32	-	3.73	4.35	-	8.51	5.59	13.67	1.86	-	17.27	18.23

Con-pro: Condensed prophase, **Clu-meta**: clumped metaphase, **Meta-pn**: Metaphase with persistent nucleolous, **C-meta**:C metaphase **M-cleft**: Metaphasic cleft, **F-meta**: Fragmentation at metaphase, **Ana-pn**: Anaphase with persistent nucleolous, **Co-ana**: Condensed anaphase, **F-ana**: Fragmentation at anaphase, **Lag**: Laggard, **Bri**: Bridge, **Binu**: Binucleolated cell

Condensation was observed at all the doses of mutagens. Metaphase with persistent nucleolous, C-metaphase, anaphase with persistent nucleolous, binucleolated condition were more frequent. Bridge was found only at 0.75% EMS treatment.

DISCUSSION

The plant parts especially the root tips are extremely useful material in biological testing, as the root tips are often first to be exposed to mutagens. The observations on the root tip system constitute rapid and sensitive method for chromosomal monitoring. The degree of cytological abberations in mitosis is regarded as one of the dependable criteria for estimating the mutagen sensitivity of the species and the effect of mutagens[2].

In our study mitotic index was found directly proportional to the doses/concentrations of mutagen Increase in mitotic index values in root meristem treated with EMS are the result of accumulation of C-metaphase configuration at zero hour recovers[3]. Stickiness was more frequent at all the doses. Stickiness has been attributed to the entanglement of interchromosomal chromatin fibers that leads to sub chromatid connection between chromosomes[4], but was considered by Patil and Bhat[5] as a type of physical adhesion involving mainly the proteinacious matrix of chromatin. Gaulden [6] postulated that stickiness may result from defective functioning of one or two types of specific nonhistone proteins involved in chromosomal separation and segregation. The altered functioning of these proteins leading to stickiness is caused by mutation in structural genes coding for them (hereditary stickiness) or by the action of mutagens (induced stickiness). According to Achkar *et al*.[7] sticky chromosomes are formed when breaks in the double strands of DNA are introduced while the intrachromatid links initiate their formation during chromosome condensation, determined by chromosome protein bond as histones. However, stickiness should be considered as mitotic disruption that is not likely to lead to chromosomal structural damage[3].

Persistent nucleolus was observed at metaphase and anaphase stage more frequently. Several workers have observed nucleolar persistence in several crops[8]. The persistence of nucleolus can be assumed to be cause by the extension of heterochromatin activity up to the stage of division. Increased frequency of persistent nucleolus may be attributed to the inhibitory effect of EMS on the synthetic process[9].

EMS treatment also induced C- metaphase and frequency of C- metaphase followed a dose dependent pattern. According to Somashekare *et al.*[10] C- metaphase is a one of the consequence of inactivation of spindle apparatus connected with the delay in division of centromere. Deysson[11] suggested that this may lead to polyploidy and cells thus formed degenerate without further division.

Laggards were observed at higher doses of mutagen. Lagging of chromosome may be either due to its attachment to very weak spindle fiber or no attachment at all. Lagging chromosomes and fragments are observed as a result of formation of acentric chromosomal fragment during exchange or due to chromosomal breaks. Das and Roy[12] were of the opinion that due to the effect of mutagens the spindle fibers failed to carry the respective chromosome to polar region and resulting in the lagging chromosome appeared at anaphase. Beside these reason delayed terminalization, stickiness of chromosome ends and failure of chromosome movements have lead to laggard chromosome. Magoon *et al.*[13] also support the above view but they specified that this could be due the change in homology of the paired chromosome. According to Gilli[14], Levan[15] formed acentric chromosome which are observed as lagging chromosome or fragment during anaphase may take the shape of a micronuclei at telophase if chromatin material involved is sufficiently large. Aurenbanch[16] stated that micronuclei are true mutageneic effect which may leads to loss of genetic material.

Anaphasic bridges were observed more frequent at the higher doses. Bridges are formed generally due to stickiness of the chromosomes at metaphase or breakage and reunion of chromosome[17,18,19]. Singh and Khanna[20] considered that anaphase bridges may be formed due to unequal exchange or dicentric chromosomes. The number of bridges depends upon the number of chromosomes taking part in the exchange[21]. Yagyu and Morris[22] also reported formation of anaphase bridges and suggested that double bridges may be seen at anaphase when there is translocation between non- homologus chromosome producing unlike arms while single bridge occurred due to fusion of sister chromatid at a common breakage point.

The other anomalies which were detected are irregular prophases, disturbed metaphase, disturbed anaphases and stray. According to Hanna *et al.*[23] the development of these abnormalities indicates that this mutagen partial inhibit the mitotic apparatus. The formation of binucleated, and multinucleated cells in treated material may be die to metabolic disorders. According to Somashekhar *et al.*[10] mutagen inhibits cytokinesis process leading to the accumulation of binucleate and multinucleate cells. Binucleate cells are otherwise interpreted as cosequence of inhibited cell cycle in which chromosome DNA is replicated but not distributed in usual way[24].

On the basis of this study it may be concluded that the EMS treatments at various concentrations affect *Catharanthus roseus* root meristem and induced number of chromosomal anomalies which lead to wide range of variations in cytological attributes. Hence EMS could be utilized for induction of genetic variability in *C. roseus*.

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