Journal of Global Biosciences
ISSN 2320-1355
Volume 4, Special Issue 1, 2015, pp. 1816-1823
Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

EMS INDUCED MITOTIC ABNORMALITIES IN *CATHARANTHUS ROSEUS* (L.) G. Don

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Abstract

Two varieties of *Catharanthus roseus* (L.) G. Don i.e. pink flower and white flower were selected for the mutagenic treatment. Fresh healthy roots were treated with 0.l%, 0.2%, O.4% & 0.5% of for 4 h. Different chromosomal abnormalities like prophase clumping, stickiness, diagonal arrangement, fragment, Tropokinesis, Triploid cell, bridge, laggard, unequal separation and binucleate cell were observed following treatment with different concentration of EMS. Mutagenic efficiency was computed on the basis of induction of aberrations. Lower dose of EMS (0.2% & 0.3%) was found to be most efficient in inducing abnormalities.

Key words: *Catharanthus roseus*, EMS, Chromosomal aberrations, mitosis.

INTRODUCTION

Catharanthus roseus (family Catharanthaceae) is an important medicinal plant with immence therapeutic applications including anticancerous property. It is a handsome garden plant which flowers throughout the year and consequently it is called Sadabahar. Due to its powerful herbal effect, the French called it as "Violet of Socerers". The period of crowning glory came for *C. roseus* when it was nicknamed "Flower that cure Cancer" due to presence of two anticancerous alkaloids viz. vincristine and vinblastin. Two varieties of *Catharanthus roseus* i.e. pink flower and white flower were subjected to chemical treatment. Cytological analysis with respect to mitotic behaviour is considered to be one of the dependable indices to estimate the potency of mutagen. Mutagens may bring changes in qualitative and quantitative characters of plants thus promising remarkable possibilities in crop improvement. Induction of chromosomal abnormalities has been regarded as one of the dependable parameters for estimating the mutagenic efficiency of a mutagen. It also provides a considerable clue to assess sensitivity of plant for different doses of EMS. The present investigation was to find out the effect of chemical mutagen ethyl methane sulphonate on the frequency and types of mitotic chromosomal aberrations and efficiency of chemical mutagen.

MATERIAL AND METHODS

Young plants of *Catharanthus roseus* of pink and white flower variety were treated with 5 different concentrations of EMS (0.l%, O.2%, 0.4% & 0.5%) for 4 hours. After treatment, plants were thoroughly washed with distilled water and were planted in separate earthen pots. New roots were cut and pretreated with 8- hydroxyl quinoline and fixed in 1: 3 acetoalcohol for 24 hours. They were preserved in 70% alcohol in separate test tube. Staining was done in 2%

acetocarmine and N Hcl solution. Slides were prepared by squash technique. The cytological aberrations were studied and recorded for M_1 and M_2 generation.

RESULTS

Ethyl methane sulphonate decreased the mitotic index as compared to control in all the concentrations (Table 1 and 2).

Various abnormalities like nuclear vacuolation, prophase clumping, stickiness of chromosome, spindle abnormality, tropokinesis, diagonal or unorientation of chromosome, laggards, bridges and binucleate cells were observed.

Nuclear vacuolation appeared in all concentrations of EMS in both the varieties. Prophase abnormalities showed clumping of chromosome. It was found maximum (2.80± 0.50) in white variety at 0.3% concentration. Stickiness was again found maximum (3.50± 0.35) in white variety at 0.1% concentration. The result showed that the frequency of stickiness decreased gradually as the concentration of EMS was increased in white flower while it was found to be fluctuating in pink flower at different concentrations. Diagonal arrangement was recorded maximum (3.12±0.35) in white flower at 0.1 percent concentration in M_1 generation.

The frequency of fragment was found maximum (1.63±0.00) in pink flower at 0.3% concentration. In white flower it was recorded only at 0.3% concentration in M_1 generation. Tropokinesis was recorded maximum (2.50±0.00) in white flower at 0.1% concentration in M_1 generation. Triploid cell (0.71±0.00) was recorded only in pink flower at 0.2% concentration in M_1 generation.

Frequency of single bridge, double bridge and multiple bridge were found maximum in pink flower at 0.3% (1.63±0.00), 0.2% (0.71±0.00) and 0.3% (1 .63±0.00) respectively.

Double bridge and multiple bridge were absent in both M_1 and M_2 generation in white variety. Laggard was found maximum (1.05±0.00) in pink flower at 0.1% concentration.

The frequency of stickiness and unequal separation was recorded maximum in pink flower at 0.1% concentration.

Diagonal arrangement was found maximum (2.50±0.00) in white flower at 0.1% concentration.

Diagonal arrangement of chromosome at telophase was recorded maximum (1.63±0.00) in pink flower at 0.3% concentration in M_l generation.

Binucleate cell was observed only in pink flower and was recorded maximum (1.04±0.30) at 0.1% concentration.

Data related to frequency of mitotic inhibition and abnormalities in pink and white variety of *C roseus* have been presented in table 3 and 4.

The frequency of aberrations recorded were maximum at 0.4% concentration of EMS in pink variety. It found that in M_l generation, relative division rate and relative abnormality rate were inversely correlated to each other in pink variety.

Relative division rate was found to be maximum (-30.70) at 0.4% concentration and Relative abnormality rate was found maximum (32.79) at 0.2% concentration in white flower in M_1 generation. Mitotic inhibition was recorded maximum (89.92) at 0.3% concentration in pink variety. The data revealed that the mitotic inhibition along with frequency of chromosomal abnormalities were decreased in M_2 generation as compared to M_1 generation.

DISCUSSION

The effect of ethyl methane sulphonate on the two varieties of *C. roseus* i.e. pink flower and white flower not only brought down the frequency of dividing cells but also produced quite large number of anomalies in the mitotic cells. A concentration dependent decrease and increase in the mitotic index was observed. Drastic lowering in mitotic index and relative division rate of treated material indicated that the drug inhibited the nucleic acid synthesis (Cummins, 1969). This has revealed the antimitotic property of the chemical mutagen, EMS. Because of this, celI was arrested at various stage of metphase perhaps malfunction of mitotic spindle (Kihlman, 1966; Meena Kumari etal, 1995). It was also assumed that the reduction of mitotic index after chemical treatment may be due to prophase poisoning which obstruct the chromosome movement from prophase to metaphase. Such findings were also supported by

Sudhakaran (1972) in *Vinca rosea* L. While comparing the mitotic index in two varieties, pink flower variety was found more sensitive and white flower variety was resistant towards the doses of EMS for both M_1 and M_2 generations.

The clumping of chromosome may be due to depolymerisation of nucleic acid present over the chromosome surface, which the chromosome to form clump. The prophase clumping was again higher in white flower variety than pink flower showing its sensitivity towards different doses of EMS.

The stickiness of chromosomes may be due to the disturbances in nucleic acid metabolism of the cell (Darlington, l942). The diagonal orientation of chromosome may be due to improper functioning of the spindle apparatus. The chemical mutagen EMS has an action on the spindle therefore, it can be considered as stathmokinetic agent (Shehab,1979). The stickiness and diagonal arrangement were found more in white flower than pink flower. The formation of fragments may be due to chromosome breakage due to the effect of chemical mutagen EMS. It was found maximum in pink flower variety.

Tropokinesis was due to complete inactivation of spindle stamokinesis. It was again found higher in white flower showing its sensitivity toward the mutagen EMS. The presence of triploid cells also indicated that EMS is a potential spindle poison. Similar results have been recoded in *vicia faba* (Chandra et al. 2002)

The anaphasic bridges may be formed due to paracentric inversion and stickiness. It also might have resulted due to disturbances at cytochemical level (Evans, 1962; Sinha, 1967). Double bridges might be due to fusion between broken ends of chromosomes. Lawely and Brookes (1963) suggested that the lagging chromosome may be explained on the basis of abnormal spindle formation and failure of chromosomal breakage by binding to DNA regions rich in GC pairs causing them to become instable.

Formation of laggards were due to unequal separation of chromosome. Fragments may be due to radiomimetic property of EMS. Arrest of cytokinesis led the formation of binucleate cells (Kihlman,1974). The inhibitory effect of the chemical mutagen EMS on mitosis in generally attributed to mitotic poison (Corbett, 1974).

While considering the abnormalities in metaphase and anaphase, white flower variety was found sensitive towards different concentrations of EMS and pink flower variety was found resistant. The efficiency of chemical mutagen at lower doses (0.2% and 0.3%) was found to be highest when compared to all other concentrations. Thus EMS at 0.2% and 0.3% was most efficient in inducing chromosomal abnormalities. While considering the two generations it was clear that there may be repair mechanism due to which there was decrease in relative abnormality rate and increase in mitotic index.

Table 1 : Types and frequency of chromosomal abnormalities during different phase of mitosis at varying concentration of Ethyl Methane sulphonate in pink variety of *Catharanthus roseus* (L.) G. Don in M_1 and M_2 generations.

Conc.	Genera-tion	Total No. of Cell observed	Resting cell	Total no. of dividing cell	Mitotic index	Prophase Abnormality (Clumping)	Percentage Metaphase abnormality						Percentage Anaphase abnormality							Telophase abnormality	
							Stickiness	Diagonal arrangement	Fragment	Tropo-kinesis	Elongated cell	Triploid cell	Single bridge	Double bridge	Multiple bridge	Laggard	Stickiness	Unequal separation	Diagonal arrangement	Diagonal arrange-ment	Binu-cleate cell
Control		950	352	598	62.94	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 %	M_1	958	863	95	9.91	-	1.24± 0.12	1.26± 0.20	1.05± 0.00	1.05± 0.00	-	-	1.05± 0.00	-	1.05± 0.00	1.05± 0.00	1.05± 0.00	1.05± 0.00	-	-	1.04± 0.30
	M_2	921	716	205	22.25	-	0.80± 0.33	0.73± 0.50	0.48± 0.00	0.48± 0.00	-	-	-	-	-	-	-	-	0.48± 0.00	-	-
0.2 %	M_1	940	800	140	14.89	0.71± 0.00	0.92± 0.25	0.71± 0.00	-	-	2.14± 1.15	0.71± 0.00	-	0.71± 0.00	-	-	-	-	-	-	-
	M_2	921	684	237	25.73	-	0.56± 0.33	0.52± 0.28	0.63± 0.50	-	-	-	-	-	-	-	0.42± 0.00	-	0.42± 0.00	-	-
0.3 %	M_1	961	900	61	6.34	-	3.00± 0.98	1.63± 0.00	1.63± 0.00	1.63± 0.00	9.83± 0.00	-	1.63± 0.00	-	1.63± 0.00	-	-	-	1.63± 0.00	1.63± 0.00	-
	M_2	914	702	212	23.19	-	0.58+ 0.25	0.70± 0.50	-	0.47± 0.00	-	-	-	-	-	-	0.94± 0.00	-	0.47± 0.00	-	-
0.4 %	M_1	959	632	327	34.09	0.30± 0.00	0.40± 0.21	0.36± 0.20	0.30± 0.00	0.30± 0.00	-	-	0.30± 0.00	0.30± 0.00	-	0.30± 0.00	-	0.30± 0.00	0.38± 0.00	0.30± 0.00	0.50± 0.33
	M_2	940	551	389	41.38	0.51± 0.57	0.41± 0.24	0.51± 0.00	0.25± 0.00	-	-	-	-	-	-	-	0.38± 0.5	0.25± 0.00	0.34± 0.33	0.25± 0.00	0.51± 0.00
0.5 %	M_1	963	808	155	16.09	1.07± 0.33	1.09± 0.26	1.45± 0.75	0.64± 0.00	0.96± 0.28	-	-	0.64± 0.00	-	-	0.64± 0.00	0.64± 0.00	0.85± 0.33	0.85± 0.33	0.64± 0.00	-
	M_2	920	759	161	17.50	0.82± 0.33	0.77± 0.25	0.77± 0.25	-	0.77± 0.25	-	-		-	-	-	0.62± 0.00	-	0.82± 0.00	-	-

Table 2 : Types and frequency of chromosomal abnormalities during different phase of mitosis at varying concentration of Ethyl Methane sulphonate in white variety of *Catharanthus roseus* (L.) G. Don in M_1 and M_2 generations.

Conc.	Generation	Total No. of Cell observed	Resting cell	Total no. of dividing cell	Mitotic index	Prophase Abnormality (Clumping)	Percentage Metaphase abnormality				Percentage Anaphase abnormality						Telophase abnormality	
							Stickiness	Diagonal arrangement	Fragment	Tropokinesis	Single bridge	Laggard	Stickiness	Unequal separation	Diagonal arrangement	Unorrientation	Binucleate cell	Diagonal arragement
Control		938	628	310	33.04	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1%	M_1	922	882	40	4.43	2.50±0.00	3.50±0.35	3.12±0.35	-	2.50±0.00	-	-	-	-	2.50±0.00	-	-	-
	M_2	929	654	275	29.60	-	0.54±0.50	0.36±0.00	0.54±0.50	0.36±0.00	-	-	0.36±0.00	-	0.36±0.00	-	-	-
0.2%	M_1	936	836	100	10.68	1.25±0.25	1.69±0.20	1.40±0.22	-	1.00±0.00	-	-	1.50±0.50	-	1.00±0.00	-	-	1.00±0.00
	M_2	919	554	365	39.71	-	0.34±0.25	0.34±0.25	0.27±0.00	0.27±0.00	-	-	0.27±0.00	-	0.27±0.00	-	-	-
0.3%	M_1	946	857	89	9.40	2.80±0.50	1.55±0.14	1.49±0.14	1.12±0.00	1.12±0.00	-	-	-	-	1.28±0.14	1.12±0.00	-	-
	M_2	908	584	324	35.68	0.30±0.00	0.46±0.18	0.30±0.00	-	-	-	-	-	-	0.30±0.00	-	-	-
0.4%	M_1	913	799	114	12.48	-	1.49±0.39	1.37±0.20	-	1.45±0.60	0.87±0.00	0.87±0.00	0.87±0.00	-	0.87±0.00	0.87±0.00	-	0.87±0.00
	M_2	919	463	459	49.61	0.21±0.00	0.21±0.00	0.32±0.00	0.21±0.00	-	-	-	-	-	-	-	0.65±2.00	-
0.5%	M_1	921	807	114	12.37	0.87±0.00	1.38±0.14	1.35±0.24	-	1.01±0.16	0.87±0.00	-	-	1.75±0.00	1.09±0.25	-	-	0.87±0.00
	M_2	916	488	428	46.72	0.31±0.33	0.29±0.25	0.29±0.25	0.23±0.00	-	-	-	0.35±0.50	-	0.35±0.50	-	-	-

Table 3 : Frequency of mitotic inhibition and abnormalities in the root tip of pink variety of *C. roseus* (L.) G. Don after treatment with EMS.

Conc.	**M_1 Generation**				**M_2 Generation**			
	Mitotic index	Relative Division Rate (R.D.R.)	Relative Abnormality Rate (R.A.R.)	Mitotic inhibition	Mitotic index	Relative Division Rate (R.D.R.)	Relative Abnormality Rate (R.A.R.)	Mitotic inhibition
Control	62.94	-	-	-	62.94	-	-	-
0.1%	9.91	-143.09	-78.95	84.25	22.25	-109.79	-132.91	64.64
0.2%	14.89	-129.65	-127.44	76.34	25.73	-100.40	-140.93	59.11
0.3%	6.34	-152.72	-37-12	89.92	23.19	-107.25	-107.90	63.15
0.4%	34.09	-77.84	-139.31	45.83	41.38	-58.17	-143.44	34.25
0.5%	16.09	-126.41	-86.29	74.43	17.50	-122.61	-126.33	72.19

Table 4 : Frequency of mitotic inhibition and abnormalities in the root tip of white variety of *C. roseus* (L.) G. Don after treatment with EMS.

Conc.	**M_1 Generation**				**M_2 Generation**			
	Mitotic index	Relative Division Rate (R.D.R.)	Relative Abnormality Rate (R.A.R.)	Mitotic inhibition	Mitotic index	Relative Division Rate (R.D.R.)	Relative Abnormality Rate (R.A.R.)	Mitotic inhibition
Control	33.04	-	-	-	33.04	-	-	-
0.1%	4.33	-42.87	21.59	86.89	29.60	-5.13	-42.83	10.41
0.2%	10.68	-33.39	32.79	67.67	39.71	9.96	-43.21	-20.18
0.3%	9.40	-35.30	22.80	71.54	35.68	3.94	-39.20	-7.99
0.4%	12.48	-30.70	10.91	62.22	49.61	24.74	-44.44	-50.15
0.5%	12.37	-30.86	20.08	62.56	46.72	20.43	-41.32	-41.40

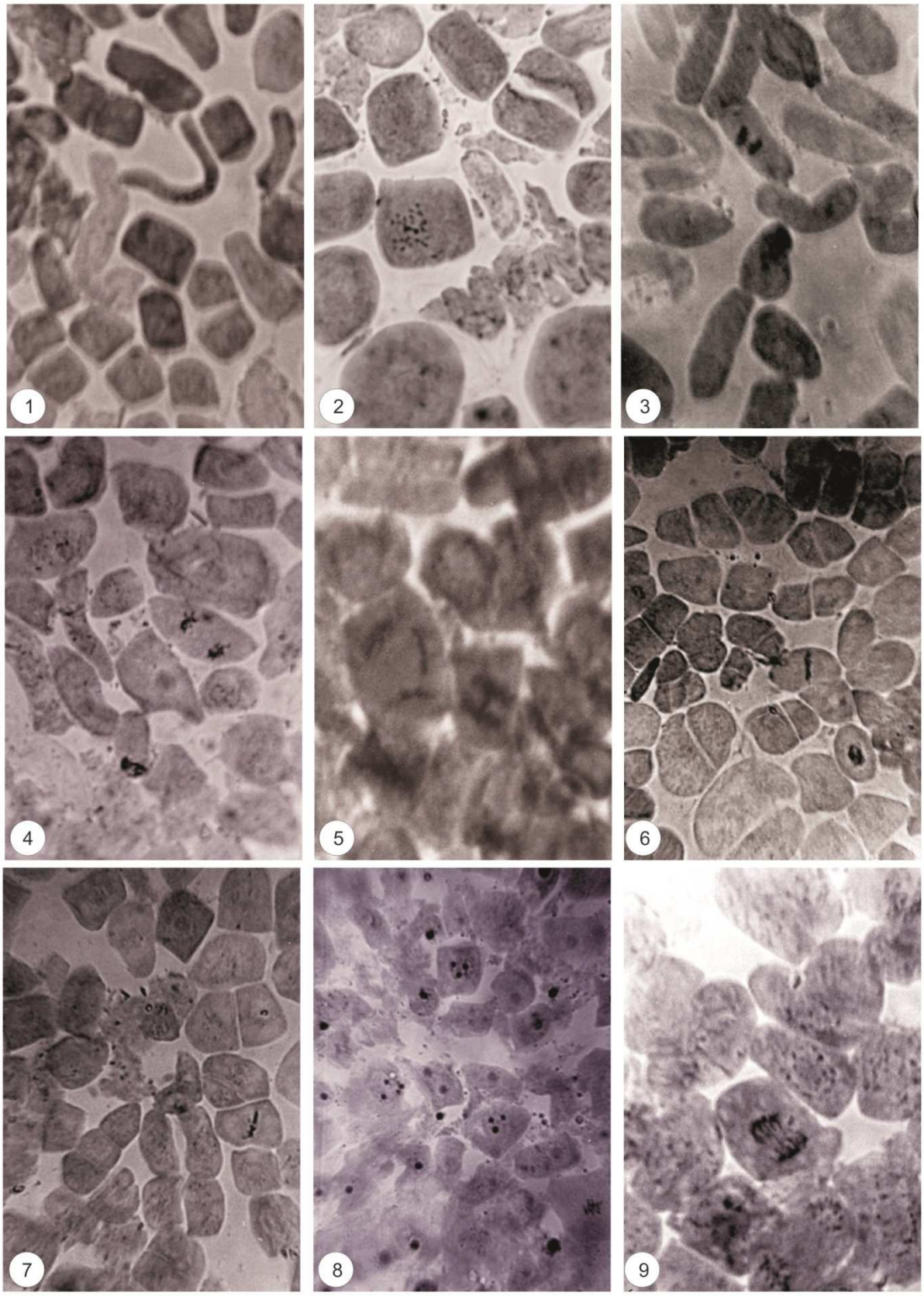

Fig 1-Tropokinesis, 2-Endomitosis, 3-Double bridge at anaphase, 4-Diagonal arrangement & stickiness at anaphase, 5-Single bridge & laggard at anaphase, 6-Stickiness at metaphase & prophase clumping, 7-Stickiness at metaphase, 8-Binucleate cell and 9-double bridge at anaphase

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