ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 13, Issue, 09, pp.18719-18722, September, 2021

DOI: https://doi.org/10.24941/ijcr.42268.09.2021

RESEARCH ARTICLE

CYTOLOGICAL STUDIES IN PEA BY THE APPLICATION OF CHEMICAL MUTAGEN

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ARTICLE INFO

Article History:
Received 25th June, 2021
Received in revised form
20th July, 2021
Accepted 19th August, 2021
Published online 30th September, 2021

Key Words:

Pisum sativum, chemical mutagen, cytology, EMS, aberrations, meristems.

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ABSTRACT

Cytological investigations have been carried out in the experimental set of individual treatment of chemical mutagen such as EMS concentration in Pea (*Pisum sativum* L.). Seeds of Pea were subjected to different concentrations of EMS. The treated root tips showed varying degree of mitotic abnormalities almost in all the concentrations of EMS. The frequency of mitotic abnormalities was found to be more in highest concentrations of EMS. The various types of mitotic aberrations such as fragments, bridges, laggards, micronuclei, early and late separation were scored in case of root meristem cells of treated materials. Taking the percentage of mitotic aberrations and germination as an index of effectiveness of a mutagen concentration proved to be the most effective.

Citation: Dr. Govind Dhulgande and Datta Ghogare. "Cytological studies in Pea by the application of chemical Mutagen*", 2021. International Journal of Current Research, 13, (09), 18719-18722.*

INTRODUCTION

The plant Pea (*Pisum sativum* L.) is classified under family Leguminosae and subfamily Fabaceae. Botanically it is known as *Pisum sativum* Linn. It is commonly known as 'matar'. It is an important nutritious vegetable crop, which occupies a very sound position due to its importance both vegetable as well as a pulse crop. Pea is an economically important food grain grown in most parts of India.

It is one of the rich sources of protein. In developing country like India, pulses still remain the only available protein source to the people. It is therefore, of utmost importance to find ways to increase their qualitative and quantitative characters. Mutation breeding seems to be a handy tool for such purposes. Therefore, different mutagens are being used in genetic improvement programmes. During the present investigation single treatment of ethyl methanesulphonate have been used to induce genetic variability in pea.

MATERIALS AND METHODS

Seeds of pea (*Pisum sativum* L.) obtained from local market of Pune (M.S.), India were treated with different concentrations of EMS (0.05%, 0.10% and 0.15%). After each treatment, seeds were thoroughly washed with running tap water for one hour and sown in petridishes along with suitable control. For the mitotic studies, root tips of appropriate size were cut and fixed in Carnoy's fixative for 24 hours and then transferred in 70% alcohol. The meristematic root tip cells were analyzed to score the chromosomal aberrations at different stages of mitosis at each concentrations of EMS. Also germination percentage was calculated during present investigation.

$$\text{Mitotic index} = \frac{\text{Number of dividing cells in microscopic field}}{\text{Total number of cells in microscopic field}} \times 100$$

$$\text{Metaphase frequency} = \frac{\text{Number of cells in metaphase stage}}{\text{Total number of cells in microscopic field}} \times 100$$

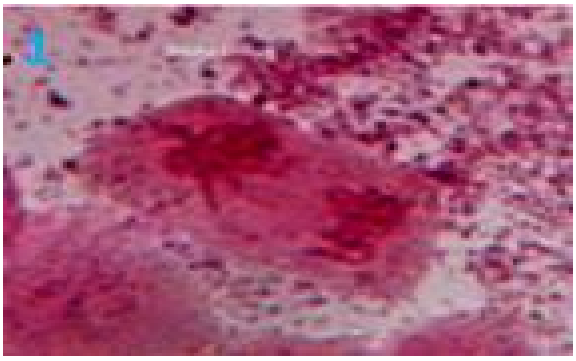

Fig.1.Sticky Bridge

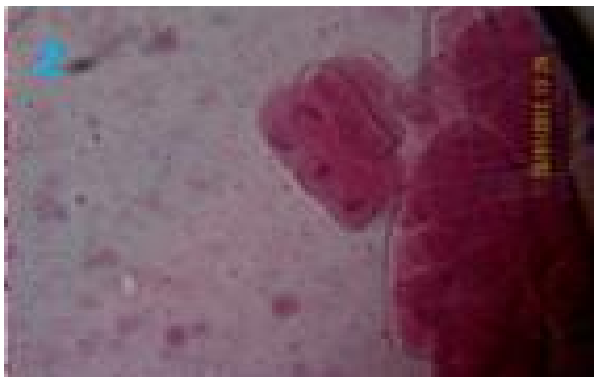

Fig.2. Early separation

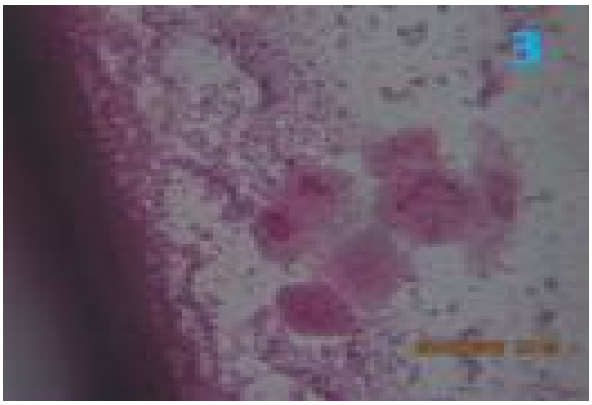

Fig.3.Late separation

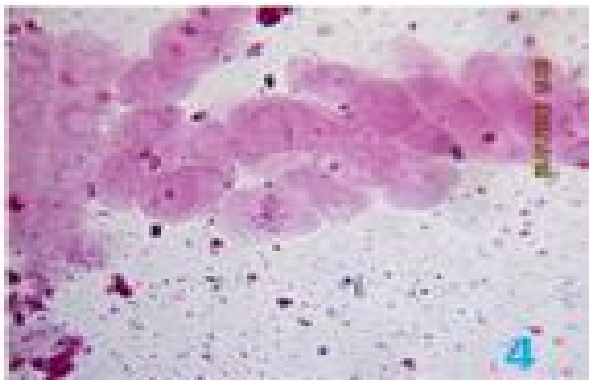

Fig. 4. Micronuclei

Fig. 5. Multipolarity

Fig. 6. Fragmentation

Fig. 7. Bivalent laggards

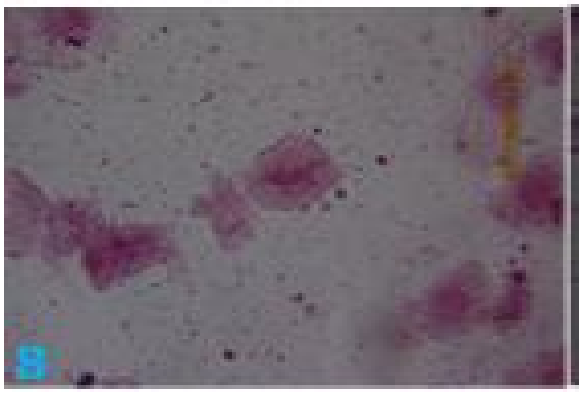

Fig. 8. Metaphase

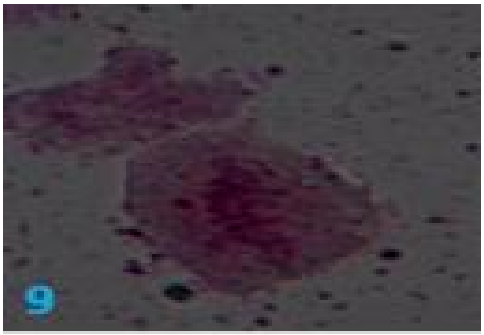

Fig. 9. Polysomaty

OBSERVATIONS AND DISCUSSION

In pea the maximum number of seeds germinated on 8th day after sowing. In the present investigation, the seed germination revealed the declining trend with an increase in concentration of EMS. The germination values ranged from 89% to 70% in case of EMS concentrations along with control. In response to EMS concentration 70% germination was recorded at 0.15% revealing a reduction of about 19% as compare to control. The reduction in germination percentage may be due to the action of mutagens in the seed.

The frequency percentage of mitotic index of metaphase was 7.37% recorded at 0.15% EMS concentration. It can be attributed to either prophase inhibition or disruption in the normal functioning of the spindle formation mechanism or due to both (Kaul, 1972). The lowering of MI might have been achieved by the inhibition of DNA synthesis at telophase (Sudhakar et. al., 2001).

Besides this, a wide range of mitotic abnormalities were also recorded in root tips of pea. All the major abnormalities registered a concentration based increased in pea. The main mitotic abnormalities were Sticky Bridge (Fig.1), fragmentation (Fig.6), polysomaty (Fig.9), early separation (Fig.2), late separation (Fig.3), micronuclei (Fig.4) metaphase (Fig.8), bivalent laggards (Fig.7) and multipolarity (Fig.5). Sticky bridge of chromosomes at metaphase and anaphase was very significant at 0.10% EMS concentration. Patil and Bhat (1992) suggested that stickiness is a type of physical adhesion involving mainly the proteinaceous matrix of chromatin material. Sticky bridges in anaphases were recorded at 0.15% EMS concentration. The formation of bridges could be attributed to chromosomal stickiness (El-Khodary et. al., 1990) and to chromosome breakage and reunion (Haliem, 1990). Induction of bridges and breaks may lead due to loss of genetic material (Salam et.al., 1993).

Table 1: Effect of mutagens on seed germination percentage in pea (*Pisum sativum* L.)

Mutagens	EMS Concentrations	Germination (%)	± S.E.
Control		89	0.54
EMS (%)	0.05%	85	0.26
	0.10%	76	1.08
	0.15%	70	1.53

Table 2: Frequency of Mitotic index induced by EMS in Pea (*Pisum sativum* L.)

Concentrations	Control		0.05% EMS		0.10% EMS		0.15%EMS	
No. of observations	I	II	I	II	I	II	I	II
Total no. of cells in microscopic field	120	150	100	100	90	100	80	95
Prophase	5	3	4	3	5	4	3	5
Mitotic index	5	2	4	2.72	5.5	4	3.75	5.26
Metaphase	7	3	5	4	6	5	6	7
Mitotic index	5.83	2	5	3.63	6.66	5	7.5	7.37
Anaphase	12	7	6	8	10	11	5	3
Mitotic index	10	4.66	6	7.27	11.11	11	6.25	3.16
Telophase	2	1	0	2	0	0	2	0
Mitotic index	1.66	0.66	0	1.81	0	0	2.5	0

S.E. = Standard error

Dhankar and Dhankar (2003) in Okra and Khawar (2006) in pea reported the reduction in seed germination percentage due to dose of gamma ray treatment. The chemical mutagens revealed inverse relationship in relation to germination percentage (Siddiq and Swaminathan, 1968; and Subramanian, 1980).Mitosis in the control root tips was normal at the entire stages exhibited mitotic index (MI) near about 11.11% at 0.10% EMS concentration in anaphase stage. There was very less mitotic index (MI) was upto zero percent at all concentrations of EMS in all the stages of mitosis. Mitotic index revealed slight fluctuations in all the concentrations of EMS. Increase in the percentage of metaphase at the expense of the other phases was observed in all concentrations.

Early separation and late separation was observed in anaphase at 0.10% and 0.15% EMS concentrations, respectively. Also fragmentation, multipolarity and ring chromosomes were recorded at higher concentration (0.15%) of EMS. The formation of small fragments can be attributed to the chromosomal breakage due to effect of mutagen. The formation of ring chromosomes may be the result of broken chromosomal ends (Kesarwani et.al. 2003). Favret (1963) proposed that the general inhibition of germination and increased lethality could be due to lowering of the rate of mitotic proliferations and the consequent delay in cell division and repair of damaged DNA (Hutterman et. al., 1978).

Bhat *et al.,* (2006) observed different types of chromosomal aberrations followed by treatment of physical and chemical mutagens. He also opined that the formation of chromatin bridges might be due to the failure of chiasmata in a bivalent to terminalise and the chromosomes get stretched between the poles. The laggards may be due to abnormal spindle formation and chromosomal breakage. The chromosomal aberrations or anomalies are the good signs of deviations in the normal mechanism of the cell cycle. Therefore, we conclude that although the chemical mutagen (EMS) can be potent mutagen and have higher mutagenic potential.

Acknowledgements

Authors are thankful to Prof. Kothekar V.S. Ex-Head, Dept. of Botany, Dr. B.A.M.U. Aurangabad, Dr. Satpute R. A. Director, Government Institute of Science, Aurangabad, Dr. Patale M.W. Head, and all colleagues of Post-graduate Dept. of Botany, Sir Parashurambhau College, Pune-30 (MS), India for their timely guidance and constant encouragement during present work.

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