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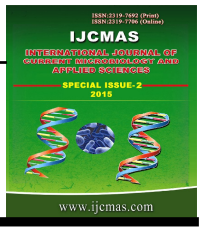


Original Research Article

Impact of Mutagenesis on Cytological Behaviour in Chickpea (*Cicer arietinum* L.)

G.S.Dhulgande*, Nilambari Jagtap, Swapnali Parchande and Sheetal Wagh

Department of Botany, Sir Parashurambhau College, Pune-411030 (M.S.) India

**Corresponding author*

A B S T R A C T

Pulses are more prone to biotic and abiotic stresses as compared to cereals. There is a serious need to develop such varieties having high resistance to the above mentioned stresses. The present study was an effort to identify appropriate dose of mutagens for the elevation of cytological behavior through induced mutagenesis in Chickpea. For present study the local variety of Chickpea seeds were subjected to different concentrations of EMS. The treated root tips showed varying degree of mitotic abnormalities almost in all the concentrations of the EMS. The various types of mitotic aberrations such as fragments, bridges, laggards, micronuclei, early and late separations were scored in case of root meristem cells of treated materials. Taking the frequency of mitotic index for effectiveness of a mutagen concentration proved to be the most effective.

Keywords

Mutagenesis, Cytology, Chromosomal aberrations, Mitotic index

Introduction

The chickpea belongs to the family leguminoceae containing 9 annuals and 31 perennial species are distributed worldwide, of which *Cicer arietinum* L. The diploid chromosome number has been reported 2n=2x=16 in the cultivar and its wild annual relatives.

Chickpea is a cool season legume crop and is grown in several countries worldwide as a food source. Seed is the main edible part of the plant and is a rich source of protein, carbohydrates and minerals especially for the vegetarian population. As in case of other legume crops, even chickpea can fix atmospheric nitrogen through its symbiotic association with *Rhizobium* sp.; thus helping in enhancing the soil quality for subsequent cereal crop cultivation. Chickpea is the third most important food legume crop and India is the largest producer contributing to 65% of world's chickpea production (FAOSTAT, 2008). Even though India is the largest producer of chickpea; it still imports chickpea from other countries.

Keeping in view, the ever-increasing demand for this legume crop; it is essential

to improve the production and area under cultivation, at the same time minimizing the stress on this crop plant.

The impact of mutagens for creating variation on crops like Chickpea is an important criterion in the contemporary world where food insecurity and malnutrition alarming at the doors of various nations. It is a fact that in the present time number of chemicals having mutagenic properties have been discovered but relatively few of them are frequently applied for practical purposes in mutation breeding.

Chromosomal aberrations induced by mutagenic agents in plants are indicator of genetic damage. Analysis of chromosomal behavior of various mitotic stages is the most dependable indices for estimation of the potency of any mutagen. Cytological analysis clarifies the specific response of different genotypes to a specific mutagen and provides significant evidences for selection of desirable traits.

Material and Methods

The seeds of chickpea (*Cicer arietinum* L.) were procured from local market of Pune (MS), India. The seeds of 1st set treated with three different concentrations viz. 0.05%, 0.10%, and 0.15% of Ethyl Methane Sulphonate.

The healthy and actively growing root-tip of each and untreated control of the germinated seeds on reaching the length about 1 to 1.5 cm in the petriplates lined with the 2-3 layer of moist filter paper. The root-tips were excised during the time interval of 10 am to11.30 am. 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. Also germination percentage was calculated during present investigation on blotting paper in petri-dishes.

Slide preparation

The slides were prepared by following standard squash technique (Sharma and Sharma, 1990). The stain 2% aceto-orcein was used in present study. Three slides of each treatment were observed under trinocular microscope (Olympus) and the random 10 counts of each slide were scored to cover maximum surface area of the slide for computing the Mitotic Index. The various abnormalities were observed such as fragments, bridges, laggards, micronuclei, early and late separations, etc.

Mitotic Index (MI)

It is computed in term of percentage frequency of dividing cells and mitotic index was calculated by scoring the total dividing cells out of total cells scored.

$$\text{Mitotic index} = \frac{\text{Total number of dividing cells}}{\text{Total number of cells scored}} \times 100$$

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

Standard Deviation and Stander Error

The data obtained was statistically analyzed as per procedure given by Panse and Sukhatme (1978).

Result and Discussion

In present study the chickpea shows maximum germination on 8th day after sowing on blotting paper in petri-dishes. It could be revealed that the declining trend with an increase in concentration of EMS. The germination values ranged from 92% to 70% in case of EMS concentration along with control. In response to EMS concentration 70% germination was recorded at 0.15% revealing a reduction of about 22% as compare to control (Table 1).

The reduction in germination percentage may be due to the action of mutagens in the seeds. Similar results were recorded by Dhankar and Dhankar (2003) in Okra and Khawar (2006) in pea. The chemical mutagens revealed inverse relationship in relation to germination percentage (Siddiq and Swaminathan, 1968; Subramanian, 1980).

The normal and abnormal dividing cells of prophase, metaphase, anaphase and telophase were counted out of the total cells scored to compute the Mitotic Index and standard deviation for all the treatments and represented in table 2. The slight fluctuations in the mitotic index were observed in all the EMS concentrations. The mitosis in the control root tips was normal at the entire stages exhibited mitotic index. The maximum mitotic index (17.94) could be seen at 0.15% EMS concentration. Increase in the percentage of metaphase at the expense of other phases was observed in control.

The observation scored in the study represents EMS induced various types of qualitative and quantitative chromosomal aberrations such as clumping ring formation, stickiness at metaphase. Chromatin bridges, laggard, multipolarity at anaphase. It is further revealed that the rate of cell division was affected which in turn the mitotic index decreased with the increase in concentration of EMS. The number of cells with various anomalies has been scored at different stages of mitosis (Kumar and Dubey, 1997).

It can be attributing to either prophase inhibition or disruption in the normal functioning of the spindle formation mechanism or due to both (Kaul, 1972). The lowering of mitotic index might have been achieved by the inhibition of DNA synthesis at telophase (Sudhakar *et al.,* 2001).

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 (Fig. 1). 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). Early separation and late separation was observed in anaphase at 0.10% and 0.15% EMS concentrations, respectively (Fig. 2 and 4).

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.

The mutagen represents mitodepressive property in relation with frequency of abnormal dividing cells. 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.

Table.1 Effect of mutagens on seed germination percentage of chickpea (*Cicer arietinum* L)

Sr. No.	Treatments	Germination (%) ±S.E.
1	**Control**	92.00±0.64
2	**0.05% EMS**	86.00±1.02
3	**0.10% EMS**	74.00±2.05
4	**0.15% EMS**	70.00±1.53

S.E. = Standard Error

Table.2 Mitotic Index of the dividing cells in Mitotic cell division of chickpea (*Cicer arietinum* L.)

Sr. No	Treatment	Mitotic index±S.E	Total cells scored	Dividing cells	Prophase	Metaphase	Anaphase	Telophase
1	**Control**	16.84±0.05	190	32	8	**12**	9	3
2	**0.05% EMS**	15.88±1.02	170	27	8	8	6	5
3	**0.10% EMS**	14.54±2.01	220	32	10	7	7	8
4	**0.15% EMS**	**17.94±0.25**	195	35	8	10	7	10

Fig.1 Sticky bridge

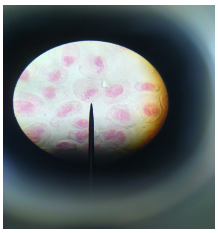


Fig.2 Early separation

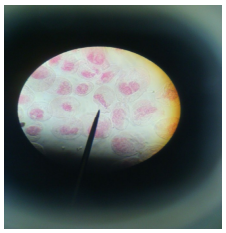


Fig.3 Laggards

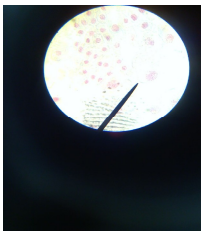
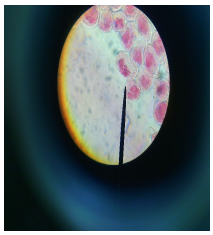


Fig.4 Late separation



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