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Research Article – Plant Cytology

Studies on the effects of mutagens on cytotoxicity behaviour in Pigeon pea (*Cajanus cajan* (L.) Millsp) Var. CO-7.

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Abstract

The cytological studies provide more information regarding the response of a genotype to the particular mutagen and also provide chances to select desirable characters. The seeds of pigeon pea were subjected to different doses and concentrations of gamma rays and ethyl methane sulphonate (EMS). The effects of different mutagenic treatments on mitosis chromosomal behavior have been studied in both the mutagens. Different types of mitotic aberrations like stickiness, precocious movement, bridge, clumping of chromosome and laggards, etc., were observed in all the treatments. However, the gamma rays treatments proved to be more effective in inducing mitotic aberrations as compared to EMS. The frequency of laggard was high when compared to other mitotic aberrations. The reduction in mitotic index and relative deviation rate frequency were observed with increase in doses and conc. of both the mutagenic treatments and gamma rays were found to be more effective than EMS treatments.

Key words: Gamma rays, EMS, Chromosomal aberration and pigeon pea.

Introduction

Pigeon pea (*Cajanus cajan* (L.) Millsp) is an important food legume predominantly cultivated in the tropical and subtropical regions of Asia and Africa. It is a diploid (2n = 22) often cross-pollinated crop with a genome size of 858 Mbp Greilhuber *et al.* (1998). Pigeon pea plays an important role in food and nutritional security because it is a rich source of protein, minerals and vitamins. Pigeon pea seeds are mainly consumed as split pea soups or 'dal' but a significant proportion is also eaten as green pea vegetable and as wholegrain preparations. The mutation breeding has become a proven way since the beginning of this century as one of the driving force for evolution besides creating genetic variation within the crop variety Singh, (1987). Induced mutagenesis has been recognized as the most efficient method for induction of morphological and genetical variability in plants especially in those with limited genetic variability.

Ethyl methane sulphonate (EMS), a chemical mutagen of the alkylating group has been reported to be the most effective and powerful mutagen and usually causes high frequency of gene mutations and low frequency of chromosome aberrations in plants (Khatri *et al*., 2005). Gamma rays, an energetic form of electromagnetic radiations are known to be the most popular mutagens for their simple application, good penetration, reproducibility, high mutation frequency and less disposal problems (Chahal and Ghosal, 2002).

Cytological analysis with respect to their mitotic and meiotic behaviour is considered to be one of the most dependable indices to estimate the potency of mutagen (Siddiqui *et al*, 1982). Cytological studies provide information regarding the response of various genotypes to a particular mutagen and provide greater chances for the selection of desired characters. The greater mutagenic potentiality of mutagen can be judged by inducing minimum chromosomal damage and physiological injuries and thus, effect of EMS and gamma rays were assessed on structural chromosomal changes in pigeon pea.

Materials and Methods

The healthy seeds of the pigeon pea variety CO-7 were obtained from Millet Breeding Station, Tamil Nadu Agricultural University, Coimbatore. Dry seeds were irradiated from a ^{60}Co source at Indira Gandhi Centre for Atomic Reserch (IGCAR), Kalpakkam with doses of 15, 20 and 25KR gamma

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rays. Another set of seeds were pre soaked in distilled water for 6 hr and was treated with different concentration of EMS (20, 25 and 30mM) to 6 hr with constant intermittent shaking. After the mutagenic treatments, seeds were thoroughly washed in running water for 10 to 15 times to leach out the residual of chemicals. The treated seeds and untreated seeds were used as control and were transferred to petri dishes containing two layers of moist filter paper for cytological investigation. The root tips collected from control and treated seedlings were fixed in 1:3 acetic ethanol. The root tip squashes were made by using Iron alum Haematoxylin squash technique (Marimuthu and Subramanian, 1960). The root tips were hydrolyzed in 0.1N HCl for 5 to 10 minutes at 60ºC and then they were thoroughly washed in distilled water and transferred to 4% iron alum for 3 minutes. The root tips were then washed in distilled water and transferred to ripened dilute haematoxylin stain and kept for 3 hours. The root tips were thoroughly washed in distilled water and then they were treated in 45% acetic acid for 1 minute to soften the tissues. Acetic acid being a de-staining agent, the time of study in haematoxylin had to be adjusted to the time required for softening in acetic acid. One or two root tips were placed on a clean slide and squashed by using a cover slip and the slide was sealed and mounted in DPX solution and then examined. The slides were observed under microscope to find out the structural changes in chromosome due to mutagenic treatment. The variation between the control and the treated mitotic abnormalities was observed. The chromosomal aberrations were examined and they were counted and micro photographed from the squashes. Mitotic index (MI) and relative division rate (RDR) were also recorded.

MI= Total number of dividing cells/
Total number of cells examined×100

RDR= Percentage of dividing cells in treated root-percentage of dividing cells in control root tips/100-Percentage of dividing cells in Control seedlings×100.

Results and Discussion

The present investigation was done to study the chromosomal changes by induced mutagenesis using Gamma rays and EMS on M_1 generation plants of pigeon pea. The metaphase chromosome number was 2n=22 in control. Mitotic studies in root tips of pigeon pea revealed a wide range of chromosomal aberration such as Anaphasic laggard, anaphasic multiple bridges, late anaphase, telophasic bridge, stickiness in metaphase chromosomes, stickiness of chromosome, Clumping of chromosome and precocious movement of chromosomes were observed in all mutagens treated plants (Table-1). Similar type of chromosome aberrations was observed earlier workers like Dhamayanthi and Reddy (2000) in Chilli, Sharma and Kumar (2004) in chickpea, Zaman and saleh (2005) in wheat, Kumar and Gupta (2009) in chilli. The maximum aberrations were recorded in higher doses of gamma rays (25KR) and higher concentration of EMS (30mM).

Dose dependent increase in frequency of different chromosomal aberrations has been reported in chilli by Salam and Thoppil (2010). Bhat *et al*. (2006) observed different types of chromosomal aberrations followed by treatment of physical and chemical mutagens. The percentage of abnormalities as an index of effectiveness of mutagen and the combined treatment has been reported to be the most effective (Kumar *et al*., 2003). Chromosomal rearrangements are one of the most frequently produced classes of mutation that result from the action of physical and chemical mutagenic agents (Gecheff, 1996).

The gradual decrease in the mitotic index was observed in all the treatments as compared to the control plants. The maximum mitotic index was observed in control plants (48.08) and lower mitotic index was recorded at 25KR of gamma rays (36.38) among all the treatments (Table-1). The total number of abnormal cells revealed increase along with the increase in the concentration of mutagenic treatment which resulted into the decrease in mitotic index. It is clearly highlighted that the higher concentrations and duration's affects notably on mitotic cell division at initial stages. A lowering in the mitotic index may be the consequence of DNA synthesis inhibition at S-phase (Sudhakar *et al*., 2001).

The frequency of abnormal cells was observed increased along the increase in the concentration of mutagenic agent. The maximum frequency of aberrant cells was observed at 25KR (25.97) of gamma rays treatments. The highest chromosomal aberration observed as laggards, followed by precocious movement, bridges, clumping of chromosome and stickiness (Table-1). Dose dependant increase in total number of abnormal cells and relative deviation rate and in increase frequency of various abnormalities in all the treatment was observed in both the mutagen. Similar results were observed by Cichoriumintybus Khan *et al*. (2009), Wani (2009) in chickpea and Dhanvel *et al*. (2008) in cowpea. Combination treatment of different mutagens increase the mutation frequency and alter the mutation spectrum, maximum high frequency of abnormal dividing cells

Table: 1 Effect of gamma rays and EMS on cell division of pigeon pea

Mutagens	Treatments dose/con.	Total number of cells observe	Number of abnormal cells					MI (%)	RDR (%)	Total number of dividing cells	Total number of abnormal cells	% of abnormal cell frequency
			BD	LD	CL	PM	ST					
Gamma rays	Control	339	-	-	-	-	-	48.08	-	163	-	-
	15KR	334	14	12	10	13	12	40.11	16.57	136	61	18.26
	20KR	320	12	15	14	13	12	37.81	19.78	121	66	20.62
	25KR	308	18	19	15	12	16	36.68	21.95	113	80	25.97
EMS	20mM	336	8	17	13	9	12	42.55	10.65	143	59	17.55
	25mM	332	14	12	13	12	11	38.85	17.77	129	62	18.67
	30mM	325	15	19	14	10	16	37.53	20.31	122	74	22.76

(BD-Bridges, LD-Laggard, CL- Clumping of chromosome, PM- Precocious movement, ST- Stickiness, MI-Mitotic index, RDR- Relative deviation rate)

followed by the combination treatment with EMS and gamma rays has been reported in chickpea (Wani and Anis, 2008).

The laggards were more abnormality found in all the mutagenic treatments and frequency was more in gamma rays as compared to EMS. According to Saylor and Smith (1966), the bridge formation could be due to the failure of chiasmata in a bivalent to terminalize and the chromosomes get stretched between the poles. Maximum stickiness of chromosomes was found at 30mM of EMS when compared to gamma rays (Table-1). Stickiness of chromosomes was resulted due to depolymerization of DNA partial dissolution of nucleoprotein (Kaufmann, 1956). The laggard chromosomes highly found in both the mutagens. The occurrence of lagging chromosomes may be due to abnormal spindle formation and as a result spindle fibres failed to carry the respective chromosomes to the polar region and resultantly lagging chromosome appeared (Tarar and Dnyansagar, 1980). Precocious movement of chromosomes is the result of inactivation of spindle mechanism. It may be due to the early terminalization or advanced movement of the chromosomes during the anaphase (Permjit and Grover, 1985).

Conclusion

In the present study, the aberrations caused by mutagens were due to partial or complete failure of spindle mechanism. The percentage of abnormal cells increased with an increase in the dose/concentration of both the gamma rays and EMS mutagens. The maximum aberrations of chromosome were observed in gamma rays than the EMS. This research findings may helps to understand the mutagenic effect on mitotic behavior and chromosomal alteration in pigeon pea.

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