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Mutagens Induced Chromosomal Damage in *Lablab purpureus* (L.) Sweet Var. *typicus*[1]

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Abstract—Cytological analysis with respect to meiotic behaviour is considered to be the one of the most dependable indices to estimate the potency of mutagens and to elucidate the response of various genotypes to a particular mutagen. Seeds of *Lablab purpureus* (L.) Sweet var. typicus cv. CO(Gb)14 were subjected to different doses/concentrations of gamma rays and EMS. The effects of different mutagenic treatments on meiosis were studied on treated and control plants. Various types of meiotic aberrations such as stickiness, clumping of chromosomes, laggards, ring chromosomes and precocious movements were observed in the mutagenic treatments. As increase in the concentration, the frequency of cells showing chromosomal aberrations shows a linear increase up to a certain level. However, the EMS treatments proved to be more effective in inducing meiotic aberrations as compared to gamma rays.

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INTRODUCTION

Lablab purpureus (L.) Sweet ($2n = 22$) belongs to the family Fabaceae. It is an ancient domesticate widely distributed in Africa, the Indian subcontinent and Southeast Asia, where it has been used as a grain legume and vegetable for more than 3000 years [1–3]. It is also used as animal fodder and green manure in mixed crop-livestock systems and, over the past 50 years, it has become an important annual forage crop in Australia and America. They are excellent quality crops for fattening both sheep and cattle, and are also regarded as good feed for milking cows. In a crop rotation program, they can significantly improve soil nitrogen levels by nitrogen fixation or by incorporation in soil as a green manure crop. *Lablab* grains and pods are rich in protein, vitamins, minerals [4, 5] and antioxidants [6].

Mutation breeding is an effective, inexpensive and dependable approach to overcome the drastic situation of food shortage and increasing world food security. A large number of desirable varieties have been developed through mutation breeding in field and horticulture crops. Varieties with better genotype and phenotype characters have been revealed to farmers as a result of induced mutations which have been used directly as new cultivars or they can be used in cross breeding programs [7].

Induced mutation has great potentials and serves as a complimentary approach in genetic improvement of crops [8–10]. So far, 3218 number of crop varieties has been developed through induced mutagenesis in varieties of crop such as cereals, oilseeds, pulses, vegetables, fruits, fibres and ornamental plants [11]. In India, 343 mutant cultivars belonging to 57 plant species have been released. A great majority of mutant varieties (64%) were developed by the use of gamma rays [12]. Gamma radiation is an electromagnetical ionizing type of radiation used in mutation experiments. This type of pathological agents can cause breaks in both strands of the DNA at a given site (double-strand break) and it is the most serious type of DNA damage because neither strand is able to provide physical integrity or information content, as would be the case for single-strand DNA damage where one strand of the duplex remains intact. The repair of such breaks usually results in an irreversible alteration of the DNA [13]. Gamma ray is used for inducing beneficial as well as harmful cytogenetic effects in many crop plants [14–17]. It is known to cause breakage, depolymerization of DNA, which consequently leads to transitions, transversions, or chromosomal anomalies [18].

Chemical mutagenesis is considered as an effective means in improving the yield and quality traits of crop plants [19, 20]. It is known that various chemicals have positive or negative effects on living organisms. Many of these chemicals have clastogenic (chromosomal damaging) effects on plants through reactive oxygen-derived radicals. Patil and Bhatia [21] reported that the localization of breaks along the chromosomes result from the affinity of EMS for guanine rich areas on the other hand the radiations can have direct effect on chromosomes. They may directly break chromosomes

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or alter one of the DNA bases or indirectly may initiate a chain of chemical reactions. The increased frequency of meiotic anomalies with increasing concentrations of mutagens was reported in several crops by many workers [22, 23].

Induced mutants in plants are often associated with cytological abnormalities. Meiotic mutants have been extensively reported in higher plants [24, 25]. Chromosomal rearrangements are one of the most frequently produced classes of mutation that result from the action of both physical and chemical mutagenic agents [26]. Chromosomal aberrations induced by mutagenic agents in plants are indicator of genetic damage [27]. Analysis of chromosomal behavior at various meiotic stages is the most dependable indices for estimation of the potency of any mutagen. Cytological studies constitute an important component in breeding program involving the development of new original forms with aid of experimental mutagenesis [28].

Mutation induced chromosomal anomalies are the primary basis of genetic changes. Thus, cytogenetical investigation is an important source of information regarding the genetical hazards due to mutagens as they deal with the genetic material, the chromosomes and more appropriately the DNA which controls the phenotype. It also provides a considerable clue to assess the sensitivity of plants for different mutagens and to ascertain the most effective mutagens and their treatment doses for a given crop to realize maximum results.Therefore, investigation on meiotic aberrations and their genetic consequences forms an integral part of most of mutation studies. They also provide a considerable clue to assess the sensitivity of plant for different mutagens [29].

Thus considering the above points in mind, the study was aimed to document and report valuable information on the cytological behavior in *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO(Gb)14, effect on chromosomal abnormalities at various meiotic stages caused due to induced mutagenesis.

MATERIALS AND METHODS

Seed Procurement and Treatment

Seeds of *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO(Gb)14 were obtained from Tamilnadu Agricultural University (TNAU), Coimbatore. Uniform healthy and dry seeds of the variety were treated with different doses/concentrations of gamma rays (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 KR) and Ethyl methane sulphonate (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 mM). Gamma irradiation was applied using ^{60}Co gamma source at a dose rate of 26.05 sec /KR in, Sugarcane Breeding Institute, Coimbatore, Tamil nadu, India. For the treatment of EMS, the seeds were presoaked in distilled water for 6 hr and then treated with different concentration of EMS for 4 hr with intermittent shaking. After mutation induction, seeds were thoroughly washed in running tap water to leach out the residual of chemicals from the seed surface before they were sown in the field. Untreated seeds pre- soaked in distilled water for 6 hours were used as control. The treated seeds were then subjected to seed germination test. Based on the reduction of 50% seed germination, LD50 value were determined. Three treatments of gamma rays (20, 25 and 30 KR) and EMS (25, 30 and 35 mM) around LD_{50} value were fixed for further studies.

Meiotic Studies

On the onset of budding, the young flower buds were collected from control and treated plants of M_2, M_3 and M_4 generations. The collected flower buds were fixed in 1 : 3 acetic ethanol. 1% acetocarmine was found suitable for meiotic studies. Anthers smear were made following the Belling's Iron-acetocarmine smear method [30]. Slides were observed under the microscope to observe different meiotic stages and various chromosomal aberrations of each treated as well as for control.

RESULTS

The effect of gamma rays and Ethyl Methane Sulphonate (EMS) on *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO(Gb)14 was studied on meiotic abnormalities. Cytological analysis of pollen mother cells (PMCs) of the control plant revealed 11 perfect bivalents (n = 11) at diakinesis (Figs. 1, 3 and 7). The abnormalities in meiotic cells of untreated (control) plants were nil. In the meiotic cells of treated population, the most frequent aberrations were ring chromosome (Fig. 2), clumping of 2 bivalents (Fig. 12), precocious movement of chromosomes at metaphase II (Fig. 5), stickiness at anaphase I (Figs. 4 and 6), stickines at metaphase I (Fig. 10), laggards at metaphase II (Fig. 9) and disturbed polarity with one laggards at telophase II (Fig. 8).

The frequency of various meiotic aberrations in each treatment of both the mutagens along with the total percentage of abnormal cells have been summarised in Table. The percentage of abnormal cell frequency varied from 2.68–15.10% in gamma rays and 4.91–18.57% in EMS treated plants of M_2, M_3 and M_4 generations. The meiotic abnormal cell frequency percentage showed linear increase with an increase in concentration. The maximum percentages of meiotic abnormalities were observed in 35 mM of EMS followed by 30 KR of gamma rays when compared to control (table). From the aforesaid results it becomes clear that EMS elicited a much greater genotoxic response than the gamma rays.

DISCUSSION

The importance of all the phases of meiosis is unquestionable but the most interesting phase of the

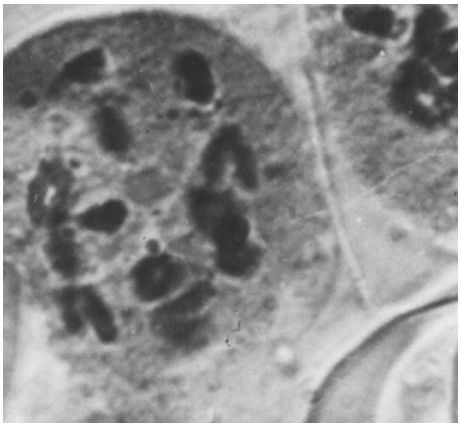

Fig. 1. 11 bivalents at diakinensis (Control).

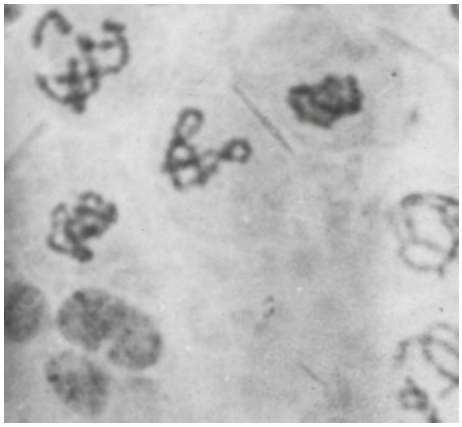

Fig. 2. Ring chromosomes (25 mM).

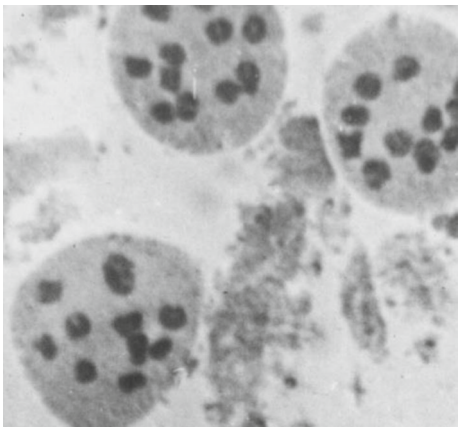

Fig. 3. 11 bivalents at diakinensis (Control).

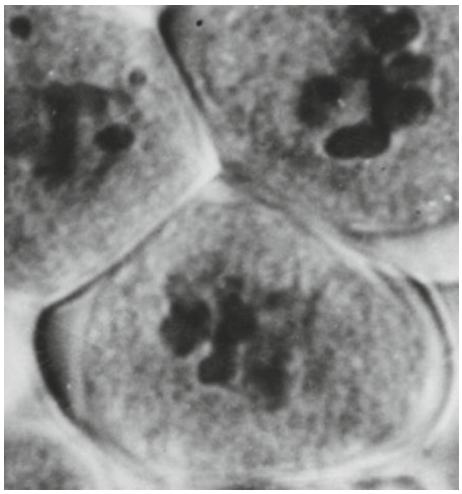

Fig. 4. Stickiness at anaphase I (30 mM).

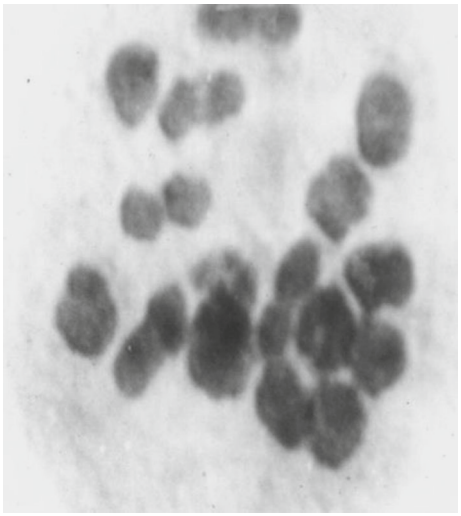

Fig. 5. Precocious movement at metaphase II (35 mM).

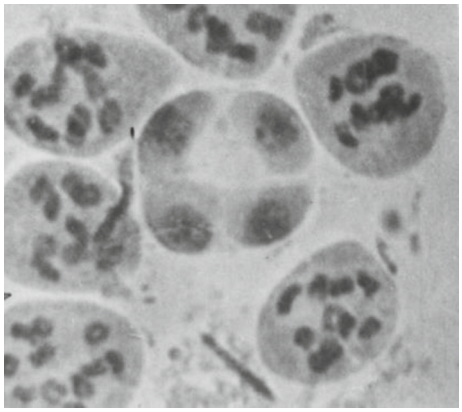

Fig. 6. Stickiness at anaphase I (35mM).

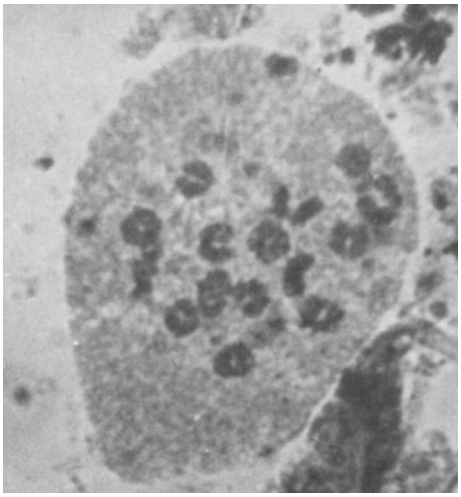

Fig. 7. 11 bivalents at diakinesis (Control).

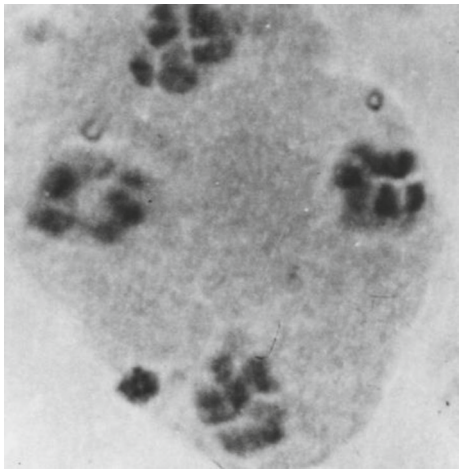

Fig. 8. Disturbed polarity with one laggard at telophase II (30 KR).

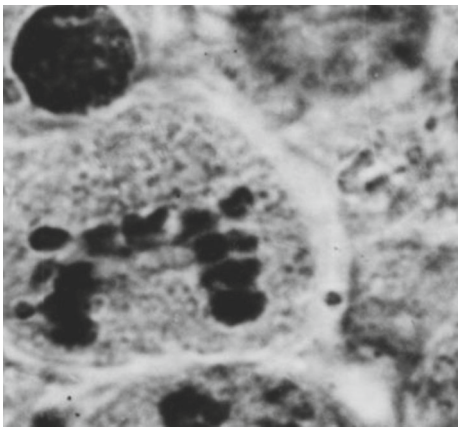

Fig. 9. Two laggards at Metaphase II (30 KR).

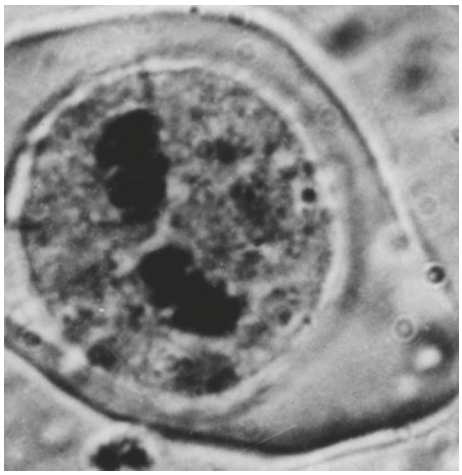

Fig. 10. Stickiness at Metaphase I (25 KR).

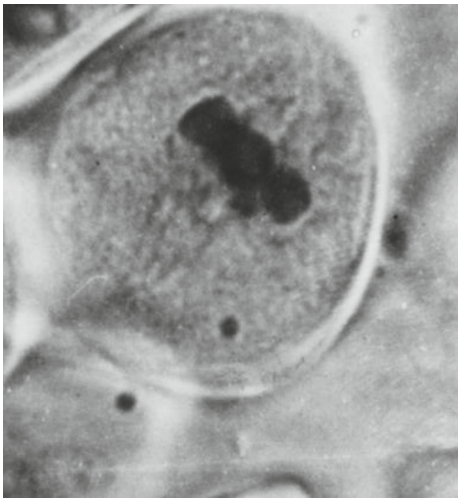

Fig. 11. Side view showing spindle in Metaphase I (20 KR).

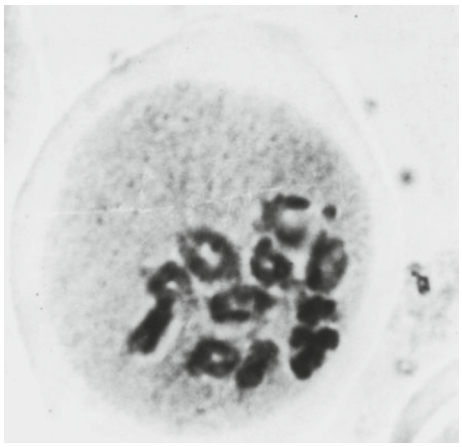

Fig. 12. Clumping of 2 bivalents (30 KR).

Frequency (%) of meiotic aberrations induced by Gamma rays and EMS in *Lablab purpureus* (L.) Sweet var. *typicus*

Generations		Treatments Dose/Conc.	Pollen Mother Cells						
mutagens			total no. of PMCs observed	no. of cells showing abnormalities				total no. of abnormal cells	% of abnormal cell frequency
				St or cl	Lg	Pr	Dis pol		
M_2	Gamma rays	Control	196	–	–	–	–	–	–
		20 KR	199	3	5	2	1	11	5.52
		25 KR	186	7	8	4	2	21	
		30 KR	192	9	11	5	4	29	15.10
	EMS	25 mM	200	4	8	5	2	19	9.50
		30 mM	190	8	10	7	3	28	14.73
		35 mM	183	10	13	6	5	34	18.57
M_3	Gamma rays	Control	210	–	–	–	–	–	–
		20 KR	194	2	4	1	–	7	3.60
		25 KR	182	5	7	2	1	15	
		30 KR	178	6	10	2	3	21	11.79
	EMS	25 mM	192	3	6	2	1	12	6.25
		30 mM	188	7	9	5	3	24	12.76
		35 mM	195	8	11	7	4	30	15.38
M_4	Gamma rays	Control	182	–	–	–	–	–	–
		20 KR	186	2	2	–	1	5	
		25 KR	174	3	4	2	1	10	5.74
		30 KR	181	5	8	2	2	17	
	EMS	25 mM	183	3	4	2	–	9	4.91
		30 mM	180	5	7	2	1	15	
		35 mM	177	4	11	4	2	21	

St—Stickiness, Cl—Clumping, Pr—Precocious, Lg—Laggard, Dis pol—Disturbed polarity.

meiosis division is the mechanism of reduction occurs between the homologous chromosomes and relationship between synapsis, chiasma formation and genetic crossing over. Any failures in the chromosomal association cause abnormalities. Cytological analysis provides a genetic basis for chromosomal behavior at different stages of cell cycle and hence provides an authentic mean to determine the efficiency of mutagens. Mutagens may cause error in the normal behavior of chromosome. Hence, any disturbance in normal cytological behavior of chromosomes (either positive or negative) reflects in phenotypic traits of plants.

Physical and chemical mutagens are known to produce chromosomal aberrations leading to abnormal chromosome behavior. In the present investigation a vast array of meiotic aberrations were recorded in the plants raised from the seeds treated with different doses/concentrations of EMS and gamma rays in *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO(Gb)14. The meiotic study of pollen mother cells of control exhibited a normal diploid status with chromosome number (n = 11), i.e. 11 bivalents at diakinesis.

The cytological study of the control plants was having normal meiosis activities in comparison to treated

sets which had ring chromosomes, clumping of bivalents, stickiness, laggards and precocious movement. Similar types of aberrations were also reported by many researchers in different plant materials after treatment with different mutagens [31, 32]. A dose dependent enhancement in the meiotic aberration due to the effect of different mutagens has also been studied by various investigators [33, 34]. In the cytological observations of the mutagenic treatments, it was found that the EMS was more effective in inducing chromosomal aberrations than gamma rays. Similar result was also obtained in *Linum usitatissimum* [35].

Laggards observed during the present cytological studies might have originated due to delayed terminalization, stickiness of chromosomal ends, or because of failure of the chromosomal movements [36, 37]. The formation of laggards may also be due to chromosomal breakage by binding to DNA at GC rich regions [38]. Disturbed polarity observed during telophase may be due to spindle disturbance. Laggards were present in almost all treatments and occurrence of laggards in the present study has also been reported previously by many workers [39–41].

Precocious movement of chromosomes may be due to spindle dysfunction. Migration of precocious chromosome to the poles may be due to the formation of univalent chromosomes at the end of prophase I or to precocious chaisma terminalization at diakinesis or metaphase. Precocious movement of chromosome caused by the effect of mutagens was also observed in Lentil [33] and Coriandar [23]. The abnormalities of laggards and precocious movement may be due to spontaneous breakage and exchange rather than the presence of a paracentric inversion.

Sticky chromosomes were first reported in maize by Beadle [42], and he attributed such irregularity to a mutation caused by a recessive gene called sticky (*st*). The phenotypic manifestation of stickiness may vary from mild, when only a few chromosomes of the genome are involved, to intense that may involve the entire genome [43]. Stickiness might be due to improper folding of chromosome fibres [44, 45]. It could also be due to the result of partial dissociation and altered pattern of organization of nucleoproteins [46] or due to polymerisation of nucleic acid caused by mutagenic treatment [47]. When affected, these proteins lead to chromosome clumping. It may also be possible that the mutagen itself reacts with the histone proteins and brings about a change in the surface property of chromosomes due to improper folding of DNA, thereby causing them to clump or stick [48]. The meiotic abnormality stickiness was reported by various workers in *Cicer arietinum* [49], *Triticum aestivum* [50] and *Vigna mungo* [51].

CONCLUSION

In the present investigation, there is no chromosomal aberration in control while in treated populations aberrations were observed. Chromosomal aberrations were increased with increase in dose/concentration of physical and chemical mutagens. Among the different dose/concentration of mutagens, EMS shows more chromosomal abnormalities than the gamma rays. Therefore, it is concluded that lower and intermediate doses/concentrations of EMS and gamma rays are effective in inducing genetic variability for the improvement of economic characters.

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