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Cytogenetics effect of gamma rays on root meristem cells of *Vigna unguiculata* (L.)

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ABSTRACT

Cytological effects of the gamma rays were investigated in root tip cells of Vigna unguiculata (L.) Walp var. CO7. The root tip of V. unguiculata seeds were treated with various doses (10, 15, 20, 25 and 30KR) of gamma rays. The radiations can have direct effect on chromosomes. They may directly break chromosomes or alter one of the DNA bases or indirectly may initiate a chain of chemical reactions. The biological effect also depends on the kind of cell and stage of nuclear cycle. The results showed dose dependent increase in mitotic indices. The chromosomal mutations like anaphasic bridge, anaphasic laggard, stickiness, C-metaphase chromosome were also observed. The chromosomal aberration increased with increased in gamma irradiation doses.

Keywords: *V. unguiculata*, root tip cells, chromosome, aberration, gamma rays.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is a grain legume grown in savanna regions of the tropics and subtropics. Its value lies in its high protein content (23-29%, with potential for perhaps 35%); and its ability to fix atmospheric nitrogen, which allows it to grow on, and improve poor soils [27]. Mutation induction is one approach for creating genetic variation in crop plants [8]. The technology of mutation induction has become an established tool in plant breeding in order to supplement existing germplasm and to improve cultivars in specific traits. Improved varieties of many crops have been released to forms as a result of induced mutations which have been used directly as new cultivars or in cross breeding programs [13, 22].

Cytogenetics studies are necessary to obtain information regarding the role and effect of mutagen and in elucidating the response of genotypes to a particular mutagen. Gamma irradiation is one of the main physical mutagens for mutation studies in plants. Mutagens have been effective to decrease the mitotic index [24]. Gamma irradiation as a mutagen can induce useful as well as harmful mutation in plants [15, 21]. The present study was undertaken to assess the mutagenic effect of gamma rays on a capable variety of cowpea CO7 by studying mitotic behavior of chromosomes after treatment.

MATERIALS AND METHODS

The dry and dormant seeds of the cowpea variety CO7 were obtained from Millet Breeding Station, Tamilnadu Agricultural University, Coimbatore. Gamma rays are one of the electromagnetic radiations, which having the low wavelength with high penetrable power. The source of Gamma rays is 60CO, one of the labeled metals, which emit the rays. The dry and healthy seeds were treated with 10, 15, 20, 25 and 30 KR of gamma rays. The treated seeds and untreated seeds were used as control was 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 [20]. 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.

RESULTS AND DISCUSSION

In the present study somatic chromosome was carried out with effect of mutagen. The metaphase chromosome number was 2n=22 in control. The numerical variation of somatic chromosomes of 2n complement was revealed mutagenic effect in the genome. Chromosomal aberrations such as abnormal distribution of chromosome, anaphase bridge, laggards, stickiness and C-metaphase were also observed in present study (Fig-1). Similar observations were reported by many workers in black gram [6], sunflower [11], chilli [10], chickpea [25, 12], wheat [3, 31] and onion [5].
The effects of physical mutagen (gamma rays) have been studied on mitotic activity of the root meristems. The percentage of dividing cells (mitotic indices) increased with increasing doses of mutagen. The frequency of total abnormalities (1.57, 4.89, 8.57, 10.11, 14.47 and 17.80) was observed in various doses (control, 10, 15, 20, 25 and 30KR) of gamma rays respectively (Table-1). The number of abnormal cells having bridge, laggard, stickiness and C- metaphase cells are gradually increased from control to 30KR onwards. The similar findings were observed by many authors [3, 2, 28, 31, 16].

Mutagens can cause physiological damage besides gene and chromosomal changes. Physiological damage, mainly manifested as growth retardation and death, is generally restricted to M_1 generation. The present study showed that gamma radiation did not have severe effect on % seed germination in cowpea. The first phase of germination is swelling of cells by hydration followed by enzymatic activation and metabolism. Seed germination which is simple growth of radicle and shoot, is apparently unaffected by embryo damage caused by irradiation treatment. However, embryo damage might become apparent only at the later stages of ontogenesis. This is evident from the results of the survival count where survival rate decreased with increasing levels of doses. A similar trend of results was obtained in cowpea [23] and other crops [4].
As expected, the height of seedlings was significantly reduced after irradiation, especially at higher doses. Such phenomenon has been attributed to changes in hormonal levels such as auxins and ascobic acid; physiological and biochemical disturbances [14, 26]; changes in enzyme activity and impaired mitosis in the meristematic zone of growing seedlings [9].

The similar results were suggested that it might also be due to a decrease in respiratory quotient in the irradiated seedlings [30]. Fragments at metaphase may be due to the failure of broken chromosomes to recombine. Fragment might have arisen due to the stickiness of the chromosomes and the consequent failure of the arrival of chromatids at the poles. Fragments may also be acentric chromosomes formed as a result of inversion [1].

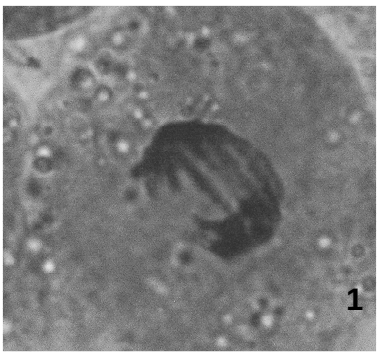
The thick sticky bridges may be due to the stickiness chromosomes. This stickiness interfered in the normal arrangement of chromosomes at metaphase and further led to their inability to separate, thus leading to sticky bridges. The spindle fibres pulled the chromosomes towards the poles these bridges were broken into fragments, which either moved towards the poles or formed laggards and micronuclei [18].

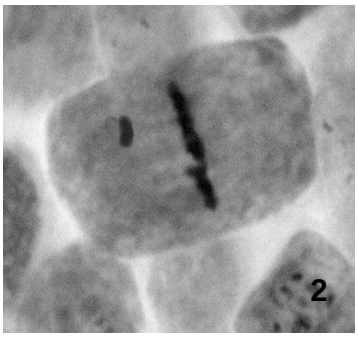
Chromosomal bridges may also be due to the chromosomal stickiness and subsequent failure of anaphasic separation or may also be attributed to unequal translocation or in origin of chromosomal fragments. Lagging chromosomes may be explained on the basis of abnormal spindle formation and failure of chromosome movement. Mutagens may have caused chromosomal breakage by binding to DNA at GC rich regions and making the DNA unstable and hence formation of fragments and laggards. Bridges and laggards with (or) without fragments were found both at anaphase and telophase, bridges without fragments were found at higher concentrations of the mutagens, both single and double bridges were found but the multiple bridges were not also rare. Multiple bridges were mostly found at anaphase and the single bridges at telophase [7]. Laggards and disturbed polarity might have appeared due to improper spindle functioning [17, 19]. The frequency of cells showing chromosome aberrations gave a linear increase with dose [29].

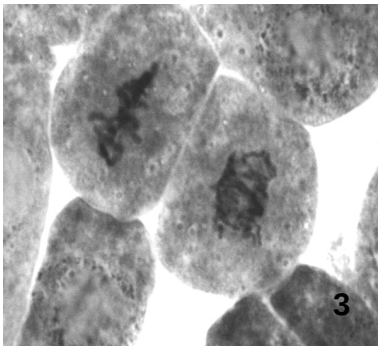
Table-1. Effect of different doses of gamma rays on cell division of root meristem of *Vigna unguiculata* L.

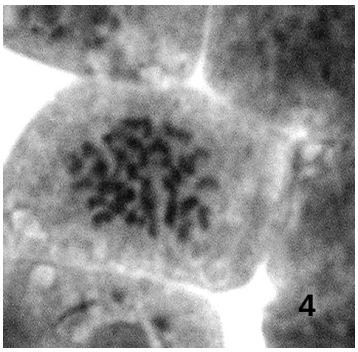
Gamma rays Dose (KR)	Total number of cells observed	Number of abnormal cells				Total number of abnormal cells	% of abnormal cell frequency
		Bridge	laggard	stickiness	C -metaphase		
Control	190	-	-	1	2	3	1.57
10KR	184	1	1	3	4	9	4.89
15KR	175	3	4	5	3	15	8.57
20KR	168	5	6	3	3	17	10.11
25KR	152	7	8	4	3	22	14.47
30KR	146	9	10	4	3	26	17.80

Fig-1. Effect of different doses of gamma rays on cell division of root meristem of *Vigna unguiculata* L.









1. Anaphasic Bridge
3. Stickiness with precocious movement

2. Anaphasic laggard
4. C-metaphase

CONCLUSION

On the basis of this study it may be concluded that the gamma rays treatments at various doses affect *Vigna unguiculata* root meristem and induced number of chromosomal anomalies which lead to wide range of variations in cytological attributes. Hence gamma rays could be utilized for induction of genetic variability in cowpea.

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