Effect of gamma rays on induced chromosomal variation in cowpea

Vigna unguiculata (L.) Walp

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Abstract

Effect of gamma rays on induced chromosomal variation in cowpea was studied using five different doses of mutagen along with a control in randomized blocked design with three replications. Gamma rays belong to ionizing radiation and interact with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect the morphology, anatomy, biochemistry and physiology of plants differentially depending on the irradiation level. These effects include changes in the cellular structure and metabolism of the plants e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds. The chromosomal variation was found to be mutagen sensitive in somatic cells of cowpea. It was found to increase with increasing the doses of gamma rays in cowpea plants. The physical mutagen like gamma rays induces high frequency of chromosomal changes like anaphasic bridge; anaphasic laggard, anaphasic bridge and clumbing of chromosome were also observed.

Keywords: gamma rays, chromosomal aberration, cowpea

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Introduction

Mutation is a sudden heritable change in an organism and generally a structural change in gene. Mutation produced by changes in the base sequences of genes (as a result of base pair transition or transversion, deletion, duplication or inversion etc.) are known as gene or point mutation. Some mutation produced by change in chromosome structure, or even in chromosome number is known as chromosomal mutation. The induced mutation is caused artificially by mutagenic factors. The agents that induce mutation are called mutagen and mutagen mainly consists of only one mutagen for radiation (physical) mutagen. Mutagen are not only beneficial to create genetic variability in a crop species, but also useful for the effective control of pests during post harvest storage (chaudhuri 2002). Practicing of induced mutation for crop improvement is known as mutation breeding. Mutation breeding is one of the possible alternatives to conventional breeding for crop improvement. Exposing plant genetic material to mutagens enhances the chance of isolation unique genetic material. In the post induced mutation have effectively been utilized in development of new and valuable alteration in plant characteristics that have contributed to increase yield potential. Induced mutation can rapidly create variability in quantitatively and qualitatively inherited traits in crops (Malusynski et al., 1995), Muduli and Mishra, 2007).

Induced mutagenesis has been used to obtain direct mutants or by using these mutants in hybridization (Ahloowalia et al., 2004) to overcome yield plateaus and generate desirable horticultural traits. Mutation breeding has contributed significantly to plant improvement, resulting in release of at least 2250 varieties of different crops. In India, at least 300 cultivars have been developed in at least 55 plant species (kharkwal et al., 2004). Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improve cultivars in cereals, fruits and other crops (Lee et al., 2002).
These mutations provide beneficial variation for practical plant breeding purpose. During the fast seven decades more than 2252 mutant varieties have been officially released in the world (Maluszynski et al., 2000). A great majority of mutant varieties (64%) were developed by the use of gamma rays (Ahloowalia et al., 2004). In India still today there are 7 mutant varieties of cowpea released by both physical and chemical mutagens (Natarajan, 2005). Hence, mutation breeding programme has provided to be a successful tool in breeding amelioration in self pollinated crops. Gamma rays belong to ionizing radiation and in tract with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect the morphology, anatomy, biochemistry and physiology of plants differentially depending on the irradiation level. These effects include changes in the cellular structure and metabolism of the plants e.g. dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds (Kovacs and Keresztes, 2002; Kim et al., 2004; Wi et al., 2005).

Cowpea (Vigna unguiculata (L.) Walp) forms major food crop of millions of people in most developing countries. It belongs to the Kingdom plantae, division Magnoliophyta, class Magnoliopsida, Order Falales, Family Fabaceae, Sub-Family Faboideae, genus Vigna and species unguiculata (Singh et al., 1997). Cowpea is one of the most important pulse crops in tropical Africa. The seeds are a major source of dietary protein in most developing countries. Induce mutation breeding has been recognized as a voluble supplement to the conventional breeding in crop improvement programme and has been least applied in grain legumes. The present investigation was made to effect of gamma rays on induced chromosomal variation in cowpea. Genotype differences exist in response of plants to physical mutagen in Gamma rays were differing in their relative capacity to induced mutation in crop plants. In the present investigation the effect of physical mutagens on morphological and cytological mutations were also studied many chromosomal aberrations such as champing of chromosome, precocious movement, anaphasic laggards and bridges were the typical effects of mutagens observed in the root tip squash studies.

**Materials and methods**

The present study on induced mutagenesis in cowpea was carried out in the Department of Botany, Annamalai University. 60Co Source in gamma chamber installed at Sugarcane breeding Institute (ICAR), Coimatore. Two sets containing 200 healthy seeds were treated with 0, 05, 10, 15, 20, 25, 30, 40, 45, 50 KR (Kilo rad) of gamma rays to determine LD50 value. The treated seeds were transferred to petridishes containing two layers of moist filter paper for germination. Ten petridishes of 10 seeds per treatment were sown and percentage germination and seedling variations for each treatment were subsequently demined. The treated seeds were then subjected to germination test. Based on the reduced growth of germination to 50 percent, LD50 value was determined. There doses of gamma rays around LD50 were fixed for further studies. Non-treated dry seeds of Cowpea variety Co7 pre-soaked in distilled water for 6 hours were used as control.

The study was carried out the nature of induced genetic variability in cowpea variety Co7 which was subjected to physical mutagenic treatments for two generations. Physical mutagen in gamma rays (5, 10, 15, 20, 25, 30 KR) the mutagenetic treated seeds were tested for 50 percent as lethal dose; laboratory and field work of M1 and M2 generations. In present investigation, the effects of different mutagens on morphological and cytological work have been carried out in this generation. The root tips collected from control and treated seedling were fixed in 1:3 acetic ethanol. The root tip squashes were made by using iron alum, haematcrxylon squash technique (Marinuthu and Subramanian, 1960). The root tips were hydrolyzed in 0.1N HCL for 5 to 10 Minutes at 60° 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 haematcrxylin stain and kept for 3 hrs. The root tips were thoroughly washed in distilled water and then they were treated in 45% 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 normal and abnormal mitotic stages were photographed.

**Results and discussion**

Cowpea *Vigna unguiculata* (L.) Walp is an important food crop throughout sub-Saharan Africa. Its grain and leaves are rich sources of high-quality protein and Vitamins which provide an excellent supplement to the lower quality cereal or root and tuber protein (Kitch et al., 1998). Mutation breeding the difference in due to genotypes of primary importance then that mutagens. The improvement crop species by resorting to mutation breeding should therefore be pursued with varieties. Outstanding in their agronomic fitness for practical breeding purposes (Scarascia-Mugnozza, 1968).

**Viable mutation**

Mutations are phenotypically classified into two groups (Gaul, 1964); macro mutations: These are easily detectable in individual plants, phenotypically visible and morphologically distinct and they are qualitatively inherited genetic changes, and occur in major genes or oligogenes; and micro mutations; These result a small effect that, in general, can be detected only by help of statistical methods and quantitatively inherited genetic changes and occur in minor genes or polygons. The viable mutants have been observed in Tall, Dwarf, bushy, leaf, early maturity and late maturity. (Plate 3). The radiation 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 physical and chemical reactions. The biological effect also depends on the kind of cell and stage of nuclear cycle. For instance, chromosomes are extremely sensitive to breakage in mitotic prophase. The frequency of mutants per viable organism often increases linearly with the dose.

**Cytological studies**

In the present study, somatic chromosome was carried out with effect of mutagens. The metaphase chromosome number was 2n=22 in control. Whereas, 20KR of gamma rays treatment showed in 2n=20 (Nullisomic) and 30 KR of gamma rays showed 2n=21(Monosomic) chromosomes. The numerical variation of somatic chromosomes of 2n complement was revealed mutagenic effect in the genome. Whereas, chromosomal aberrations such as abnormal distribution of chromosome, anaphase bridge, laggards, multiple bridges, late anaphase, precocious movement of chromosomes, unequal separation of chromosomes and clumping of chromosomes etc. were also observed in present study. Similar observations were reported by many workers in black gram (Bandyopathyay and Bose, 1983) sunflower (Elangoven and selvaraj, 1995) Chilli (Dhamayanthi and Reddy, 2000) wheat (Zaman and saleh 2005), Onoin (Matobole, 2010).

**Fig. 1.** Chromosomal aberrations. (A) Metaphase (2n=22 control), (B) Anaphasic chromosome (10KR gamma rays), (C) Monosomic (15KR gamma rays), (D) Nullisomic (20KR gamma rays)

Gamma radiations at the lower concentration of gamma rays 5, 10, 15KR was a lowest mutation frequency was slightly earlier i.e., before then control but when the exposure increase with dose/concentrations 20, 25 and 30 KR was highest mutation frequency and chromosomal variation observed. Radiation inducing chromosome stickiness was reported to be the result of partial disassociation of the nucleoprotein and alteration in their pattern of organization (Evans, 1962). The bridges occurring in this study might be attributed to the breaking and reunion of the chromosomes or to the stickiness of the chromosomes at metaphase. Jackson (1988) suggested that
some of the bridges may have been caused by a crossover between a paracentric inversion heterozygote loop and the centromere and another in the loop of the same bivalent. In addition, the asserted that chromatin bridges without fragments may have resulted from linear disjunction of multivalent some of these are unstable and are not transmitted through successive cellular generations e.g., achromatic gaps, breaks of one or both chromatid arms, Chromatid interchanges, acentric fragments ring and Dicentric chromosomes. Stable structural modifications that are transmissible are inversions, translocations and some small deletions. These genetic factors clearly play important roles in many plant disorders (Gregor, 1998). Bridges were commonly observed at anaphase and telophase stages in present study. The occurrence of lagging chromosomes may be due to abnormal spindle formation and as a result spindle fibers failed to carry the respective chromosomes appeared (Tarar and Dnyansagar, 1980; Badr, 1983).

**Fig. 2.** Chromosomal Aberrations. (A) Anaphasic chromosome (20KR gamma rays), (B) Anaphasic bridge (25KR gamma rays), (C) Anaphasic bridge & Clumping of Chromosome (25KR gamma rays), (D) Anaphasic Laggard (30KR gamma rays)

**Conclusions**

The chromosome aberration increased with increased in gamma irradiation doses. Such as chromosomal ir-regulation can affect the fertility, yield on competitive ability of the exposed plants. Cytological studies provide greater chances for the selection of desired characters. The improved variety of Cowpea Co7 responded more and more viable and chromosomal aberrations for highly productivity. Cowpea was cytological studies provide information regarding the response of Cowpea a particular mutagen and provide greater chances for the selection of desired characters.

**Fig. 3.** Viable Mutants. (A) Tall (10,15 and 20KR Gamma rays), (B) Dwarf (30 and 35KR Gamma rays), (C) Bushy (20 and 25KR Gamma rays), (D) Compound leaves 25,30KR Gamma rays), (E) Early Maturity (20,25KR Gamma rays), (F) Late Maturity (35KR Gamma rays).

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