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REGULAR ARTICLE

Gamma rays, EMS and DES induced changes on cytology of bhendi [*Abelmoschus esculentus* (L.) Moench]

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Abstract

Mutation breeding in crop plants such as bhendi (*Abelmoschus esculentus* (L). Moench) is a successful approach in change of product having narrow genetic base. In the present study to the determine the effect of physical mutagen such as gamma rays and chemical mutagens such as Ethyl methane sulphonate (EMS) and Diethyl sulphate (DES) were used. The seeds were treating with different doses/concentration of Gamma irradiation (10KR, 20KR, 30KR, 40KR, 50KR and 60KR), EMS and DES (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6%) for six hours were applied to 200 seed sample of each concentration and one respective control on bhendi. plants of four generations viz., M_1, M_2, M_3 and M_4. The cytological analysis, for example, chromosomal number was watched and recorded for images. The maximum changes of chromosome were observed in 50 KR of gamma rays and 0.4 % of EMS treatments than the other physical and chemical mutagens. The length and shape of chromosome for varied in treated plants than the untreated plants.

Key words: Bhendi, gamma rays, irradiation, treatment, mutagen, chromosome

Introduction

Among the conventional plant breeding strategies, mutation breeding is widely trusted method. The radiations are the best tools to induce genetic variability within a very short span of time. Chemical mutagens for the most part deliver induced transformations which cause base pair substitutions, especially G.C→A.T which brings about amino acid changes in this way adjusting the capacity of proteins, yet they don't dispense with their capacities as happen in deletions or frame shift mutations (Van der Veen, 1966). The mutants created by mutagens of any sort are helpful for the isolation, recognizable proof and cloning of potential genes, which assume a part in enhancing crop yield, stress tolerance and various other subjective and quantitative attributes (Ahloowalia and Maluszynski, 2001; Al-Qurainy et al., 2011). Induced mutation is exceedingly viable in upgrading natural genetic resources and has been utilized as a part of creating enhanced cultivars many crops (Lee et al., 2002).

Bhendi (*Abelmoschus esculentus* (L.) Moench) is mainly cultivated as vegetable. Bhendi is particularly esteemed for its delicate delicious fruits which contain iodine, Calcium, iron and vitamin A, B and C. Aside from its nutritive esteem, developed fruits containing rough fiber which is utilized as a part of paper industry. Mucilaginous concentrates of the green stem and roots are regularly utilized in India for clearing up the sugarcane Juice before it is changed over into Jaggery and sugar. Dry seeds of Okra contain 18 to 20% oil and 20 to 30% unrefined protein (Savello et al., 1980). A few times the seeds are cooked and utilized as a substitute for Coffee (Lamont,

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1999). The therapeutic estimations of Okra are related with genito-Urinary issue, and constant diarrhea (Bose and Som, 1970).

Bhendi is one the world's oldest cultivated crops. The Egyptians made the first recorded reference to bhendi in 1216 A.D. It is originated in tropical Africa and was also grown in Mediterranean region and its wild forms are found in India. The immature fruit is eaten green either fresh or prepared, by boiling or frying and used in soups and stews (Bleasdale, 1984). Its nutritional value lies in its high amount of phosphorus, calcium and some minerals. It also contains protein, carbohydrate and fat and some types of vitamins (Tindall, 1986).

Transformation reproducing supplement ordinary plant rearing as a source of expanding changeability and could present particular improvement without fundamentally adjusting its satisfactory phenotype (Ojomo et al., 1979). The mutagen induced abnormally of the chromosome is the primary structure of genetic change; therefore, investigations on the mechanism of chromosome chromosomal aberrations, breakage of chromosome, and their genetic consequence (Zeerak, 1992). Induced mutagenesis has been perceived as the most proficient strategy for induction of morphological and genetically variability in plants particularly in those with constrained hereditary variability.

Cytological investigation concerning their mitotic behavior is thought to be a standout amongst the most trusted way to check the intensity of mutagen. Cytological studies give data with respect to the reaction of different genotypes to a specific mutagen and give more prominent opportunities to the choice of desired characters (Gnanamurthy and Dhanavel, 2014).

A chromosome irregularity, anomaly or deviation reflects a regular number of chromosomes or a basic variation from the norm in at least one chromosomes. A karyotype refers to a full arrangement of chromosomes from an individual which can normal karyotype for the species via genetic testing. A chromosome abnormality might be distinguished or affirmed in this way. Chromosome abnormalities for the most part happen when there is a mistake in cell divisions taking after mitosis. There are many sorts of chromosome inconsistencies. They can be sorted out into two essential gatherings, numerical and basic inconsistencies (Bhat et al., 2007).

Mitotic studies revealed a wide range of chromosomal aberration such as nullisome, anaphasic bridge with laggard, anaphasic multiple bridges and laggards, anaphasic bridge, late anaphase, clubbing of chromosome and precocious movement of chromosomes. The chromosome studied was observed in treated plants such as both physical and chemical treatments. The 40% chromosome aberration was high for M_1 generation than the M_2 generation.

Materials and methods

The dry seeds of Bhendi (*Abelmoschus esculentus* (L.) Moench) variety Arka anamika were subjected to both physical and chemical mutagens. The physical mutagens namely, gamma rays were given at six different dose (10, 20, 30, 40, 50 and 60KR), The physical treatments were induced at sugarcane breeding institute (ICAR), Coimbatore. The chemical mutagens like Ethyl methane sulphonate and Diethyl sulphate were used in different concentration (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 %) for six hours. The chemically treated seeds were presoaked in distilled water for 6 hrs to ensure complete hydration of the seeds. The seeds were treated with solution of EMS, and DES for duration of 6 hrs. After, the seeds were thoroughly washed in running tap water for 8 to 10 times. The treated seeds were sown in the field along with the control in a randomized block design with three replications. M2, M3, and M4 generation were used in ten randomly selected plants. The treated and untreated (control) seeds were germinated. Three or four days old actively growing root tips were collected and thoroughly washed in tap water and pretreated in 0.002% Hydroxyquinolin at 4 to 10°C for 3 hours. The pretreated root tips were then washed in distilled water and stored in acetic alcohol for further study. Root tip squashes were made following the Iron alum haematoxylin squash technique (Marimuthu and Subramanian, 1962). From the mitotic squash slides, the metaphase stage and chromosomal aberrations of the cell division were found and micro photographed for chromosome analysis. Each treatment was repeated at least three times as described above. The data were recorded till four generations.

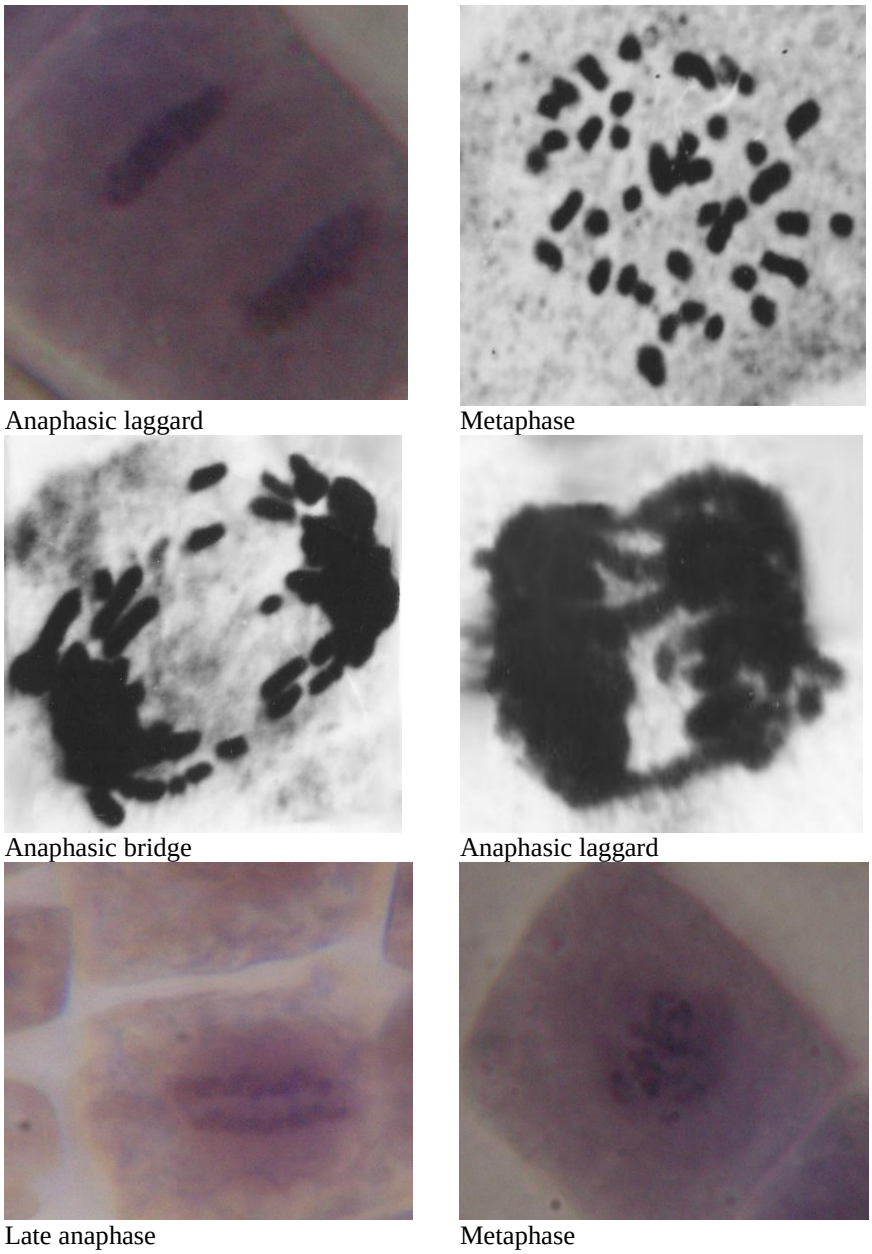


Plate I. Gamma rays, EMS and DES induced changes on cytology of bhendi.

Results and discussion

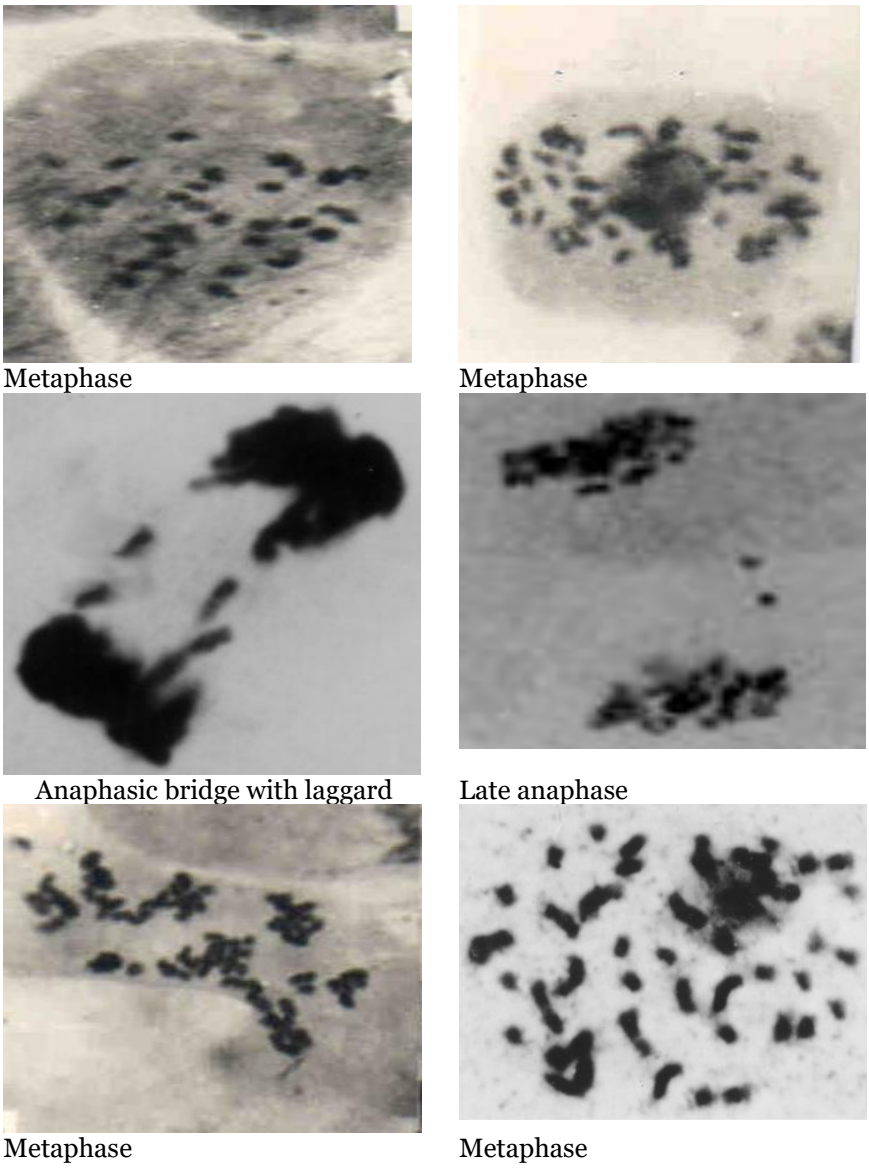
Mitotic studies

The physical mutagens like gamma rays and chemical mutagens like Ethyl methane sulphonate and Di-ethyl sulphate was induced many mitotic abnormalities. The observations were photographed and given in plate I & II.

Physical and Chemical mutagen

The physical mutagens namely, gamma rays were used in 10, 20, 30, 40, 50 and 60KR ranges, chemical mutagens like EMS and DES (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 %) was used. The somatic chromosome was well scattered and the constriction of chromosomes were clearly seen. The somatic chromosome number 2n=160 from a normal division, the less chromosome number such as nullisomic 2n=158 and anaphasic laggards, late anaphase, precocious movement of chromosome and clumping of chromosome were observed in the present study. The bhendi basic diploid chromosome number 2n = 160 (Sen and Vidyabhusan, 1960). The detailed chromosome studies were carried out understand it cytology (Biswas, 1977; Ahmad et al., 1983). In the present study somatic chromosome was

carried out with effect of mutagens. The metaphase chromosome number was 2n=160 in control. Whereas, 50kR of gamma rays treatment showed 2n=158 (Nullisomic) and 0.4% of EMS showed 2n=160 chromosomes (Plate I). The numerical variation of somatic chromosomes of 2n complement was revealed mutagenic effect in the genome. The tetraploid chromosome number showed was due to the absence of well-organized spindle in metaphase (Dusane et al., 1991) caused by effect of DES. 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). Whereas, chromosomal aberrations such as anaphasic bridge with laggards, multiple bridges, laggards, late anaphase, precocious movement of chromosomes and clumping of chromosomes were observed in present study (Plate I & II). Similar observations were reported by many workers in wheat (Alam et al., 1980), black gram (Bandyopadhyay and Bose, 1983), sunflower (Elangovan and Selvaraj, 1995), chickpea (Sharma and Kumar, 2004; Ganai et al., 2005), maize (Kumar and Rai, 2007) and chilli (Kumar and Gupta, 2009).



Metaphase | Metaphase

Anaphasic bridge with laggard | Late anaphase

Metaphase | Metaphase

Plate II. Gamma rays, EMS and DES induced changes on cytology of bhendi.

Chromosomal bridge may be formed due to the breakage and fusion of chromosomes. The bridge formation cab be due to the general stickiness of chromosome of metaphase stage (Ahmad and Yasmin, 1992).

Bridges and laggards with or without sections were discovered both at anaphase and telophase, bridges without pieces were found in lower concentration while as bridges with parts were found at higher concentrations of the mutagens, both single and twofold bridges were found yet the various extensions were not likewise uncommon. Different bridges were for the most part found at anaphase spindle bridges at telophase (Bhat et al., 2007).

Precocious development of chromosomes is by all accounts an appearance of despicable spindle working. The presence of spindle and multiple bridges might be because of the event of dicentric chromosomes shaped accordingly of breakage combination bridges cycles (Fluminhan and Kameya, 1997). The inhibition of seedling growth seemed to be well correlated with the amount of chromosomal damage. The EMS was found to react with the genetic material by alkylating DNA bases and phosphate groups (Thengane, 1984). The radio sensitivity was related to nuclear volume and interphase chromosome volume (Constantin and Love, 1967). Chromosomal and extra chromosomal materials were reported as the primary site of damage in the irradiated seed (Inoue et al., 1975).

Conclusion

In the present study, the deviations created by mutagens were because of partial or complete failure of spindle system. The percentage of abnormal cells increased with an increase in the dose gamma rays (40KR and 50 KR) and 0.4 % of EMS treatment.

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