

EFFECT OF GAMMA RAYS ON MITOTIC CHROMOSOME BEHAVIOUR OF ROOT TIP CELLS IN *Catharanthus roseus* (L) G. Don.

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Abstract

The mitotic effect of physical mutagen gamma rays was observed in the root tip cells of *Catharanthus roseus*. The Chromosome analysis has been showed as an important tool for establish variability of the plant seed by use of physical mutagen gamma rays. The gamma rays have of low wavelength and high penetrable power. The plant has tremendous medicinal values and it is known from ancient times. The dry and well matured seeds of *C.roseus* were irradiated with different doses of gamma rays viz., 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 KR respectively. The chromosome number of control plant is $2n = 16$. The gamma rays affect the normal cytological behaviour of *C. roseus* species. The chromosomal aberrations increase with increase in the doses of gamma rays to optimum level of 30 KR, because it causes changes in the chromosome structure, cellular structure and metabolism of plants. The chromosome aberration like, Sticky metaphase, Precocious moment chromosome, Fragments, Anaphasic bridge, Anaphasic laggard, Telophasic laggard. The present investigation was carried out to study the cytogenetic analysis of the species *C.roseus*.

Key words: *Catharanthus roseus*, Chromosome aberrations, Gamma rays and KR (kilo rad)

1. Introduction

The Periwinkle ($2n = 16$), (Apocynaceae), native of south eastern and eastern Madagascar, has been popularly known for its considerable medicinal value. Some of the features of *C.roseus* that make it a suitable genetic system are the biannual, seed cyclable, herbaceous perennial habit, diploidy ($2n = 16$;). Its medicinal values were known even in 50 BC (Jaleel *et al.*, 2006). It possesses largest number of alkaloids in plant kingdom (Magnotta *et al.*, 2006). More than 170 alkaloids of the indole and dihydroindole groups have been isolated and characterized from different organs (Mishra and Kumar, 2000). The

medicinal importance of this plant has increased considerably because of the discovery of six anti-cancerous activity containing alkaloids. Among them only vincristine and vinblastine are active in human system. *C. roseus* also owns its importance due to the presence of antihypertensive alkaloids such as ajmalicine and serpentine in root (Debnata *et al.*, 2006; Geerlings *et al.*, 2001). The annual world demand for vincristine, vinblastine and ajmalicine is production as evident from recent reviews relatively very little work has been done on conventional approaches for development of periwinkle varieties with high alkaloid contents (Facchini and St-Pierre, 2005; Pandey-Rai *et al.*, 2003). Among the conventional methods mutation breeding is a promising approach for approaches for creation of genetic variability and useful for

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the development of 'idiochemovars' in medicinal plants (Pandey-Rai and Kumar, 2000; Gecheff, 1996). The aim of this review is to summarize the all efforts of mutation breeding which were made world widely for the improvement of *C. roseus*.

Mutation is a sudden heritable change in an organism generally the structural change in genes. The term mutation was first introduced by (Hugo De Vries, 1901.) in *Oenothera lamarckiana*. Mutation produced by changes in base sequences of genes (as a result of base pair transition or transversion, deletion, duplication or inversion etc.) are known as gene mutation or point mutations. The agent that induce mutation are called mutagen. Gamma rays are one of the physical mutagen used in the field of mutation breeding. The technology of mutation breeding is a conventional method of plant breeding in order to supplement existing germplasm and to improve cultivars in specific traits. Improved varieties of many plants have been released through mutation breeding. Any mutagen that induces single base pair mutation or small deletion/ insertion is effective for tilling.

Gamma ray is one of the physical mutagen with low wavelength and high penetrating power and causes gene mutation in living organisms. Mutations are randomly distributed in the genome. A high degree of mutation saturation can be achieved with a mutagen like gamma rays that does not cause a lot of collateral DNA damage.

2. Materials and Methods

The material used for this cytological study was "Pink Rosea" variety of. Periwinkle. The dry and dormant seeds of variety of *Catharanthus roseus* were collected from J.P Laboratories, Virudhunagar District and Tamil Nadu. Two sets containing 100 well Dry seeds were irradiated from a 60 Co source at Dry and matured seeds with 12 per cent moisture content (50 kr numbers for each dose) of the cultivar was irradiated with different doses of gamma rays 5 kr, 10 kr, 15 kr, 20 kr, 25 kr, 30 kr, 35 kr, 40 kr, 45 kr and 50 kr viz., from Cobalt-60 (⁶⁰Co) using the Gamma

Chamber at Indra Gandhi Centre for Atomic Research, Kalpakkam, Tamil Nadu. The treatments were undertaken in the evening, one day before sowing. After the treatment, the irradiated seeds were sown in raised nursery beds the next day morning along with the control (untreated) seeds.

The periwinkle root tips about 3 cm in length were excised, fixed in glacial acetic acid: alcohol (1:3 ratio) solution for 48 hrs. Then root tip squashes were made by using iron alum, haematoxylin squash technique (Marimuthu and Subramanian, 1960). The most frequent chromosomal aberrations were stickiness, precocious movement, bridges followed by laggards was more frequent. Frequency of chromosomal aberration was expressed as the number of cells with chromosomal aberration per 100 scored cells, out of approximately 1000 examined cells taken from 10 separate seedlings for each dose/concentration.

3. Results and Discussion

In the present study somatic chromosome was carried out with effect of mutagen. The metaphase chromosome number was $2n = 16$ 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). Chromosomal observation were reported by many workers, Sunflower (Elangovan, 1995), Black gram (Bandyopadhyay and Bose, 1983), Chilly (Dhamayanthi, 2000), Chick pea (Sharma 2004) and the physical mutagen gamma rays induced many mitotic abnormalities in Soybean (Pavadai, 2013). The mutagen can cause physiological damage besides gene and chromosomal changes. Physiological damage, mainly manifested as growth retardation and death, is generally restricted to M1 generation. Such a phenomenon has been attributed to changes in hormonal levels such as auxins and ascorbic acid. Physiological and biochemical disturbances (Gunckel, 1954, Singh, 1974),



change in enzyme activity and impaired mitosis in the meristematic zone of growing seedlings (Blinks, 1952). Similar results were suggested that it might also be due to a decrease in respiratory quotient in irradiated seedlings (Woodstock and Justics, 1967). In the view of these contradictory reports, the present investigation was conducted in Ashwagandha with sole objective to comprehend chromosome behavior by passing the gamma rays in the seeds. Mitotic aberrations were recorded by many authors in plants raised from seeds treated with different dosages of the gamma rays.

Precocious movements of chromosomes might have occurred due to disturbed homology for chromosome pairing, disturbed spindle mechanism or in activation of spindle mechanism (Agarwal and Ansar 2001) in *Vicia faba*. Laggards were present in almost all treatments and occurrence of laggards in the present study has also been reported previously by many workers such as (Singh *et al.*, 1999) in *Vigna radiata* (Iqbal and Datta 2007), (Khan *et al.*, 2009). 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 (Dhanavel *et al.*, 2012) in Cowpea. Delayed terminalisation and or failure of chromosomal movements, following spindle fibre discrepancies have lead to lagging chromosomes. The fragments which appeared on the breakage of bridges, as result of spindle fibres functioning to pull the chromosomes towards poles, formed laggards (Kumar and Gupta *et al.*, 2009) in Aswagantha.

Bridges occur due to sister chromatid chromosomes exchange followed by delayed or failure of their separation at later stages. Bridge formation observed by (Ahmad and Yasmin 1992), (Utsunomiya *et al.*, 2002) and (Cequea *et al.*, 2003). Bridges might have occurred as a result of delayed terminalization (Bipasha and Shella, 1992), unequal separation of chromosomes (Iqbal and Datta, 2007). The thick sticky bridges may be due to the stickiness of chromosomes. The 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 at metaphase and further led to their inability to separate, thus leading to sticky bridges. The spindle fibres pulled the chromosome towards the poles these bridges were broken into fragments, which either moved towards the poles or formed laggards and micronuclei (Kumar, 2003). 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 chromosome may be explained on the basis of abnormal spindle formation and failure of chromosome movement.

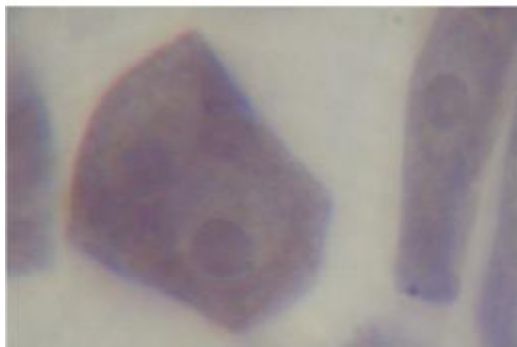
The fragments at metaphase may be due to the failure of broken chromosome to recombine. The Fragments might have arisen due to the stikiness of chromosome and the consequent failure of the arrival of chromatids at the poles. Fragments may also be acentric chromosomes formed as a result of inversion (Agarwal, 2001). Stickiness was one of the abnormalities found both in mitosis and in meiosis. It occurs due to disturbances of cytochemically balanced reactions by the effect of alkylating agents (Chidambaram *et al.*, 2008). Bridges and laggards with or without fragments were found both at anaphase and telophase, bridges without fragments were found at higher dosages of mutagen.

Chromosomal rearrangements are one of the most frequently produced classes of mutation that result from the action of both physical and chemical mutagenic agents (Gecheff, 1996). In higher plants, chromosome aberrations induced by radiation have been utilized for many years in classical genetic studies (Mcclintock, 1984) and more recently to provide starting material for gene isolation and mapping (Liharska *et al.*, 1997; Bhatt *et al.*, 2001). In studies with gamma rays, mainly seeds were used for the induction of chromosome variation (Brock, 1980; Friebe *et al.*, 1991; Sanamyan *et al.*, 2000).



Chromosome studies

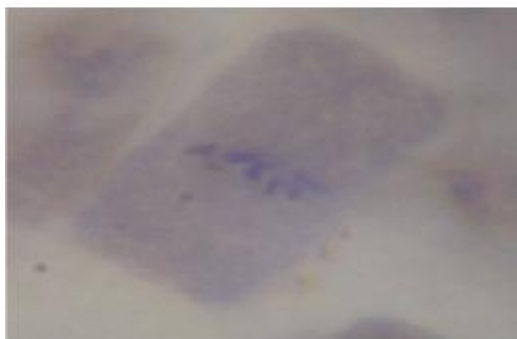
Multi nucleus stage



Anaphasic bridge



Precocious movement



Metaphase stage



However, they can yield chimeras, which hinder the analysis of mutations of the M1 generation (Brock, 1980). By contrast studies like these have been conducted in some species, like *Nicotiana tabacum* (Kramer, Reed 1988), *Capsicum annuum* (Kumar *et al.*, 1998), *Cucumis sativum* (Mustafa *et al.*, 1999), *Arabidopsis thaliana* (Vizir Muliigan, 1999) and *Gossypium hirsutum* (Sanamyan *et al.*, 2000). In previous studies on maize, the doses applied ranged from 30 to 1500 Gy and their consequences were also variable (Brock Pryor, 1996; Romanova, 1998; Lysikov, 1999; Ikhim, 2000). In addition, the high number of nonviable plants after mutagenic treatment can hinder, in some cases, the transmission of the alterations, the selection of mutants and consequently the reliability of genetic

and molecular analysis (Kumar Dubey, 1998). Thus, the goal of this study was to induce chromosome- altered maize plants without viability loss, by means of gamma irradiation of pollen grains.

4. Conclusion

A dose dependent increase in the mitotic aberrations was observed in root tips of *Catharanthus roseus*. It is clear from the observation that gamma rays caused more chromosomal damage in *Catharanthus roseus* and also a dose dependent reduction was observed in seed germination, seedling survival and plant height was also observed. Although gamma rays showed inhibitory effect on seed germination, plant height and seedling survival but the gamma



rays have enough potential for induction of genetic variability and to exhibit quantitative and qualitative variations which can be favorably exploited by cytogenetic and plant breeders in improving the genotype of *Catharanthus roseus* (L.) G. Don and to improve the yield characters by cause of gene mutation.

5. References

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