Ionizing radiation mediated cytological manifestation in microsporogenesis of *Brassica campestris* L. (Brassicaceae)

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Abstract

Purpose of the present work is to investigate the mutagenic effects of ionizing radiations (gamma rays) on ***Brassica campestris*** L. accession no - IC363713. Homogeneous seeds of ***Brassica*** were irradiated at four doses of gamma rays i.e. 150 Gy, 300 Gy, 450 Gy and 600 Gy by the gamma-chamber type ^{60}Co at the dose rate of 2 second/Gy. During microsporogenesis, meiotic analysis of young floral buds was carried out in irradiated as well as non-irradiated plant materials. Meiotic study clearly revealed the meiotic malfunctioning of pollen mother cells (PMCs) that had shared copious count of cytological abnormalities namely unorientation, stickiness, precocious movement or fragmentation, secondary association of bivalents, asynchronous division, laggards, tripolarity and chromatin bridge. These aberrations were found to be distributed in all the phases of male meiosis. However, this impairing during meiosis has found to be collinearly associated with doses i.e. inclining tendency of abnormality percentage alongwith increasing doses were registered. Perhaps aforementioned chromosomal aberrations may be introduced by asymmetrical distribution of chromatin material in PMCs, had definitely compromised with pollen fertility, resulting the increased frequency of pollen sterility. Hence, pollen fertility registered, simultaneously, a moderate to sharp fall depending upon the intensity of doses.

Keywords**:** ***Brassica campestris*** L., cytological aberration, gamma rays, Ionizing radiation, pollen fertility

Introduction

Unlike non renewable energy providing source (coal, petroleum etc.) utilization of ionizing radiation (radioactive isotopes) for ease of obtainment of million fold of energy by means of nuclear technique has been tremendously increased. Especially in developed countries it has become very frequent and has caused sudden surge of nuclear reactors which are highly responsible for the release of harmful ionizing radiations in the environment. Consequences of such ionizing radiations are long lasting even for years which may be responsible for inducing point-mutations on organisms existing in a radiation contaminated environment. Recently nuclear techniques are widely applied in agriculture for improved genetic variability despite conventional breeding procedures which involve the production of new genetic combinations from already existing parental genes (Majeed, et al., 2009). Hence, studies on biological effects of ionizing radiations and their utility in induced gene mutations have been a topic of immense interest of cytogeneticists (Shukla nee Tripathi and Kumar, 2010).

Moreover for successful mutagenesis, selection of efficient mutagen and treatment is a pre-requisite as mutagens are the potent tools for bringing direct improvement and certain valuable changes in crop plants (Paul and Dutta, 2006). Gamma rays, class of ionizing radiations are extremely potent physical mutagen which can induce beneficial as well as deleterious mutations in crop plants. It is therefore, of utmost importance to recognize an advantageous dose which is competent to increase qualitative as well as quantitative traits in plants like Shah, et al. (2001), developed a new oil seed ***Brassica napus*** L. cv. ABASIN-95 by induced mutation at 1.0 Gy, 1.2 Gy and 1.4 Gy gamma irradiation thereby resulting a new variety which was high yielding, resistant to alternaria blight and white rust.

Brassica campestris belongs to family Brassicaceae is a crop of immense economic importance and is among the oldest cultivated crop known from the early human civilization and has been used as oilseed crop and condiments. It is reported that ***Brassica*** spp. are actively hyper-accumulatory and efficiently competent to take up that metals which releases ionizing radiation in the environment after natural decay such as uranium and thorium. Many researchers such as Hung, et al. (1998) in ***brassica*** ap. and Huhle, et al. (2008) in ***Brassica juncea***, reported the phytoextraction of some rare earth metals viz. uranium and thorium (radioactive metals) that are extensively utilized in nuclear reactors for the production of ionizing radiations. Since these radiations are extremely harmful and considered as highly potent mutagen for inducing point mutations hence, responsible for enforcing unusual cytological behavior of treated materials of ***Brassica campestris***. Basi, et al. (2006) mentioned that the analysis of PMCs for meiotic study is inevitable to generalize the background of the mutants and this information is essential for a conceptual approach and to introduce the induced variations from and to the subsequent lines.

Thus, keeping above points in mind, an effort has been made to explore the effect and extent of meiotic abnormalities as a consequence of ionizing radiations (gamma irradiation) during microsporogenesis of ***B. campestris*** L. and its impact on the viability of pollen grains.

Material and methods

Seeds procurement - Certified seeds of ***Brassica campestris*** L., accession no.-IC363713 procured from National Bureau of Plant Genetics Resources (NBPGR), New Delhi.

Treatment methods- healthy, dry and homogeneous seeds were irradiated with gamma-rays at 150 Gy, 300 Gy, 450 Gy and 600 Gy from a ^{60}Co source at National Botanical Research Institute (NBRI), Lucknow with dose rate of 2 sec/Gy and immediately after irradiaton seeds were soaked in distilled water for 6 hours alongwith one set of untreated sets and sown in triplicates to raise population for mutation breeding experiment.

Meiotic preparation – young flower buds of ***Brassica campestris*** L. were collected in vials at an early winter's morning and were fixed in carnoy's fixative (glacial acetic acid – alcohol, 1:3) v/v for 24 hours at room temperature i.e. 25±2ºC and transferred to 70% alcohol and refrigerated at 4ºC until use. The anthers were squashed in 2% standard aceto-carmine stain, the debris and the stain excess were removed and the slides were covered with cover slips following gentle tapping. Slides were observed under optical microscope (***Olympus CH20i***) and photomicrographs were captured by Pinnacle PCTV software. Five plants were randomly selected per dose alongwith controls.

Pollen fertility - Mature floral buds were collected and anthers having pollen grains were dusted over glass slide and stained with freshly prepared glycerol-carmine mixture, covered and observed under optical microscope. Frequency of fertile and sterile pollen grains was registered to estimate pollen fertility.

Results

Brassica campestris L., exhibits 20 chromosomes as normal somatic complement i.e., 2n=20. Meiotic behavior of chromosomes was almost normal except very low percentage of stickiness in the control set during microsporogenesis. PMCs of untreated plant showed 10 bivalents at metaphase-I (Fig.1A) and separation of bivalents into 10:10 at anaphase-I (Fig.1B). In cytological plate Fig.1C and Fig.1D showed normal second division of meiosis such as metaphase-II and anaphase-II, respectively. Analysis of PMCs of irradiated sets at varying stages of division viz. metaphase-I/II and anaphase-I/II showed a copious count of cytological anomalies namely stickiness (Fig.1E, 1G, 1H, 1I, 1J,1L and 1M), precocious movement or fragmentation of chromosomes (Fig.1J), unorientation (Fig.1E and Fig.1I), secondary association of bivalents at metaphase-I (Fig.1F), asynchronous division (Fig.1K and Fig.1N), laggards (Fig.1H), tripolarity at anaphase-II (Fig.1M) and chromatin bridge formation. Their frequency of occurrence was asymmetrical and varied from moderate to high depending upon the extent of doses ranging from 9.17% to 20.96% at the treatment doses from 150 Gy to 600 Gy, respectively (Table 1).

For control, 0.22% of chromosomal stickiness was registered that might be due to the environmental stress during the growth of plants. In PMCs of treated sets, stickiness as a result of liqueidization of chromosomes being the most prominent abnormality shared the greatest score of aberrant cells which was 5.67% at 600 Gy while least count was scored at 300 Gy (2.74%, except control) at metaphase-I/II and during anaphase-I/II it was found to be 1.09% at 600 Gy while 0.93% at 300 Gy. As far as, generalization can be made, anomalies were dose dependent i.e., percentage of abnormalities increased with the increasing dose. , In present case, although many abnormalities followed similar trend viz., precocious movement or fragmentation, secondary association and chromatin bridge yet, rest of the abnormalities deviate from the aforementioned trend and their sporadic occurrence was documented. Another anomaly that prevailed after stickiness was precocious movement which scored minimum as 1.17% and maximum as 4.44% at 150 Gy and 600 Gy, respectively. Laggards have also been observed at varying degrees during anaphase-I/II however, complete dearth of this phenomenon was documented at 150 Gy dose of ionizing radiation (Table-1).

*Table 1- Gamma rays induced meiotic abnormalities and pollen fertility in pollen mother cells of ***Brassica campestris*** L.

Doses (Gy)	Total PMCs Observed	Metaphase I/II (%)					Anaphase I/II (%)						Total abnormality (%)	Pollen fertility (%)
		Pm	Un	Asd	St	ScA	Lg	Bg	Tp	St	Asd	Oth		
Ctrl	236	-	-	-	0.22	-	-	-	-	-	-	-	0.22	99.95±0.04**
150	226	1.17	1.00	1.29	3.71	-	-	-	0.58	1.03	-	0.93	9.71	99.60±0.18
300	292	1.71	1.12	1.33	2.74	1.05	1.34	-	0.34	0.93	0.68	0.78	12.02	93.46±0.06
450	242	2.75	1.50	0.65	3.02	0.84	2.47	0.55	0.67	1.00	0.70	0.45	14.60	88.73±0.32
600	270	4.44	1.87	1.47	5.67	0.98	2.22	0.98	0.73	1.09	0.74	0.77	20.96	78.47±1.03

***Abbreviations:** PMC- Pollen mother cell, Pm- Precocious movement, Un- Unorientation, Asd- Asynchronous division, St-Stickiness, ScA- Secondary association, Lg- Laggards, Bg- Bridge, Tp- Tripolarity, Oth- Others.

**- Mean±SE

Moreover, share of asynchronous division at meiosis was found to be varied during metaphase I/II and anaphase I/II and measured maximum (1.47%) at 600 Gy during metaphase I/II while the least (0.27%) was counted at 450 Gy at anaphase-I/II. Even though unorientation was not among the most frequently occurring abnormalities nevertheless highest percentage of PMCs contributing unorientation was recorded at 600 Gy (1.87%) and the lowest percentage was found to be at 150 Gy (Table 1). Further considerable amount of chromatin bridge, tripolarity and secondary associations of bivalents were also registered.

Pollen-fertility was estimated to be 99.95±0.04% in controls while in the irradiated materials, it was found to be decreased with the increasing spectrum of ionizing radiations. The lowest dose i.e., 150 Gy has shown the moderate difference of pollen fertility as against control which was 99.60±0.18% although, gradual decrease in the pollen fertility was documented at dose of 300 Gy onwards (Table 1).

DISCUSSION

Results clearly revealed the ionizing radiation mediated induced disturbed male meiosis and its impact on viability of pollen grains. Although, it was really difficult to count exact number of bivalents since the chromosomes of ***Brassica*** species were very small, poorly differentiated and highly condensed. Thus their identification through the ordinary cytogenetic techniques was extremely difficult (Ahmad, et al., 2004).

As previously discussed that ionizing radiation has induced considerable range of meiotic aberrations. Many interpretations have been made to account for the occurrence of such cytological anomalies such as Raj, et al. (1972) stated that the cytological and genetic effects of radiation of ^{60}Co are mostly as a result of chromosomal aberrations that might have occurred at microscopic and submicroscopic levels. The radiation can induce chromosomal breakages not only by direct hits but also indirectly by the reaction chemical products produced in water surrounding the chromosomes (Basi, et al., 2006). Thus in the present case, worth noting effects of gamma rays were observed at entire spectrum of ionizing radiation which revealed the occurrence of wide assortment of chromosomal deformities and their frequency harmonized with doses of radiation i.e. the percentage of impaired PMCs increased with the increasing spectrum of doses.

The increment in the chromosomal aberrations might perhaps be due to the interactions of ionizing particles with the protoplasm, mediated through the excitation introduced by radiation that ultimately has increased the aberration frequency (Shukla nee Tripathi and Kumar, 2010). At different stages of meiosis such as metaphase-I/II followed by anaphase-I/II, plentiful cytological manifestations have been encountered explicitly unorientation, asynchronous division, precocious movement of chromosomes, laggards, chromatin bridge formation, secondary association of chromosomes and stickiness i.e. liquidization of chromatin material. Precocious movement of chromosomes at metaphase and stickiness in all the stage of meiosis was the most dominating aberrations registered. Though the stickiness is one of the phenomena in chromosomal behavior that has been recorded for almost century, yet the adequate explanations are still lacking (Kumar and Rai, 2008). Phenomenon of liqueidization of chromosomes was earlier identified by Koernicke (1905) and later termed by Beadle (1932) as stickiness,

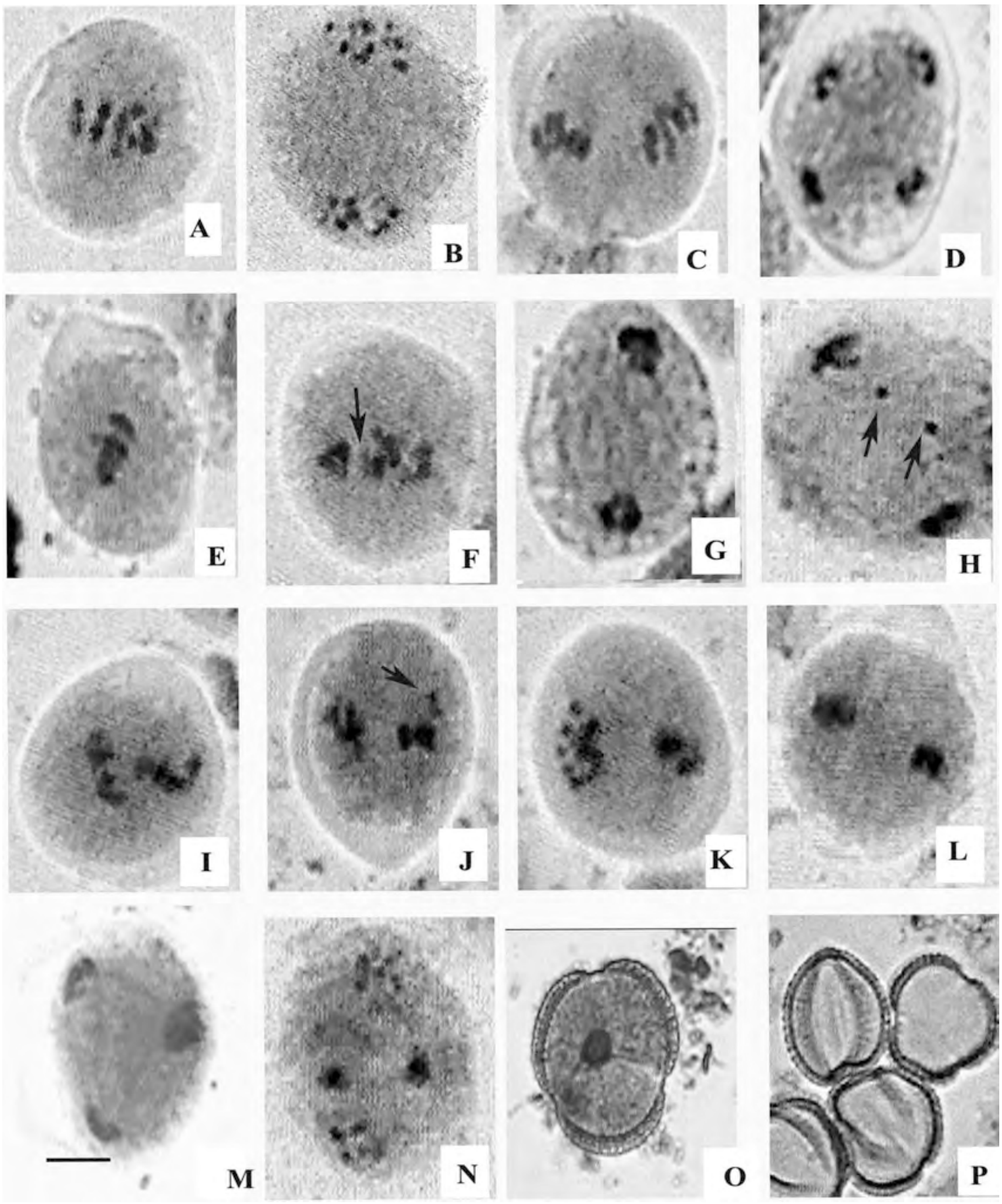

Figure1. A. Normal metaphase-I, B. Normal anaphase-I, C. Normal metaphase-II, D. Normal anaphase-II, E. Unorientation at metaphase-I, F. Secondary association at metaphase-II (arrow), G. Stickiness at anaphase-I, H. Laggards at anaphase-I (arrow), I. Unorientation at metaphase-II, J. Precocious movement of chromosome at metaphase-II (arrow), K. Asynchronous division at metaphase-II, L. Stickiness at metaphase-II, M. Tripolarity at anaphase-II, N.Asynchronous division at anaphase-II, O. Normal (stained) fertile pollen grain, P. Sterile pollen grains. (40X, scale bar=4.2µm)

when he described the sticky aspect of chromosomes that had suffered a mutation in maize. Considerable causes of stickiness may be either environmental stress or genetic and also it would be both as already noticed in control plants having some extent of stickiness that must be due to the environmental factors. However, the treated sets displayed the induced genetic stickiness that may inherit to the subsequent generations.

Another abnormality that preponderance after stickiness was the precocious movement of chromosomes, which might be formed due to breakage of chromatids or chromatin erosion (Fiskesjo, et al., 1993) during metaphse. However, such breakage or fragmentation of chromatids at metaphase might have divided the meiocyte into two cells of imbalanced genome content resulting in to aborted pollen grains. In some of the PMCs of metaphase-II and anaphase-II, however, we observed asynchronous behavior of chromosomes where two groups of chromosomes found to be in different state of division. As generalization can be made, during division chromatids of both groups divides simultaneously i.e. they remain cytologially synchronous, however, in asynchronous PMCs , one set/group of sister chromatids was at the present stage of division, whereas others group of sister chromatids simultaneously entered in to the next or upcoming stage of division, suggesting the meiotic malformation.

Occurrence of secondary associations in plants was interpreted by Swanson (1946) suggesting that they might have arisen from crossing over in overlapping inversions, thus preserving both the centric and terminal regions intact while eliminating median portions of both the arms responsible for loose pairing of chromosomes and this type of loose pairing is most often found at meiosis in the organisms with small chromosomes. Similarly, the present test crop having extremely small chromosomes displayed the secondary association of bivalents at metaphase-I.

Presence of lagging chromosomes may be due to the failure of chromosomes to reach the pole before the chromosomes relaxes to uncoil to form daughter nuclei or they disintegrates to form micronuclei (Basi, et al., 2006) that may generate unbalanced gametes. Literature also supported the changeable degree of laggards in the PMCs of various other plants treated with ionizing radiations such as ***Trigonella foenum graecum*** (Sudhakaran, 1971; Raghuvanshi and Singh, 1974).

Unorientation might be due to inability of microtubule to work properly on account of direct hits of stored ionizing energy of gamma rays on spindle fiber. This could be attributed due to improper flux of binding microfilaments during orientation of chromosomes. Chromosomal mutations, such as inversions of chromatids can cause the formation of chromatin bridges during meiotic anaphase between the segregating chromosomes (Darlington, 1937) and they were infrequently found in PMCs. Disturbed polarity or tripolarity might be due to spindle disfunctioning.

Conclusion

In view of above investigation, realization can be made that the spectrum of cytological anomalies is dose dependent yet few chromosomal aberrations displayed their sporadic occurrence. Conclusively, all the doses induced some extent of aberrations in PMCs whereas the highest dose had shared a greatest score (20.96%) while dose of 150 Gy possessed it in least frequency (9.71%) consequently responsible for abnormal gametogenesis. Moreover, aftereffect of abnormal gametogenesis might be the production of aborted pollen grains by asymmetrical distribution of chromosomes due to meiosis impairment leading to pollen sterility although; survival of abnormal pollen grains may produce imbalanced progeny bearing some peculiar genetic traits.

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References

Ahmad, H., Hasnain, S., Khan, A., (Jul - Dec, 2003) & 3-4 (Jan - Jun, 2004) Genome biology of the cultivated ***Brassica*** quarterly science vision, 9, 1-2.

Basi, S., Subedi, L. P., KC, G. B., Adhikari, N. R., (2006) Cytogenetic effects of gamma rays on indica rice radha--4, J. Inst. Agric. Anim. Sci., 27, 25-36.

Beadle, G.W., (1932) A gene for sticky chromosomes in ***Zea mays.*** Indukt Abstamm Verebung, 63, 195-217.

Darlington, C.D., (1937) Recent advances in cytology. 2nd ed. J. A Churchill, London.

Fiskesjo, G., Levan, A., (1993) Evaluation of the first ten MEJC chemicals in the ***Allium*** testa Alta), 21,125-133.

Huhle, B., Heilmeier, H., Merkel, B., (2008) Potential of ***Brassica juncea*** and ***Helianthus annuus*** in phytoremediation for uranium. Uranium, mining and hydrogeology. 307-318.

Hung J.W., Blaylock, M.J., Kapulnik, Y., Ensley B.D., (1998) Phytoremediation of uranium-contaminated soils: role of organic acids in triggering uranium hyperaccumulation in plants. Environ. Sci. Technol, 32(13), 2004-2008.

Koernicke, M., (1905) Uber die wirkung von Rontgen- und radium strahlen auf pflanzliche Gewede und zellen. Dtsch Bot Ges, 23, 404-415.

Kumar G., Rai, P.K., (2010) Genetic repairing through storage of gamma irradiated seeds in inbred maize ***(Zea mays*** L.) Turk J Biol, 33, 195-204.

Majeed, A., Muhammad, Z., Ahmad, H., Khan, A. R., (2009) Gamma irradiation effets on some growth parametersof ***Lepidium sativum*** L. American-Eurasian journal of sustainable agriculture, l3 (3), 424-427.

Paul, R., Dutta A.K., (2006) Mutagenic efficiency and effectiveness of gamma rays and EMS in some seed spices. Journal of cytology and genetics, 7, 91-99.

Raghuvanshi, S. S., Singh, A. K., (1974) Studies on the effect of gamma ray on

Trigonella foenum-graecum L. Cytologia, 39, 473-482.

Raj, A.Y., Raj, A. S., Rao, G.M., (1972) Mutagenic studies of gamma rays on ***Oryza sativa*** L. Cytologia, 37, 469-477.

Shah, S.A., Ali, I., Rahman, K., (2001) 'Abasin-95', A new oilseed rape cultivar developed through induced mutation. Mut. Breed. Newsletter, 45, 3-4.

Shukla nee Tripathi, R., Kumar, G., (2010) Comparative effect of ageing and gamma irradiation on the somatic cell of ***Lathyrus sativus .*** JCEA, 11(4), 437-442.

Sudhakaran, I. V., (1971) Meiotic abnormalities induced by gamma rays in ***Vinca rosea*** Linn. Cytologia, 36, 67-79.

Swanson, C.P., (1946) Secondary Association of Fragment Chromosomes in Generative Nucleus of ***Tradescantia*** and Its Bearing on Their Origin., Botanical gazette, vol.105.