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Evaluation of mutagenic efficiency and effectiveness of gamma irradiation doses in two cultivars of bread wheat (*Triticum aestivum* L.)

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Abstract

Seed irradiation before sowing processes is one of the most efficient tools to improve the plant genetic diversity rapidly. The present research was carried out to evaluate the mutagenic efficiency and effectiveness of different gamma irradiation doses in bread wheat (Triticum aestivum L.). For this, healthy seeds of two varieties of bread wheat (Lok-1 and WH-147) were treated with various gamma irradiation doses (5, 10, 15, 20, 25, 30, 35, 40, 50kR) at Bhabha Atomic Research Center (BARC), Mumbai. The biological damage was evaluated in M1 populations by collecting the data related to plant lethality, seedling injury, meiotic aberrations and pollen sterility. Biological damage in M1 progenies and chlorophyll mutations in M2 populations were also estimated to measure the mutagenic efficiency and effectiveness of gamma rays. The results obtained showed that mutagenic effectiveness boosted along with increased dose of gamma irradiations whereas the efficiency of gamma ray treatments revealed irregular tendency depending on the biological parameter undertaken for its estimation. The lower as well as intermediate irradiation doses were successfully found to be much effective in producing populations with less genetic damage and with greater amount of macro-mutations. Among the bread wheat cultivars used, var. Lok-1 was found to be more sensitive to gamma-irradiations as compared to WH-147.

Keywords: *Triticum aestivum,* gamma irradiation, mutagenic efficiency, effectiveness, injury.

Introduction

Induced mutagenesis, for its well-known status in modifying the morphogenetic structure of the crop plants, can be utilized as a principal tool in pertaining desirable traits in different plant species plant including wheat. The technology of induced mutagenesis has become are putable technique in mutation breeding programmes in order to enhance the current germplasm and to improve specific traits in cultivars. The main aim of mutation induction is to rapidly broaden the genetic pool of crop plants for their future improvement. The agricultural traits of F1 populations significantly provides a base for assessing the efficiency and effectiveness of mutagen, besides isolating the plant progenies with maximum variability in genetic structure which have more likelihood of possessing the higher frequency of micro or point mutations in following F2 and F3 generations. Many physical mutagens, for example, X-rays and gamma rays, have got much attention from past few decades as the most effective mutagenic agents in higher plants especially[1]. However, the application of gamma irradiations as a mutagenic agent is of huge importance not only to analyse any gene functions by isolating novel mutants, but also to improve global situations by providing new high yielding crop varieties with their beneficial traits. The gamma rays generally induce DNA lesions by producing free radicals, as a result produce chromosomal rearrangements[2,3] which in turn can damage or modify important chemical components and physiological processes of plant cells[4].

Bread wheat (*Triticum aestivum* L.) is one of the premier food crops with respect to its acreage, antiquity, and grain production in the world[3]. After rice, wheat is next most important food grain in Indian sub-continent. Relatively, the higher protein content in wheat food grains makes them very significant source of human diet, hence its improvement is of principal importance for feeding an ever increasing population. Bread wheat (family: Poaceae) is self-pollinating allo-hexaploid cereal species (BBAADD) that possesses the homoeologous genome combination of three diploid ancestral species. The presence of higher ploidy level (*i.e.* hexaploid), in bread wheat has great genetic significance in mutation breeding programmes as it can abide higher levels of meiotic anomalies often displayed due to higher gamma irradiation doses. As a result, this can bound the limitations of phenotypic manifestations of novel mutant populations owing to the presence of multiple genetic factors[5]. Therefore, estimating the suitable or proper irradiation dosage of gamma rays is most crucial step for creating desirable morphogenetic populations of this cereal species rapidly. Many attempts were made previously for isolating populations with desirable agronomic mutations affecting yield linked as well as seed related traits in various cereal species including wheat[6,7].

Therefore, the present research has been carried out to comprehend the response of two wheat cultivars to gamma irradiations with the aim to identify the proper dose required for maximizing mutation frequency in bread wheat (*Triticum aestivum* L.). In addition, the study also identified the suitable genetic system for rapid appraisement in future mutation breeding programmes.

Material and methods

Healthy and certified seeds of Lok-1 and WH-147 bread wheat cultivars, procured from KVK Ujjain, were used in present study; these varieties are well acclimatized to agro-climatic conditions of Madhya Pradesh, India. The dry seeds of these bread wheat cultivars were subjected to different doses of gamma rays at Bhabha Atomic Research Centre, Mumbai. Treatment doses of gamma rays were 5, 10, 15, 20, 25, 30, 35, 40 and 50 kR. At least 500 seeds were used for each application. The irradiated and untreated seeds were subsequently sown in experimental fields with three replicates for each dose according to randomized block design to raise the M_1 progenies. Each treatment trail as well as control comprised of 150 seeds.

In seed irradiated M1 populations, experimental data on different biological traits, *viz*. seedling injury, frequency of lethal seeds, frequency of sterile pollens as well as total meiotic abnormalities were critically analyzed and documented. The seeds of both M1 treated progenies and control ones were harvested and collected in separate bags and stored for next year at room temperature. In next season, a random sample of at least 150 seeds from each irradiation treatment were selected from a bulk of collected seeds and thereafter, sown in field with three replicates in completely randomized block design in order to raise the M2 populations. The control and treated plant populations were critically analyzed for mutations displayed inphenotypic traits during the whole growth period in the experimental field. In order to categorize the various kinds of chlorophyll mutations, the principal methodology of Gustafsson[8] was employed. Likewise, for estimating the efficiency and effectiveness of gamma irradiation doses in creating genetic mutations, the formula given by Konzak et al.[9] was employed. Mutation frequency, mutagenic effectiveness and mutagenic efficiency were estimated by employing the following formulae.

$$\text{Mutation frequency} = \left(\frac{\text{Number of mutated plants}}{\text{Total number of plants}}\right) \times 100$$

$$\text{Mutagenic effectiveness} = \left(\frac{\text{Mutation frequency (Mf)}}{\text{Dose in kilo Roentgen (kR)}}\right) \times 100$$

$$\text{Mutagenic efficiency} = \frac{\text{Mutation frequency (Mf)}}{\text{\% lethality (L) or injury (I) or sterility (S) or meiotic abnormalities (M)}} \times 100$$

For meiotic characterization, young panicles about to come out from flag leaf from 10-15 randomly selected plants were collected in the early morning and fixed immediately in a freshly prepared 1:3 acetic alcohol Carnoy's solution for at least 24 hours and subsequently transferred to 70% alcohol for future preservation. The anthers from appropriate buds were excised and crushed in a drop of 2% iron acetocarmine by putting them on cleaned glass slide. The investigations were made and appropriate pollen mother cells were photographed from the temporary slides under Olympus microscope with the help microscopic camera by using the field of 40X objective lens and 10X eyepiece. The meiotic phases for various chromosomal aberrations were examined in more than 300 micro-sporocytes for each treatment. The easily identified chromosomal aberrations in dividing cells in each irradiation dose were recorded and used for the calculation of percent abnormality.

Results and discussion

Mutagenic effectiveness was assessed in order to determine the frequency of induced mutations by exploiting the dormant seeds with different doses of gamma irradiations. This can be understood with the critical examination of Table-1, which gives a clear idea about the influence of gamma ray doses in causing the genetic effects. After the data was assembled, all the biological traits taken under consideration (for example, lethality, seedling injury, pollen sterility and meiotic anomalies) displayed constant rise along with increasing gamma irradiation doses. In M2 generation, the calculated values of effectiveness showed regular reduction especially at higher gamma irradiation intensities. In case of Lok-1 cultivar, the maximum effectiveness value (0.43) has been marked at 40kR and lowest (0.14) at 10kR of gamma irradiation doses. Similarly, in WH-147 variety, the highest value (0.47) of mutagenic effectiveness was observed in 35kR and 40kR however the lowermost value (0.14) was detected in 5kR gamma irradiated populations. The higher doses of gamma irradiations proved to be more effectual in instigating the mutagenic effectiveness as compared to lower doses. After the data was compared, the response to the mutagenic effectiveness among the two cultivars was perceived greater in the WH-147 cultivar than the Lok-1 cultivar.

Mutagenic efficiency is the actual ratio of rate of mutations displayed in M2 populations to various biological injuries revealed in M1 populations. The experimental data on efficiency of gamma irradiation doses with respect to injuries occurred in various biological traits is also shown in Table-1. The efficiency of gamma irradiation doses revealed distinct trend depending on the parameter taken under consideration for its assessment and this variable propensity also holds similar for different gamma ray treatments in case of calculating the degree of efficiency (Figure-1).

The efficiency calculated on the basis of pollen sterility was found, in general, higher (1.55) which is followed by the efficiency of lethality (0.77) with that of meiotic aberrations (0.66) and injury (0.40) in variety Lok-1. Similarly, in case of WH-147 cultivar, the maximum efficiency was displayed in terms of pollen sterility (1.26) followed by meiotic abnormalities (0.71), lethality (0.56) and injury (0.19). In general, the mutagenic efficiency recorded in present case showed a variable trend with respect to irradiation doses for all the parameters (Table-1, Figure-2).

Table-1: Mutagenic efficiency and effectiveness of gamma irradiation in two cultivars of bread wheat (*Triticum aestivum* L.).

Irradiation dose	Lethality (L)	Seedling injury (I)	Pollen sterility (S)	Meiotic aberrations (M)	Percent M2 plants mutated (Mf)	Mutagenic effectiveness Mf/dose	Mutagenic efficiency			
							Mf/L	Mf/I	Mf/S	Mf/M
Var. Lok-1										
5kR	-0.73	0.14	0.67	1.97	1.36	0.27	-1.87	0.71	2.03	0.69
10kR	0.73	3.03	1.56	5.49	1.38	0.14	1.89	0.45	0.88	0.25
15kR	0.73	9.10	1.54	3.42	2.74	0.18	3.76	0.30	1.78	0.80
20kR	-	11.30	2.93	6.41	5.67	0.28	-	0.50	1.94	0.89
25kR	9.42	27.98	5.25	18.44	8.76	0.35	0.93	0.31	1.67	0.48
30kR	13.04	38.32	7.93	22.37	12.69	0.42	0.97	0.33	1.60	0.57
35kR	27.53	44.18	10.11	23.00	13.95	0.40	0.51	0.32	1.38	0.61
40kR	48.55	53.27	12.60	23.49	17.07	0.43	0.35	0.32	1.36	0.73
50kR	53.62	59.96	15.52	20.77	19.83	0.40	0.37	0.33	1.28	0.95
Pooled Mean	15.29	24.91	5.81	12.70	8.34	0.32	0.77	0.40	1.55	0.66
Var. WH-147										
5kR	3.55	9.46	0.71	1.45	0.68	0.14	0.19	0.07	0.95	0.47
10kR	4.96	15.57	2.05	3.38	2.08	0.21	0.42	0.13	1.02	0.62
15kR	7.80	31.53	3.39	5.66	6.34	0.42	0.81	0.20	1.87	1.12
20kR	9.22	41.24	4.25	9.41	7.41	0.37	0.80	0.18	1.74	0.79
25kR	13.48	53.97	6.80	17.38	9.23	0.37	0.69	0.17	1.36	0.53
30kR	22.70	62.48	10.80	22.97	13.39	0.45	0.59	0.21	1.24	0.58
35kR	27.66	68.28	15.07	23.73	16.53	0.47	0.60	0.24	1.10	0.70
40kR	36.88	72.19	17.53	24.88	18.85	0.47	0.51	0.26	1.08	0.76
50kR	52.48	75.47	21.96	25.77	20.83	0.42	0.40	0.28	0.95	0.81
Pooled Mean	19.86	47.80	9.17	14.96	10.59	0.37	0.56	0.19	1.26	0.71

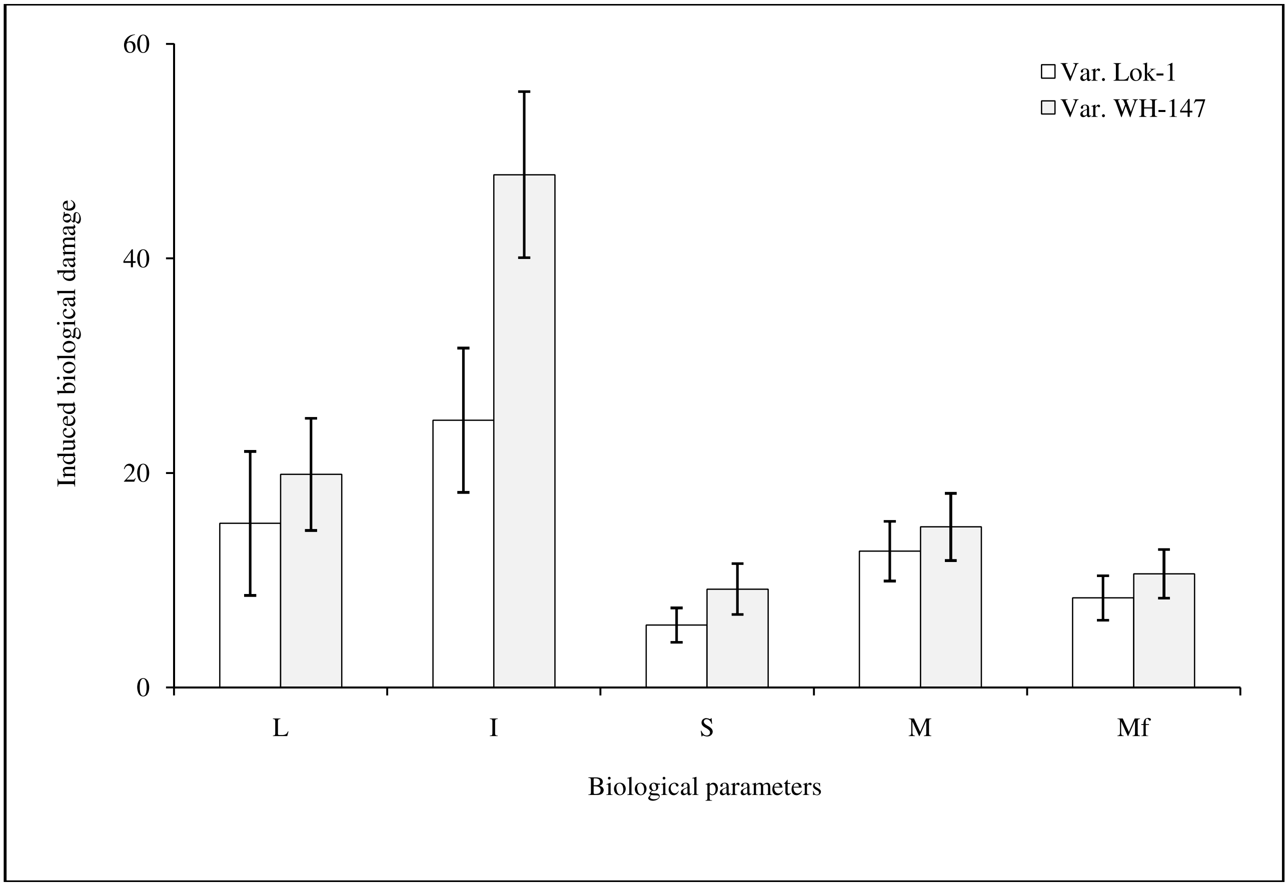

Figure-1: Gamma irradiation induced biological damage in two cultivars of *Triticum aestivum* L.

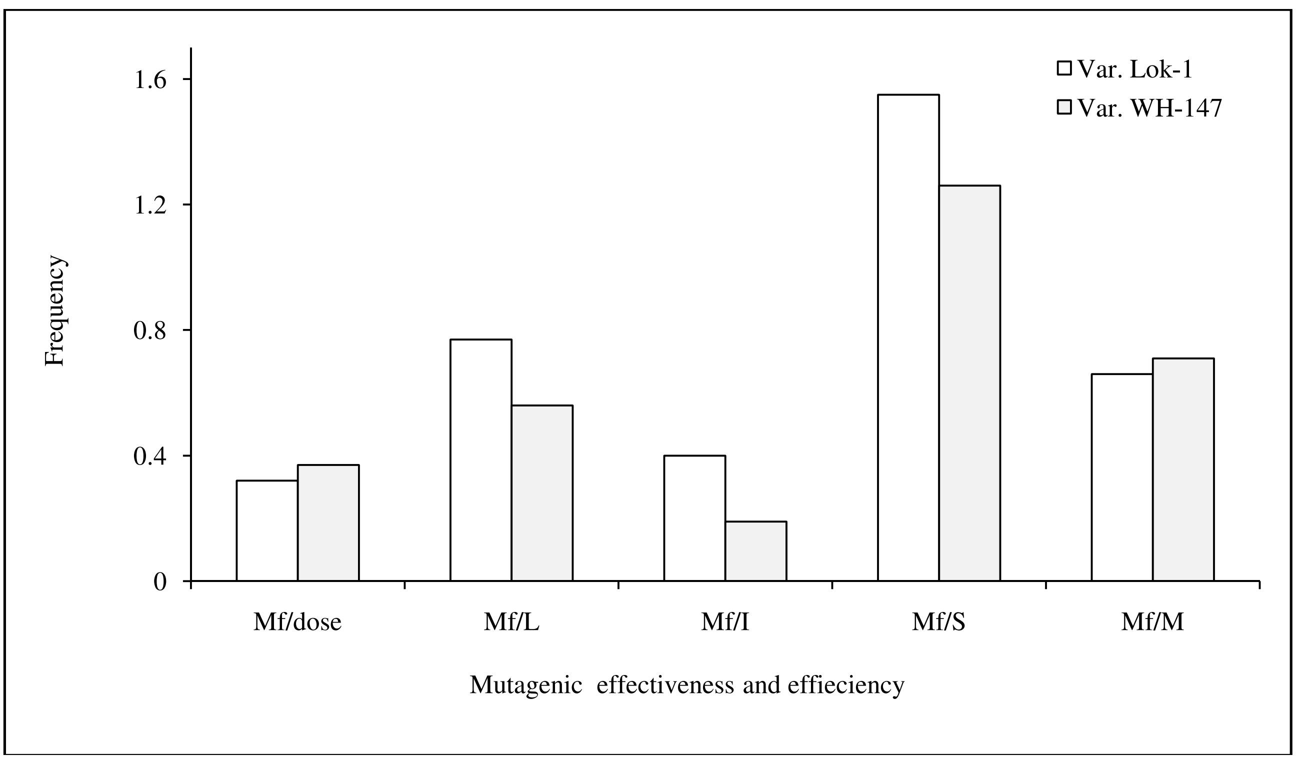

Figure-2: Frequency of mutagenic efficiency and effectiveness of gamma irradiation doses in two cultivars of *Triticum aestivum* L.

Numerous studies were made previously by various workers in order to estimate the efficiency as well as effectiveness of different mutagenic agents[10-16]. During the present investigation, the magnitude of efficiency and effectiveness indicated deviations between the irradiation doses. As a result, the median gamma ray doses demonstrated to be most prominent in inducing mutations. The reduction in effectiveness at lower irradiation doses may be ascribed to the insignificant stimulation of any kind of micro mutations at DNA level such as transitions or transversions. As already mentioned above, it is mostly accepted that polyploids with their repeated arrays of gene dosages are less sensitive to mutagenic treatments than their diploid counterparts. Therefore, the higher doses ranging from 25kR to 40kR are more effective in inducing mutagenic efficiency. The gamma irradiation doses often led to production of desirable chromosomal interchanges[17] that causes formation of new gene combinations and ultimately affects phenotype of plant. It could be well stated here that gamma irradiations could be significantly utilized to a greater level for creating mutations in common wheat germplasm as majority of its cultivars released so far through the technique of induced mutagenesis principally belong to gamma irradiations.

Conclusion

Conclusively, an outline of the above discussion indicates that the two cultivars of bread wheat can be efficiently utilized for production of mutant population through gamma irradiations which in turn could be beneficial for further breeding programs. In addition, the irradiation doses ranging from 25kR to 40kR are here recommended for generating the rapid morphogenetic variability as well as isolating favourable phenotypes in the presently investigated cultivars of bread wheat (*Triticum aestivum* L.).

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