Gamma irradiation induced chromosome aberrations in meiotic cells of bread wheat (*Triticum aestivum* L.)

ABSTRACT

Background and Objectives: Due to the ever-increasing human population and rapid urbanization, the available agricultural land becomes limited for the production of food grains. As a result, there is an urgent need for creating genetic variability upon which improved varieties could be made. The technology of induced mutagenesis plays an essential role in inducing genetic variations among crop species where hybridization is very challenging. Therefore, the present study has been designed to assess the mutagenic impact of gamma irradiation on the cytological behaviour of *T. aestivum* L in M1 and M2 generation.

Materials and Methods: Healthy seeds of *Triticum aestivum* var. Raj-1482, were treated with various gamma irradiation doses at BARC, Mumbai. Treated seeds were sown in field followed by morphological and meiotic characterization. For meiotic study, immature spikes were fixed in Carnoy's solution for 24 hours. Squash technique and 2% acetocarmine were used for cytological preparation.

Results: The results displayed a progressive elevation in the chromosomal aberrations along with a significant influence on seedling emergence, plant survival and pollen fertility. The most frequent meiotic aberrations detected include chromatin clumping, univalents and early chromosome segregations at metaphase and lagging chromosomes, chromatin bridges and chromosome clumping at anaphase/telophase while unorientation, scattering, polarity disturbances and micronuclei were also noticed. In addition, the frequency of these chromosome aberrations significantly decreased in M2 generation depicting the reclamation in genomic structure.

Conclusion: The results revealed that the higher treatment doses are toxic whereas lower doses of gamma irradiations seem quite beneficial in generating promising traits with less toxicity.

Significance of study: The present investigation supports the discovery that gamma irradiations are very effective in creating rapid genetic variability in crop plants including *Triticum aestivum* which could be essentially exploited for future mutation breeding programmes. In this study, the cytotoxicity has increased along with gamma irradiation doses and therefore, the genetic structure of the selected bread wheat cultivar is highly affected, which will possibly create new favorable genetic changes in the following generations that would be useful for plant breeders for its improvement.

Keywords: Gamma irradiation, chromosome aberrations, meiosis, mutations, mutagen, Triticum aestivum, crop improvement

1. INTRODUCTION

Induced mutagenesis, for its well-known status in modifying the genetic structure of the crop plants, can be exploited as a principal tool in pertaining desirable traits in different plant species including wheat. The technology of mutation induction via physical and chemical mutagens has become are substantial approach in mutation breeding programmes to enrich the current germplasm and to improve specific traits in cultivars. In addition, mutation induction aims to rapidly broaden the genetic pool of important crop plants for their future improvement. Several physical mutagens, for example, *x*-rays, gamma rays and particle radiation, have got much attention from the past few decades as the most effective mutagenic agents in higher plants. At present, gamma irradiations are one of the most used mutagens due to the lower cost (easy availability) and better efficiency (higher penetration into matter) compared to other mutagenic agents [1]. The gamma rays generally induce DNA lesions by producing free radicals and lead to the formation of chromosomal rearrangements which can modify important chemical components and physiological processes of plant cells [2]. In addition to gross chromosomal aberrations, gamma rays also cause point mutations in the specific genes which intensify the polygenetic changes in segregating generations and significantly increase the choice for selection [3]. Therefore, mutation induction in crops gives a better opportunity for rising favourable plant genotypes through effective selection methods among mutagen treated populations. Numerous efforts were made before for isolating populations with desirable agronomic mutations affecting yield linked as well as seed related traits in various cereal species including wheat [4].

Bread wheat (*Triticum aestivum* L.), belonging to the family Poaceae, is a self-pollinating allohexaploid grass species (BBAADD) that possesses the homoeologous genome combination of three diploid ancestral species. Bread wheat is one of the most extensively adapted food crops globally, providing approximately 30% of global grain production and about 20% of the calories consumed by the human population [5]. In India, it is the second most important cereal crop and plays a vital role in the food and nutritional security of the country [6]. The creation of high-yield cultivars is one of the necessary targets of modern wheat breeding programs worldwide due to the ever-increasing global human population and narrow land for agricultural expansion. Bread wheat, being a polyploid, offers several opportunities for generating mutations, genetic recombinations and thus increasing polygenetic variations in quantitatively inherited traits. The presence of a 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 generated after 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 [3]. Assessment of the clastogenic potential of a mutagen by scrutinizing the induced meiotic anomalies signifies an efficient way to quantify the cytotoxicity by the mutagen. Therefore, investigations on cytological abnormalities and their consequences on the genetic system of a species become a fundamental part of most mutation breeding studies [3,7,8].

The present investigation was undertaken to examine the mutagenic effect of gamma irradiations in bread wheat in terms of assessing the chromosome aberrations in meiotic cells and their subsequent impact on seedling emergence, survival as well as pollen fertility. The present study is useful in identifying and understanding the suitable gamma irradiation dose for inducing rapid genetic variations in this cereal crop and can facilitate the genetic improvement of varieties with high yield.

2. MATERIAL AND METHODS

2.1 Study area

The present study was carried out at Department of Botany, Shri JJT University, Jhunjhunu, Rajasthan from October 2019 to May 2021.

2.2 Methodology

Dry and healthy seeds of *Triticum aestivum* var. Raj-1482, procured from the market were treated with various doses (10kR, 20kR, 30kR, 40kR and 50kR) of gamma irradiation at Bhabha Atomic Research Center (BARC), Mumbai. For each gamma rays treatment, 300 seeds along with a control of both the cultivars were used. Treated seeds were sown in the field in a complete randomized block design in triplicate with 100 seeds per replicate. Recommended agriculture practices were followed throughout the cropping period. The data on seedling emergence and plant survival was collected after three and six weeks of sowing, respectively. The data on seed germination was recorded right from the emergence of the first shoot in each treatment including control. The recorded data were subjected to statistical analysis as outlined by Williams and Abdi [9] to assess the extent of induced variations.

2.3 Cytological studies

For cytological studies, young panicles of about twenty five randomly selected from both M1 and M2 plants (for each replicate) separately during the morning hours were fixed in freshly prepared Carnoy's solution (absolute alcohol and acetic acid in the ratio of 3:1) for at least 24 h and subsequently transferred to 70% alcohol. Anthers were squashed in 2% acetocarmine and the chromosome behaviour at different meiotic stages in pollen mother cells was analyzed. Traces of ferric chloride were added to improve the stainability of chromosomes. The chromosome aberrations were assessed in more than 1000 meiocytes for determining the percent abnormality.

2.4 Pollen fertility

Pollen fertility was calculated in 10 randomly selected plants per irradiation dose. The pollen grains from freshly dehisced anthers were also stained with 2% iron acetocarmine. Pollen grains that stained fully were considered to be fertile, while the empty, partially stained and shriveled ones were considered sterile. At least, ten slides per treatment were assessed and fertile pollens were counted and averaged and, consequently the fertility percentage was estimated.

3. RESULTS AND DISCUSSION

3.1 Seedling emergence and survival

From the data collected, the percentage of seedling emergence and plant survival showed continuous reduction as the gamma-ray doses increased (Table 1). Maximum germination was recorded at control (92.67%) which declined from 91.67% (10kR) to 64.33% (50kR) in M1 generation and 91.67% (20kR) to 85.67% (50kR) in M2 generation. For plant survival, irradiation doses ranging from 30-50kR were having more adverse impact on the survival of plants. This finding has been previously observed in wheat, where the irradiated seeds showed a decline in germination and total survival capacity to the non-irradiated seeds [10-14]. This biological behaviour suggests harmful effects of mutagens on genes that control this trait, by inhibited DNA repair mechanisms, deceleration of mitotic division in meristematic cells, and deleterious impact on other metabolic processes involved in germination and growth processes. In addition, the decrease in seed germination and survival caused by gamma radiation might be due to their injurious effects on genetical and

cytological processes coupled with the changes induced in physiological and biochemical processes. The impact of gamma irradiation on seedling emergence and survival was found higher in M1 generation than in M2 generation. This might be the result of genomic repairment in M2 plants followed by the normal functioning of metabolic processes.

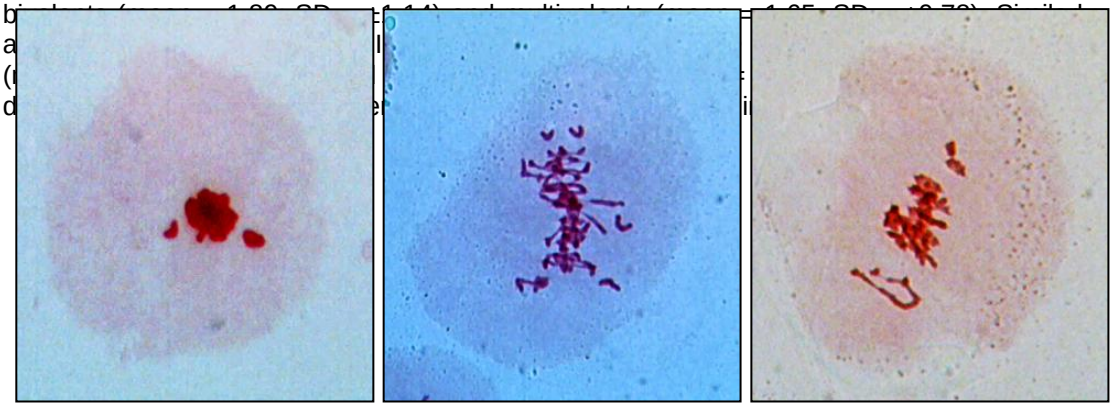
Table 1. Effects of gamma irradiation doses on seedling emergence and plant survival of *T. aestivum* L. in M_1 and M_2 generation

Treatment	Germination (Mean ± SD)	Plant Survival (Mean ± SD)	Pollen fertility (%)
0kR	92.67 ±1.25	100.0 ±1.25	96.35
M_1 generation			
10kR	91.67 ±1.70	98.18 ±2.16	92.91
20kR	87.67 ±2.62	95.44 ±2.05	89.70
30kR	83.33 ±3.40	89.60 ±3.40	86.93
40kR	78.00 ±2.94	85.47 ±2.05	82.89
50kR	64.33 ±3.30	80.31 ±2.05	74.41
M_2 generation			
10kR	92.67 ±1.25	100.00 ±1.25	95.93
20kR	91.67 ±1.25	99.27 ±1.41	95.02
30kR	91.00 ±0.82	97.80 ±0.82	92.90
40kR	87.33 ±1.70	96.18 ±2.16	90.03
50kR	85.67 ±1.70	96.11 ±1.25	90.41

3.2 Cytological studies

By using gamma irradiation, an extensive spectrum of meiotic abnormalities was observed in meiotic cells of wheat in M1 and M2 populations. The presence of a high spectrum of chromosomal aberrations in microsporocytes revealed that gamma irradiations have the substantial potential of modifying the genetic structure of *T. aestivum* L. It was observed that, the rate of aberrant cells increased along with the gamma irradiation dose. Though the range of aberrations was more or less similar for all the treatments, there was a substantial difference in the frequency of particular anomalies. Gamma irradiation effects on chromosomes in meiotic cells were previously witnessed in different plants including wheat [2,3,6,7,15]. Cytological investigations in mutation studies for analyzing the mitotic or meiotic behavior are supposed to be one of the effective indices to estimate the strength of any mutagen.

The frequency of chromosomal aberrations gets elevated from 6.66% (10kR) to 38.79% (50kR), and 3.0% (10kR) to 17.49% (50kR) in M1 and M2 generation, respectively. In this study, the irregularities in chromosome behavior of meiotic cells were recorded as Total Abnormality Percentages (TAB%) and subsequently summarized in Table-2. In M1 generation, frequent chromosome abnormalities in meiotic cells at metaphase I/II observed include chromatin stickiness (mean = 3.08; SD = ±1.89), precocious movement of chromosomes (mean = 2.24; SD = ±1.38), univalents (mean = 2.19; SD = ±1.27), stray bi



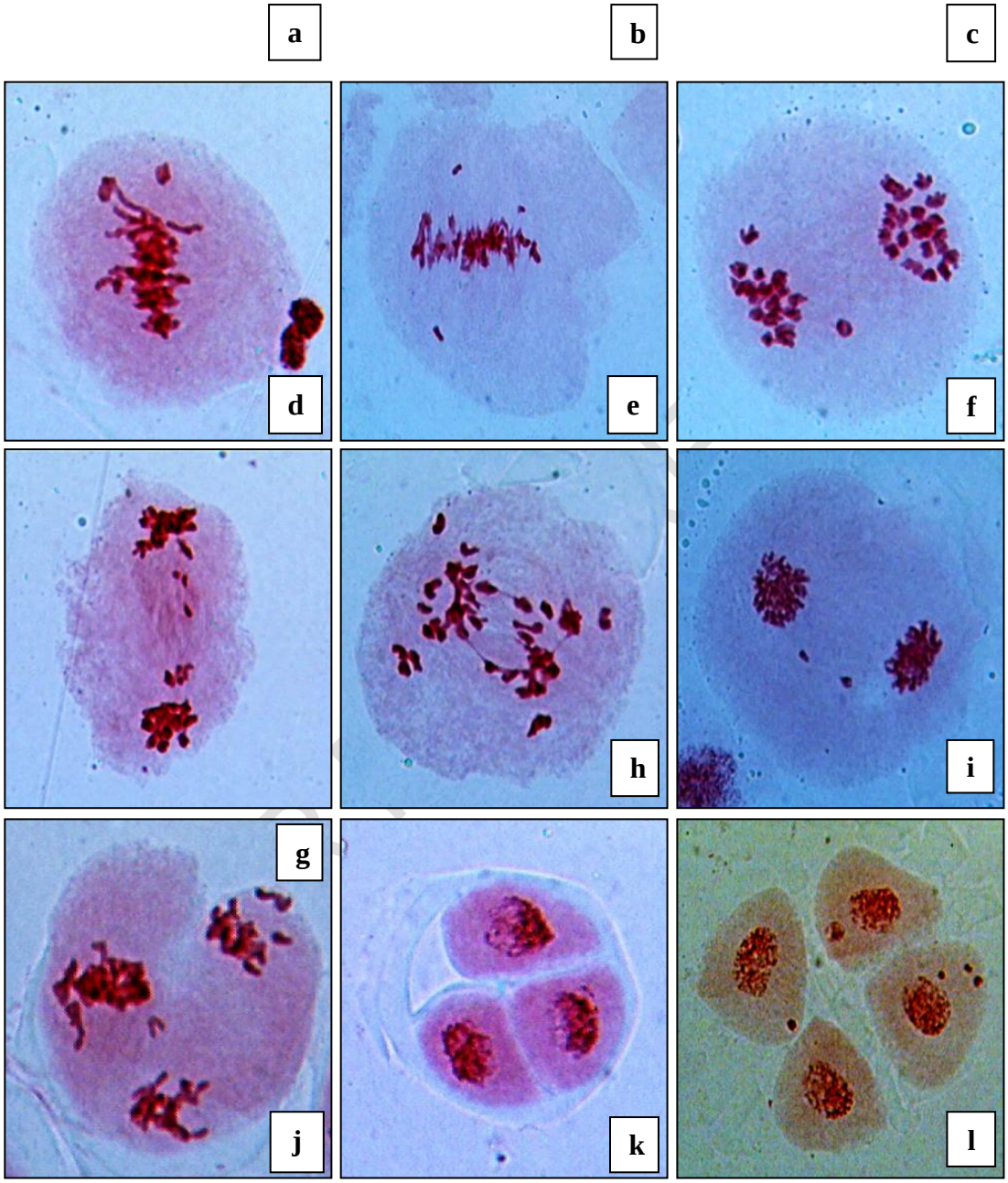


Fig. 1a-l. Meiotic cells of *T. aestivum* L. after gamma rays treatments. **a)** Chromosome stickiness **b)** Univalents **c)** Multivalent **d)** Unoriented chromosomes **e)** Precocious segregation **f, g)** Lagging chromosomes **h)** Multiple chromatin bridges **i)** Acentric fragments **J)** Disturbed polarity **k)** Triad **l)** Tetrad with micronuclei. Scale bar = 10µm.

Table 2. Frequency of aberrations in the meiotic cells of *T. aestivum* L.in M_1 and M_2 progenies after gamma irradiation treatments.

Treatment	Frequency of abnormal PMC's at metaphase-I/II (%)						Frequency of abnormal PMC's at anaphase/telophase-I/II (%)							Total abnormality percentage (TAB)
	PMC's scored	Uni.	Mult.	Stick.	Prec.	Stray.	PMC's scored	Lag.	Frag.	Brid.	Stick.	Dis.	Mic.	
0kR	1122	-	-	0.27	-	-	1023	0.39	-	0.29	0.20	-	-	1.15
M_1 generation														
10kR	1092	0.27	0.46	1.10	0.37	0.55	1046	1.24	-	1.05	0.57	0.19	0.86	6.66
20kR	1038	1.35	1.06	1.35	1.54	1.06	1079	2.22	0.37	2.04	1.11	0.28	1.30	13.68
30kR	1042	2.59	1.92	2.78	1.73	1.54	1035	3.48	0.58	3.09	2.03	0.97	1.84	22.55
40kR	1055	3.03	2.27	3.89	3.22	2.56	1058	4.16	0.76	3.88	2.74	1.23	2.27	30.01
50kR	1019	3.73	2.55	6.28	4.32	3.73	1089	5.23	0.92	4.68	3.40	1.56	2.39	38.79
M_2 generation														
10kR	1042	-	0.29	0.86	-	-	1081	1.11	-	0.65	-	-	0.09	3.00
20kR	1072	0.28	0.75	1.12	0.47	0.37	1038	1.25	-	1.06	0.29	-	0.67	6.26
30kR	1063	0.47	1.03	1.13	0.56	0.85	1022	1.86	0.29	1.76	1.08	0.29	1.17	10.49
40kR	1086	1.01	1.47	1.75	1.10	1.29	1018	2.26	0.29	2.36	1.38	0.59	1.67	15.17
50kR	1048	1.05	1.62	2.10	1.15	1.34	1075	2.60	0.19	2.88	2.05	0.74	1.77	17.49

Abb.: Uni. = Univalents, Mult. = Multivalents, Stick. = Stickiness, Prec. = Precocious segregation, Stray. = Stray bivalents, Lag. Laggards, Frag. = Fragments, Brid. = Bridges, Dis. = Disturbed polarity, Mic. = Micronuclei

polarity (0.85), and acentric fragments (0.53) were also observed as predominant abnormalities at anaphase and telophase I/II in M1 generation. In M2 generation, the dominant abnormalities observed at metaphase include stickiness (mean = 1.39; SD = ±0.46) and multivalents (mean = 1.03; SD = ±0.48) whereas laggards (mean = 1.82; SD = ±0.57), bridges (mean = 1.74; SD = ±0.82) and micronuclei (mean = 1.07; SD = ±0.63) showed constant dominance.

Chromosome stickiness at metaphase I/II (Fig. 1a) is found in most of the mutation studies although its proper and significant cause is still unknown. It seems that chromosome stickiness results from changes in specific non-histone proteins (topoisomerase II and peripheral proteins) that are essential components of the chromosome and they have a significant effect on the separation and segregation of chromatids. In addition, chromosomal breakage has also been proposed to be a conspicuous origin for clumping between the chromosomes [2]. Univalents, marked among gamma-irradiated progenies (Fig. 1b), arise from partial or complete lack of homologous chromosome pairing [2]. The normal pairing at diakinesis revealed that most of the unpaired chromosomes observed at metaphase I must have resulted from desynapsis, rather than asynapsis.

The multivalents observed (Fig. 1c) may have occurred as a result of breakage followed by an exchange of chromosome segments among non-homologous chromosomes as has been reported in many earlier studies [16,17]. In addition, chromosome stickiness occasionally led to multivalent associations. The unoriented or stray chromosomes (Fig. 1d) may have been observed due to disturbances of the spindle activity which causes anomalous chromosome alignment during metaphase [3]. The early polar movement of chromosomes (Fig. 1e) may have occurred due to desynaptic mutation or failure of chiasma to hold homologues as well as early chromosome disjunction.

At anaphase and telophase I/II, the dominant aberration observed was laggard formation (Fig. 1f, g). Lagging of chromosomes may be either due to attachment to very weak spindle fibre or no attachment at all. Kolar et al. [18] opined that due to the effect of mutagens, spindle fibres failed to carry the chromosome movement towards poles properly and resulting in the lagging chromosome appearing at anaphase. In addition, delayed terminalization, the stickiness of chromosome ends and failure of chromosomes segregation has led to laggard chromosome formation. Anaphasic bridges were also found as the dominant anomaly at anaphase (Fig. 1h). Bridges are more generally due to stickiness of the chromosomes at metaphase or breakage and reunion of a chromosome. Badr et al. [19] opined that chromatin bridges at anaphase or telophase are the product of unequal exchange of dicentric chromosomes. The chromosome number taking part in exchange determines the number of bridges. Paracentric inversions also lead to the occurrence of chromatin bridges along with acentric fragments (Fig. 1i)

Disturbed polarity observed (Fig. 1j) might have occurred due to spindle disturbances and the presence of high frequency of univalents [3]. Here in the present case, stickiness and the presence of a multivalents could also be a possible cause of disturbed polarity in the cells. In disturbed polarity, the PMCs could lead to the formation of tripolarity due to failure of chromosome disjunction at anaphase in meiosis II. Sometimes triads (Fig. 1k) instead of tetrads are formed due to disturbed polarity and abnormal anaphasic disjunction. Micronuclei found in tetrads (Fig. 1l) are caused due to various meiotic anomalies at metaphase and anaphase. Mostly, the laggards and unoriented chromosomes (whether univalents of bivalents) fail towards the poles during telophase stages and subsequently constitute micronuclei.

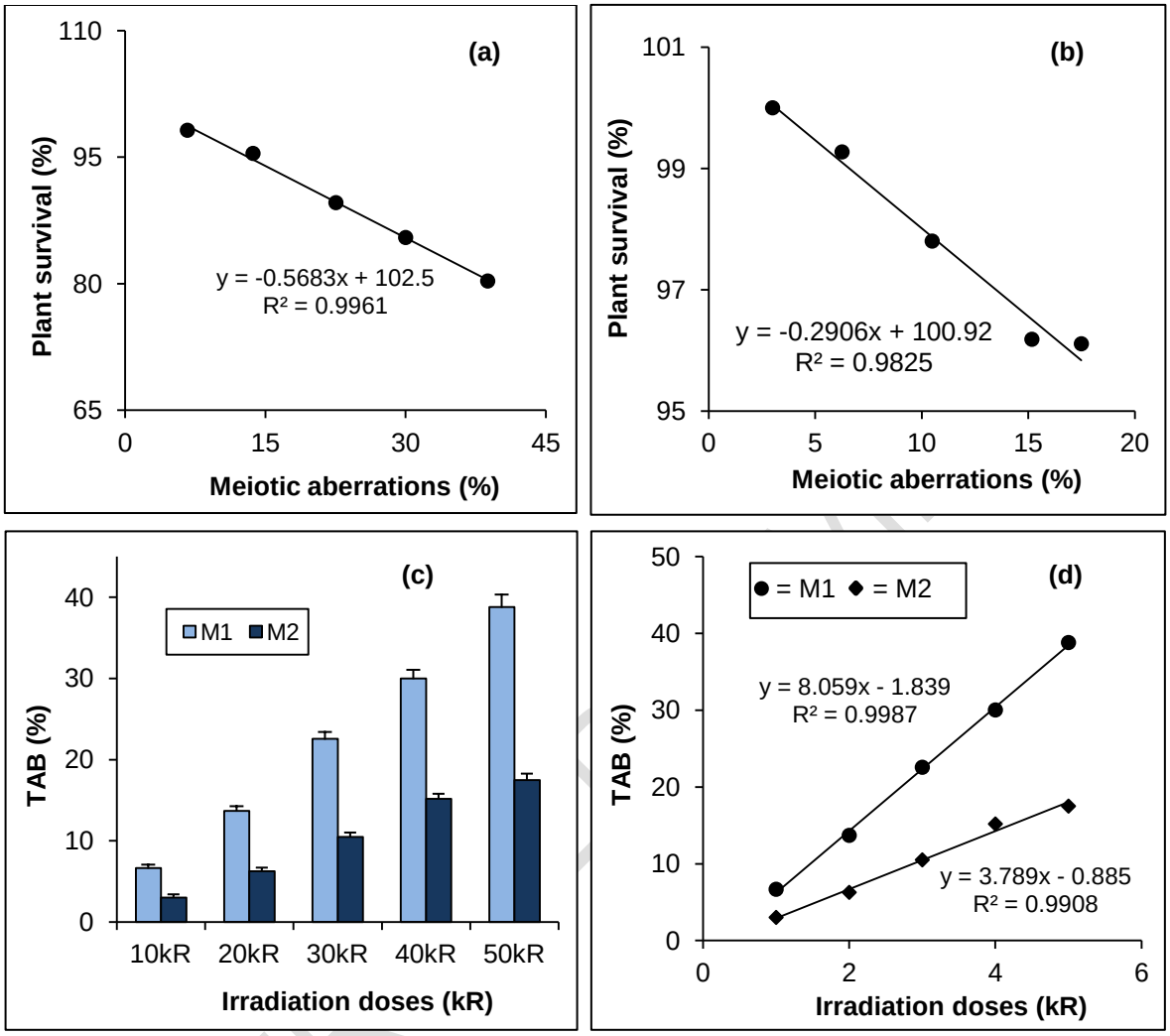


Figure 2(a-d): Graphical representation of various statistically analyzed parameters. Correlation between meiotic aberrations and plant survival in M1 (a) and M2 (b) generation. Comparative account of Total Abnormality Percentage after gamma irradiation treatment in meiotic cells of common wheat in M1 and M2 generations (c, d).

In this study, we observed a highly significant and negative correlation between the total chromosome aberrations and plant survival percentage in both M1 (r^2 = 0.9961) and M2 (r^2 = 0.9825) generations (Fig. 2a-b). This reveals that the increased percentage of meiotic aberrations observed here in mutagenic treatments became a significant cause of reduction in the survival percentage of wheat plants. The comparative trend of TAB (%) against gamma irradiation treatments in bread wheat in M1 (r^2 = 0.9987) and M2 (r^2 = 0.9908) generations was presented by Fig. 2 (c, d). Pollen fertility has been found decreased in mutagenic populations. The reduction in pollen fertility is chiefly attributed to genomic damage caused by huge array of meiotic aberrations induced by gamma rays.

4. CONCLUSION

In this study, the effect of gamma irradiations on seed germination, plant survival and meiotic behaviour of bread wheat was studied in M1 and M2 generation. The gamma rays decreased the seed germination and plant survival while increased the chromosome aberrations in meiocytes. Comparatively, the higher doses showed deleterious impact on growth due to severe cytogenetic damage. The impact was found higher in M1 generation than M2 generation.

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