Chapter

Gamma Irradiation as Tool for Mutation Breeding in Wheat

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Abstract

Mutation breeding is used to modify a specific character of a plant, while all other characteristics remain the same. Adaptation obtained through mutation breeding to biotic (disease and insect pest resistance) and abiotic (aluminum toxicity, drought, high temperature, salt tolerance) stresses leads to better harvest growth, yield and quality. The main aim is to promote the efficiency of energy conversion into growth as a tool for the prediction of the optimal gamma irradiation dosage for mutation breeding in wheat. Cytogenetic analysis done on *Triticum turgidum* ssp. *durum* cv. Orania will be presented in the form of nucleolar activity to determine incomplete mitosis as well as in the form of bridges, fragments, micronuclei and ring chromosomes that will be compared with the efficiency of energy conversion into growth. Studies done on two *Triticum aestivum* cultivars, namely Ratel and Kwartel, included observation of double spikes, reduction in fertility and determination of the window for the optimal dosage for mutation breeding. Cultivars/breeding lines that are more resistant to gamma irradiation have a wider window for the optimal dosage range for mutation breeding. The ideal gamma irradiation dosage range for the three cultivars, namely, Orania, Ratel and Kwartel were determined.

Keywords: chromosomal abnormalities, double spike, efficiency of energy conversion into growth. fertility, gamma irradiation, ideal dosage range for mutation breeding, incomplete mitosis, mitotic index, mutation breeding, nucleolar activity, sterility

1. Introduction

Mutation breeding is used to modify a specific character of a plant, while all other characteristics remain the same. Adaptation to biotic (disease and insect pest resistance) and abiotic (aluminum toxicity, drought, high temperature, salt tolerance) stresses leads to better growth, yield and quality of the harvest. Better resistance has been obtained for rust, powdery mildew and *Fusarium* head blight resistance [1–7]. Higher yield had been obtained due to increased salt, drought and aluminum tolerance [8–12]. Higher yield combined with better spaghetti quality in *Triticum turgidum* ssp. *durum* L. [13, 14] and better bread baking quality including amino acid composition changes in *Triticum aestivum* L. [15–21] has also been obtained. Dwarf and semi-dwarf mutations, induced by irradiation, reduced fall over of wheat [22–24]. Enhancement

of androgenesis and plant regeneration has also been obtained by making use of gamma irradiation [25].

Gamma irradiation is widely used as a modification agent for improving genetic diversity in agriculture for breeding purposes due to its high penetration ability. Gamma irradiation of kernels is regularly performed as a method to induce mutations [26, 27]. Its exploitation in agriculture is limited due to uncertainty in the dosage of irradiation, which varies for different crops and applications [28, 29]. However, mutation induction treatment is convenient because large quantities of kernels can be irradiated in one session, and irradiated kernels can easily be stored and shipped [30].

Research has shown that high gamma irradiation dosages given to kernels slow down the physiology of the seedlings resulting in lower growth rate. Modest stress can be absorbed by the plants without the flexibility capability being restricted [31]. It is still unclear what causes the slower growth rate [32]. Various studies have been undertaken to document the effects of gamma irradiation on the development and growth of seedlings. Large differences in shoot and root lengths among Basmati rice cultivars were observed after gamma irradiation of the seed [33]. Root growth was also found to be more sensitive than shoot growth in gamma irradiation of onion seed [34] and wheat seed [35].

Interphase chromosome volume and DNA content per chromosome influence the sensitivity to gamma irradiation. Different diploid species have demonstrated different sensitivities to gamma irradiation [36]. Interphase chromosome volume had a positive association with the ranking of resistance to gamma irradiation in *Triticum monococcum* L. and various *Aegilops* species [37, 38]. However, a negative correlation was found to exist between DNA content per chromosome and the LD_{50} values for ten species of plants by Baetcke et al. [39]. In contrast, Degani and Pickholtz [40] could not find a correlation between nuclear volume and irradiation sensitivity. Evaluations of low gamma irradiation dosages on the development of seedlings are well-studied. Hormesis is a term used to describe the positive response or stimulus of biological systems by low doses of an agent that is poisonous when the dosages increase. Hormesis can also be defined as "any physiological effect that occurs at low doses which cannot be anticipated by extrapolating from toxic effects noted at high doses" [41]. Adaptive protection that follows low-dosage irradiation causes DNA damage prevention, repair and immune stimulation in animals [42]. An increase in shoot growth in *Triticum turgidum* ssp. *durum* L. was observed at 50 Gy gamma irradiation by Von Well et al. [43]. This is supported by an increase in mitotic index in root apical meristem at 50 Gy gamma irradiation [43]. Comparable results were observed in hexaploid wheat with low gamma irradiation dosages increasing and high dosages leading to retardation in plant growth and development [44, 45]. Inter-cultivar differences to gamma irradiation were observed for the growth parameters.

Polyploid wheat species are, as a norm, more resistant to gamma irradiation in comparison to diploid wheat. The effect of gamma irradiation is dependent on and described according to what you want to irradiate. A gamma irradiation dosage that stimulates growth in a tetraploid wheat, while decreasing growth in a diploid wheat, can be described as low with regards to the tetraploid and medium to high in the diploid [46]. Genetic redundancy is the accumulation of genomes and together with different combinations of genomes that differ in susceptibility/resistance to gamma irradiation make the polyploid species more resistant. Differences in gamma irradiation resistance between diploid and polyploid species of the same genus as well as differences among these species and cultivars of the same species are well documented [47–53].

There are two methods used to predict the ideal gamma irradiation dosage for mutation breeding: (1) 50% growth reduction of seedling height (GR_{50}) and (2) M_{max}. Both are used to obtain the ideal dosage when dormant kernels with a moisture content of 14% are given gamma irradiation. GR_{50} is generally used because it is easy to be determined. In wheat (grown in sowing trays), shoot height is measured when seedlings are 10 to 14 days old with a shoot height of 11–20 cm. Measurements are taken from soil level to the end of the primary leaf. Studies over the years have shown that GR_{50} predicts a value that is higher than ideal. In *Triticum turgidum* ssp. *durum* L. the predicted ideal gamma irradiation values by using GR_{50} is 350 Gy – 500 Gy, but better results with mutation breeding were obtained by using gamma irradiation dosages of 150 Gy – 300 Gy [54]. Secondly, determination of M_{max} is a different viewpoint for the prediction of the ideal dosage for mutation breeding by using the following equation [55]:

$$M_{max} = k_m D_{max} \tag{1}$$

where the maximum mutation rate (M_{max}) is the product of mutation rate per unit dose (k_m) and the maximum dosage applicable (D_{max}).

M_1 trades that are measured for the determination of M_{max} are germination rate, seedling growth, root growth, survival rate, number of spikes and seed fertility. Higher mutagen dosages given to an object result in more severe damage. These injuries become fatal and place a threshold to the increase in dosage that leads to the prediction of the value of D_{max} [55].

We created the "efficiency of energy conversion into growth" as a predictor of the ideal dosage for mutation breeding to replace seedling height (GR_{50}) and the M_{max} [35]. It makes use of a reduction in growth as well as the amount of energy (reserved food in the caryopsis) used to obtain that growth. Respiration rate measures the effect of an external factor on the efficiency of energy usage for growth. It is calculated by making use of the following formula [56]:

$$\begin{aligned}\text{Respiration rate} = {} & \text{initial kernel dry weight} - (\text{actual dry weights of the shoot} + \text{roots} \\ & + \text{caryopsis during a specific period of growth})\end{aligned} \tag{2}$$

Respiration rate is dependent on metabolic activity that decreases with an increase in gamma irradiation dosage and can, therefore, not be used to determine the ideal dosage for mutation breeding [35]. The efficiency of energy conversion into growth can be calculated using the following formula:

$$= \frac{(\text{combined shoot and root dry weight}) - (\text{original embryo dry weight})}{(\text{original caryopsis dry weight}) - (\text{actual dry weight of caryopsis at a particular point of time})} \tag{3}$$

By dividing the actual growth rate by the energy used to obtain the specific growth rate, the decreased metabolic activity due to increased gamma irradiation dosages does not influence the prediction of the efficiency of energy conversion into growth. The (GR_{50}) makes only use of shoot length growth, while the efficiency of energy conversion into growth makes use of shoot length and width growth as well as root growth, making the efficiency of energy conversion into growth more sensitive to

gamma irradiation [35]. The growth period for the determination of the efficiency of energy conversion into growth is taken from 60 hours (when active growth of roots and shoots and therefore energetic metabolic activity have commenced) to 132 hours (recovery of the lower gamma irradiation dosages have taken place and deterioration of the higher dosages) of growth.

A threshold value determines the maximum damage that meristematic cells can handle for complete repair of the cells. Above the threshold value incomplete repair takes place and the damaged cells may stop dividing and may even be eliminated. The efficiency of energy conversion into growth depends on the effectiveness of the recovery process. Full repair of meristematic cells leads to an initial decrease in the efficiency of energy conversion into growth, followed by a recovery of the efficiency of energy conversion into growth. As the percentage of meristematic cells that are repaired and actively divide during mitosis of the cell division cycle decreases, so does the efficiency of energy conversion into growth decreases. To measure the damaging effect of the gamma irradiation, the effect on firstly: nucleolar activity, chromosomal abnormalities (bridges, fragments, ring chromosomes, and micronuclei), mitotic activity and incomplete mitosis as studied in tetraploid *Triticum turgidum* ssp. *durum* L. cv. Orania will be discussed. Secondly, the effect of gamma irradiation on double spikes, fertility and mutations concerning resistance to stem rust in two *Triticum aestivum* L. cultivars (Ratel and Kwartel) will be discussed. The relationship between the efficiency of energy conversion into growth and the above-mentioned characteristics will be discussed as well as the determination of the ideal dosage range for mutation breeding.

2. Nucleolar activity in *Triticum turgidum* ssp. *durum*

Nucleolar activity during interphase can be used as a measurement of metabolic activity [57–61]. Nucleolar organizing regions (NORs) are responsible for the synthesis and processing of ribosomal RNA (rRNA) in the nucleolus. Nucleoli are prevalent in interphase cells, from telophase (**Figure 1a, b**) to late prophase (**Figure 1c, d**), during which time active transcription of ribosomal genes (rDNA) occurs [62–64]. During the S phase, the DNA goes through a process of unfolding and DNA replication takes place. This puts a temporary halt on rDNA transcription. After DNA replication, rDNA transcription recommences in S phase. Due to the unfolding of the DNA, rDNA loci that have been inactive and completely folded during the G_1 phase can now become active, as observed in the activation of the 5D NORs by Von Well and Fossey [57] in *Triticum aestivum* L. after DNA replication. Most of the rDNA transcription takes place during S and G_2 phases. During early prophase the rDNA transcription decreases and terminates during late prophase. At this stage, the nucleoli are not discernible because of the disassociation of the nucleolar subcomplexes. During late telophase, the nucleolar subcomplexes reassemble and rRNA transcription resumes [62–67].

NOR activity differs between the A, B and D genomes as well as between chromosomes of the same genome in the wheat polyploid complex. The allopolyploidization process was accompanied by asymmetric epigenetic modification and elimination of certain rDNA sequences between different donor genomes to produce stable allopolyploid wheat with increased differentiation and diversity [68, 69]. These changes lead to the deletion of part of the 1A and 5A NORs so that the 1A NOR is inactive in the polyploid species and the 5A NOR is not functional in the polyploid species. In the tetraploid *T. turgidum* ssp. *durum* L. (AABB) the NOR of 1B is dominant over 6B,

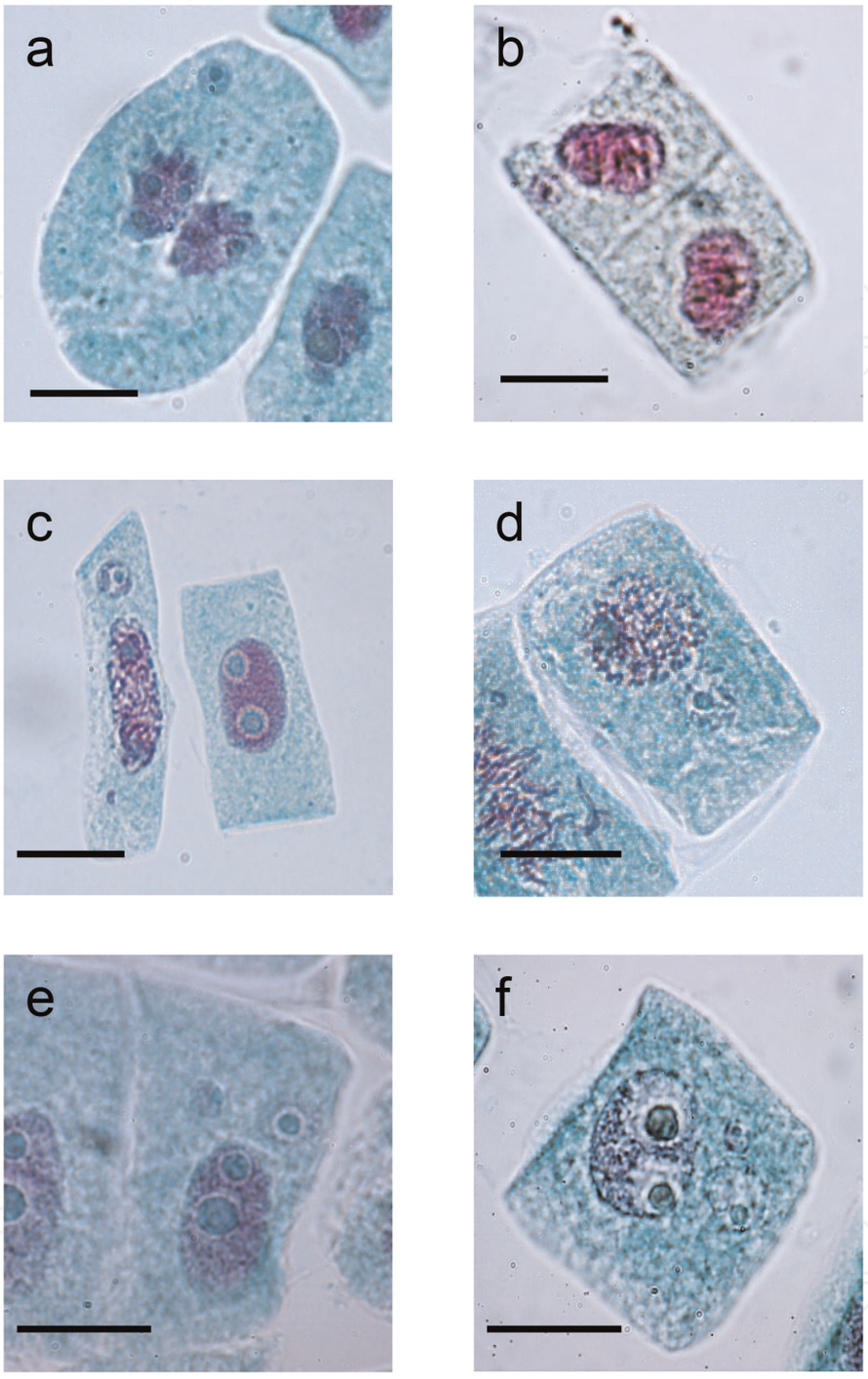

Figure 1.
Nucleoli in the nucleus and in micronuclei in Triticum turgidum *ssp.* durum *cv. Orania. Nucleoli in micronuclei during telophase (a, b). Nucleolus in micronucleus during early prophase (c, d). Large fused and medium sized nucleoli in nucleus and one medium and one small-sized (1A) nucleoli in separate micronuclei (e). Two large nucleoli in the nucleus, as well as a medium size nucleolus in a micronucleus and one small nucleolus (1A) in a micronucleus (f). Bar = 10 μm.*

while the 1A NOR remains dormant due to methylation of cytosine of the NORs. A maximum of four nucleoli are therefore present in *T. turgidum* ssp. *durum* L, [70, 71]. The genes that are responsible for the inactivation are located on the short arm of chromosome 1A, the long arm of chromosome 6B and at least 13 other nonnucleolar chromosomes. The methylation process and the function of the genes that are part of

it is still unclear [71]. Intergenic ribosomal spacer variability in length and repetitions can also play a role in activity of different NOR sites [72, 73].

The effect of gamma irradiation on nucleolar activity was studied in *Triticum turgidum* ssp. *durum* L. cv. Orania. NOR's are fully functional in micronuclei and the NOR on chromosome 1A is active in micronuclei (**Figure 1e, f**). This study showed that all the NOR sites on chromosomes 1B, 6B and 1A are active in micronuclei. This also showed that the number of nucleoli formed by the 1B and 6B NORs in the nucleus could be used to observe incomplete mitosis.

Nucleolar activity at higher dosages is associated with a drop in the number of nucleoli than observed in control, indicating a decline in rDNA transcription in these cells (**Figure 2**). The decrease in metabolism/growth and cellular activity is also reflected in retarded onset of mitosis in irradiated material [74, 75].

The appearance of the Chromosome 1A nucleoli is only a qualitative observation since no markers for the 1A NOR site were used. We can only conclude that it is active since we observed more than eight and a maximum of 11 nucleoli per cell. The NOR on chromosome 1A is active in the micronuclei since it does not have to compete with the longer intergenic ribosomal spacers that occur in more repetitions on chromosomes 1B and 6B [73]. The major NORs on chromosomes 1B and 6B have adjacent regions distal to them that may play a crucial role in their autoregulation as well as silencing of minor NORs [76].

Micronuclei with an active nucleolus indicate that the proteins that bind to the DNA to keep it unfolded after DNA replication are present in the NOR sites as well as the proteins that make up the rDNA transcription complex. Eight percent of micronuclei contained nucleoli over the whole spectrum of dosages, indicating that chromosomal breakages occurred at the rate for chromatin regions containing NOR sites as well as for regions without NOR sites.

The activation process of dormant 1A NORs in micronuclei can be explained by: (1) 1A NORs are activated in the absence of suppressor genes that are responsible for methylation of the RDNA as well as in the absence of NORs with a competitive advantage [71, 73, 77]. Activation of the dormant 1A NORs takes place during the S phase after DNA replication in the micronuclei. The findings of Klein and Grummt

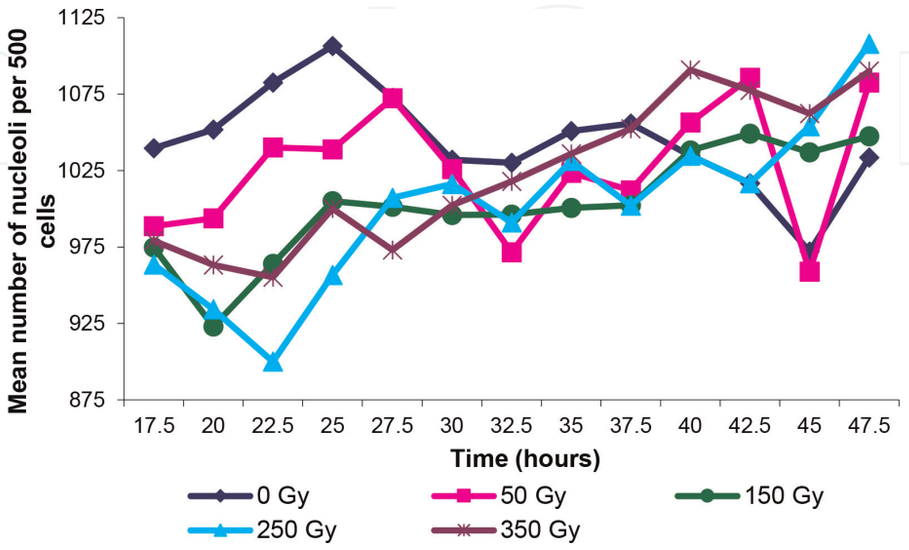

Figure 2.
Mean number of nucleoli in nuclei and micronuclei combined in 500 interphase cells in Triticum turgidum *ssp.* durum *cv. Orania.*

[66] that most of the rDNA transcription takes place during S and G_2 phases of the cell cycle supports the activation of 1A NORs during the S phase. The newly synthesized DNA remains unmethylated due to the absence of suppressor genes and remains unfolded by the binding of regulatory proteins to promoters and A Repeats in intergenic regions. This forms the platform for the binding of transcription factors and polymerase I so that rDNA transcription can be initiated [78].

3. Mitotic index and incomplete mitosis in *Triticum turgidum* ssp. *durum* L.

The mitotic index (MI) measures mitotic activity and is positively or negatively affected by gamma irradiation. The effect of gamma irradiation on MI and incomplete mitosis was studied in *Triticum turgidum* ssp. *durum* L. cv. Orania. The onset of mitosis was retarded in all the gamma irradiation dosages (50 Gy, 150 Gy, 250 Gy and 350 Gy) in comparison to the control as indicated by the dosage by time interaction on the total number of nucleoli and the mitotic index (**Table 1**; **Figure 3**). This may be due to checkpoints in the G_2 phase for the repairing of damaged DNA before the cells enter mitosis, as observed in studies of the effect of irradiation treatments on cancer cells [79–81]. There are two G_2 checkpoints for the repair of damaged DNA. The first checkpoint is for the repairing of cells that are in the G_2 phase during the irradiation treatment and is temporary and dosage independent. The second checkpoint is for the repairing of cells that are in G_1 and S phases during irradiation treatment and is dosage dependent. The accumulation of cells in G_2 takes place later than the accumulation due to the first checkpoint. Accumulation due to both checkpoints was observed in the present study. This study is the first to report on the second checkpoint in plant material.

The onset of mitosis was differently affected by the DNA damage that needed to be repaired because of the gamma irradiation spectrum. Fifty Gy gamma irradiation treatment was less affected by the retardation effect of the first G2 blockage, due to fewer cells needing to be repaired (**Figure 3**). This is supported by (1) a higher MI and (2) higher nucleolar activity at 50 Gy as well as (3) a larger number of micronuclei containing nucleoli and a larger number of micronuclei per cell containing nucleoli in the higher dosages. The first G_2 checkpoint led to a later peak in prophase cells at almost the same time in 150 Gy and the higher irradiation dosages compared to the control and 50 Gy (**Figure 4**). Another study in wheat also found a decrease in NOR activity associated with a decrease in MI [82]. The dosage-dependent second G_2 block resulted in a second increase and peak in prophase at different times for the different irradiation dosages as well as the increase in the number of nucleoli at 35 hours.

Incomplete mitotic division (where the DNA content of the nucleus is twice as much as normal after mitosis) occurs in all the gamma irradiation dosages. There is an increase with an increase in irradiation dosage [83]. This is supported by the occurrence of up to six nucleoli per nucleus and up to eleven nucleoli per cell (**Figures 4** and **5**). The number of nucleoli above four (1B NORs +6B NORs = 4 nucleoli) in the nucleus is unlikely to be activated by the NOR sites on 1A, as previously explained. Incomplete mitosis is also supported by the highly significant reduction in cells in telophase when comparing the control with the irradiation dosages (**Figure 4**). The time and time by dosage interaction on MI can also be responsible for the reduction of cells in telophase (**Table 1**). In a similar study, an increase in prophase and a decrease in anaphase and

Source	DF	Type 1SS	Mean Square	F Value	Pr > F
Nucleoli in nucleus and micronuclei combined					
Block	4	4094.7815	1023.6954	0.97	0.422
Time	12	230241.5323	19186.7944	18.25	<0.01
Dosage	4	85009.0892	21252.2723	20.22	<0.01
Time x Dosage	48	329029.3908	6854.7790	6.52	<0.01
Mitotic index					
Block	4	357.3200	89.3300	0.59	0.672
Time	11	41989.5467	3817.2315	25.09	<0.01
Dosage	4	1213.1533	303.2883	1.99	0.096
Time x Dosage	44	23959.4867	544.5338	3.58	<0.01
Incomplete mitosis					
Block	4	2.776	0.694	1.13	0.34
Dosage	4	108.816	27.204	44.28	<0.01
Time	9	27.636	3.071	5.00	<0.01
Dosage × Time	36	75.664	2.102	3.42	<0.01

Table 1.
Two-way ANOVA showing sources of variation for the average number of nucleoli in the nucleus and micronuclei combined and for the mitotic index in Triticum turgidum *ssp.* durum *cv. Orania.*

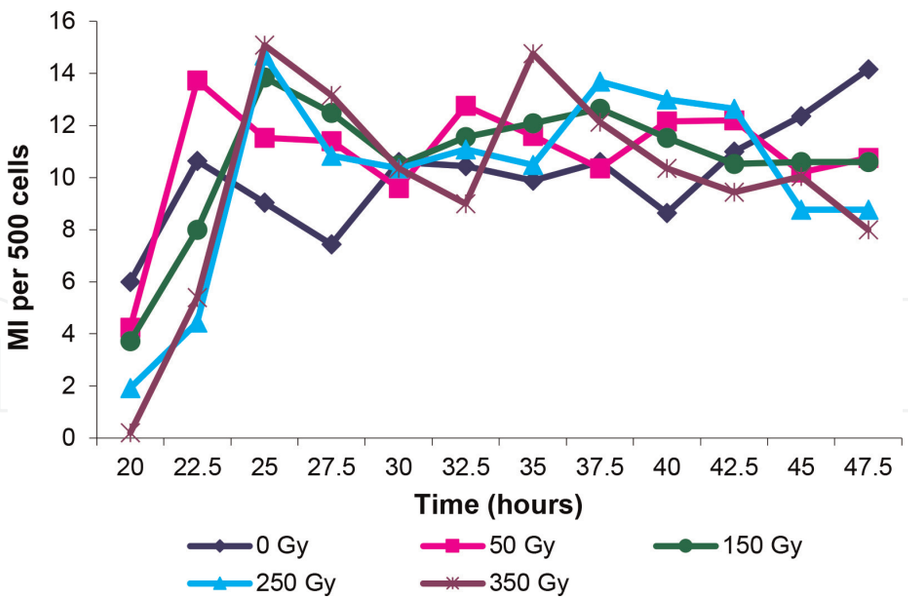

Figure 3.
Mitotic index at specific times at different gamma irradiation dosages in Triticum turgidum *ssp.* durum *cv. Orania.*

telophase together with cells with twice the normal amount of chromatin were observed which supports this study [84].

A decrease in growth at high gamma irradiation dosages can be attributed to: firstly, reduced mitotic activity partly caused by radiation-induced senescence

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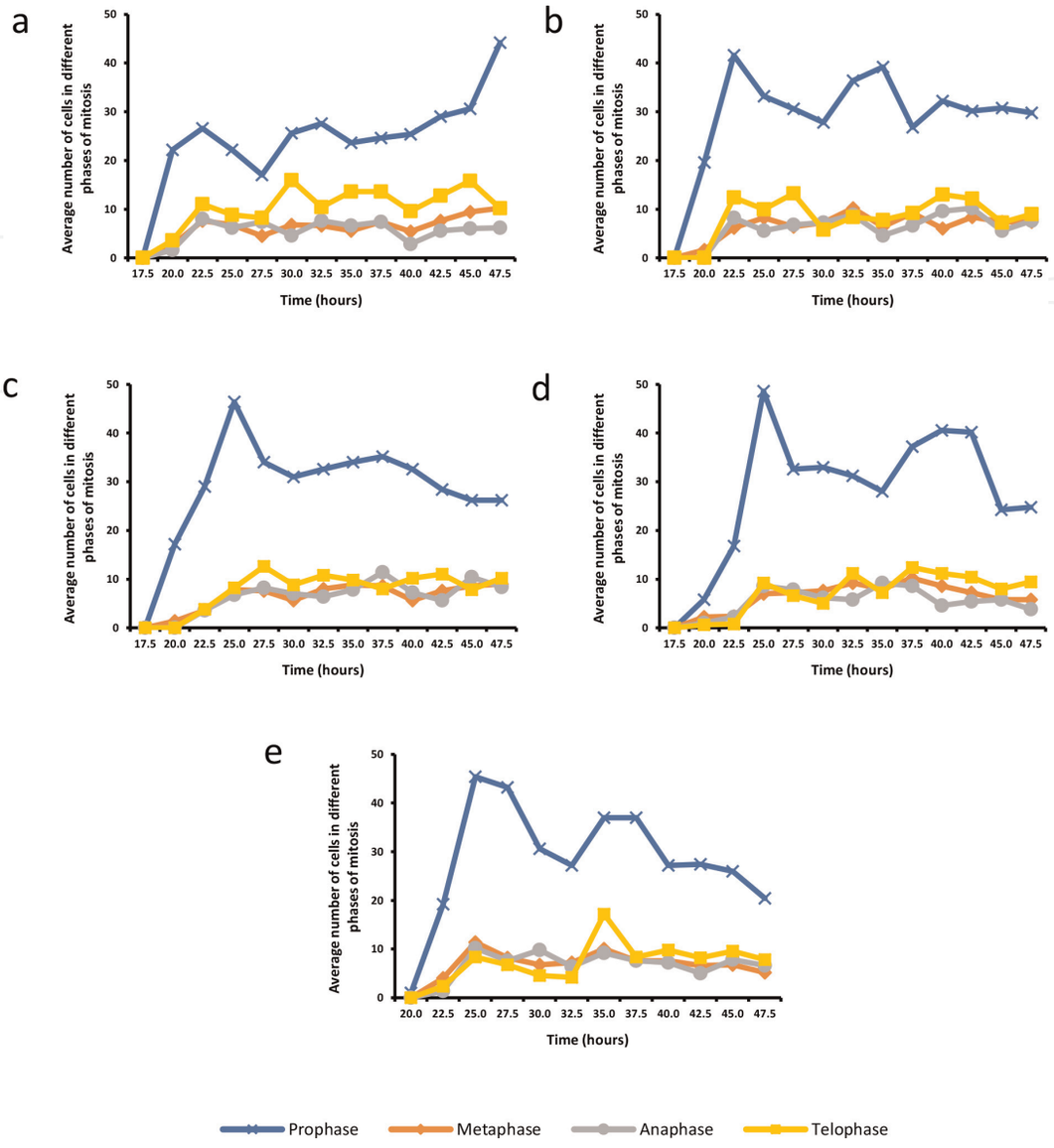

Figure 4.
Frequencies of cells in mitosis in (a) 0 Gy, (b) 50 Gy, (c) 150 Gy, (d) 250 Gy and (e) 350 Gy gamma irradiated material in Triticum turgidum *ssp.* durum *cv. Orania.*

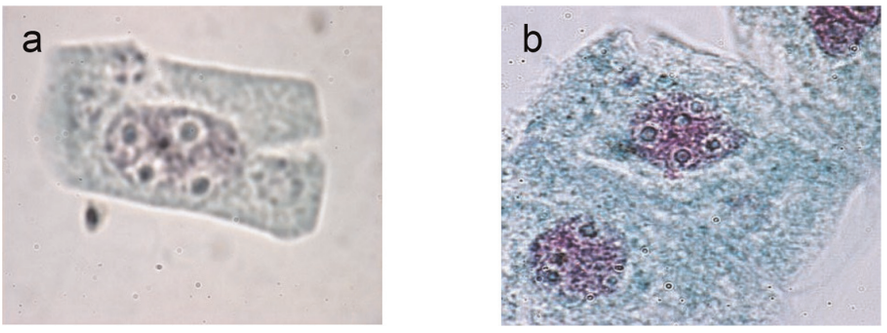

Figure 5.
Nucleoli in interphase cells in Triticum turgidum *ssp.* durum *cv. Orania. (a) Five nucleoli in the nucleus as well as small nucleoli in micronuclei. (b) Five nucleoli in the nucleus. Bar = 10 μm.*

(a condition of permanent cell cycle arrest induced by irradiation) in meristematic tissues and secondly, mitotic catastrophe (cell death, which arises after cells that are not able to perform correct mitosis enclose their condensed metaphase chromosomes in many small chromatin fragments [85]). Mitotic catastrophe takes place over a period and was not present during the cytogenetic investigation period, as seen in the occurrence of interphase cells with twice the number of chromosomes than normal cells as well as no observed fragmentation of chromatin. Mitotic catastrophe can occur over a few days [45, 86–89].

Avanzi and Deri [90] determined the durations of the mitotic cycle in two *Triticum turgidum* ssp. *durum* L. cultivars as 14 hours for Aziziah and 14 hours and 15 minutes for Capelli. Kaltsikes [91] measured a mitotic cell cycle duration of 13 hours and 45 minutes in a *Triticum turgidum* ssp. *durum* L. cultivar. According to the mitotic activity of the control, the control started with a second mitotic cycle (**Figure 4**). At the end of the 132 hours for the determination of the efficiency of energy conversion into growth, the control would have completed 6–7 mitotic cycles, while 50 Gy, 150 Gy, 250 Gy and 350 Gy would have completed 4–6 cycles. These cycles are enough for mitotic catastrophe to take place by means of apoptosis, as seen in the deterioration of the efficiency of energy conversion into growth as observed in 250 Gy and 350 Gy over time.

4. Cytogenetic abnormalities in *Triticum turgidum* ssp. *durum* L.

Gamma irradiation effects on chromosomal level, which can lead to physiological effects as well as cell death, are observed by means of cytogenetic analysis. The following abnormalities can be observed during mitosis in plants after X-ray and gamma irradiation: (1) stickiness and clumping of chromosomes, (2) diplochromosomes or pseudochiasma, (3) ring(s), (4) fragment(s), (5) bridge(s) with or without fragment(s), (6) micronuclei, (7) giant cells, (8) cellular shape deformities, (9) nuclear shape deformities, disrupted equatorial plate and (10) uncoiling chromosomes at metaphase [83, 92]. Over time micronuclei can be extruded from the cell, reincorporated in the nucleus, degraded or persisted in the cytoplasm of the cell. Ring chromosomes are also carried over separately from one cell cycle to another (**Figure 6**f; [93, 94]). These micronucleated cells may survive several cycles of mitosis or are eliminated by means of apoptosis [95].

The effect of gamma irradiation on the presence of chromosomal abnormalities was studied in *Triticum turgidum* ssp. *durum* L. cv. Orania. The presence of bridges (**Figure 6a, b**), fragments (**Figure 6c**), ring chromosomes (**Figure 6d–f**) and micronuclei (**Figure 6g, h**) because of chromatid and chromosome breaks due to gamma irradiation treatments were observed. Anaphase bridges (**Figure 6a**) result from chromosome (when cells are in S and G_2 phase) and chromatid (when cells are in G_1 phase) breaks caused by gamma irradiation/X-rays that join as sticky ends resulting in chromosomes with two centromeres. These bridges break in late telophase and can be observed as broken bridges (**Figure 6b**). Acentric fragments (**Figure 6c**) are chromosomes without centromeres and become micronuclei at late telophase when nucleus membranes are formed. There is a highly significant increase in bridges and micronuclei at 150 Gy, 250 Gy and 350 Gy in comparison to 50 Gy (**Table 2**). Ring chromosomes originate by breaks in both chromosome/chromatid arms and increase at a lower level than bridges and fragments over the dosage spectrum. There is a highly significant increase at 250 and 350 Gy gamma irradiation in ring chromosomes in comparison with

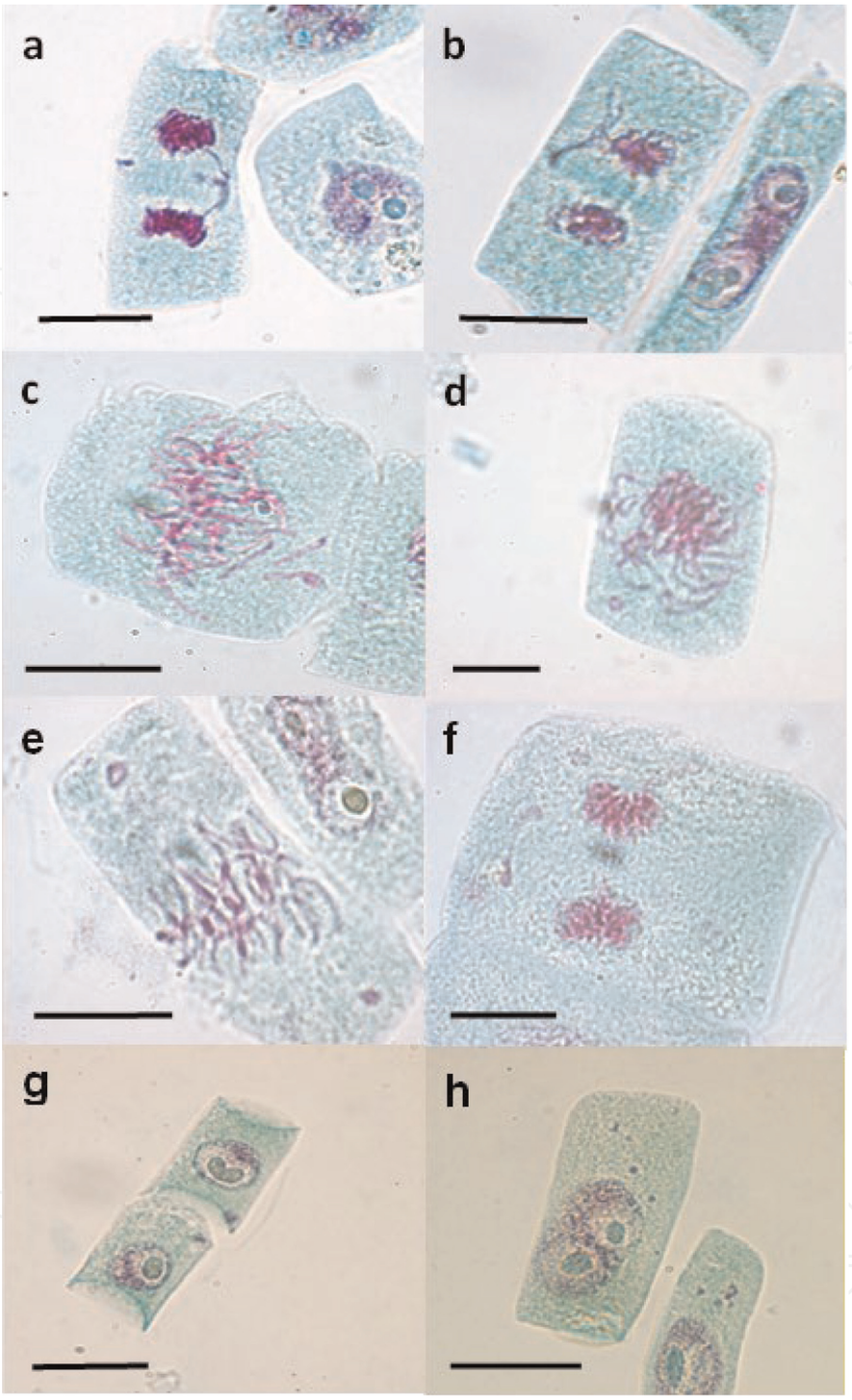

Figure 6.
Bridges, fragments, ring chromosomes and micronuclei in Triticum turgidum *ssp.* durum *cv. Orania. (a, b) bridges in telophase. (c) Fragments in metaphase. (d – f) ring chromosomes in prophase, metaphase and late anaphase, respectively. (g – h) micronuclei in interphase cells. Bar = 10 μm.*

50 Gy (**Table 2**). These results are supported by a study making use of eight winter wheat cultivars and gamma irradiation dosages of 100, 150, 200 and 250 Gy. The more susceptible cultivars had a larger reduction in mitotic activity and more chromosomal aberrations [96]. Oney-Birol and Balkan [92] also observed a decrease in mitotic index in two out of three bread wheat cultivars over a dosage range of 100 Gy–300 Gy gamma irradiation. Azer [97] concluded that a dosage less than 100 Gy was needed to induce

P values of least squares means comparison										
T. turgidum ssp. *durum* L. cv. Orania										
	Average number of Bridges					Average number of Ring Chromosomes				
Average:	**0.00**	**1.33**	**4.47**	**7.15**	**9.13**	**0.00**	**0.16**	**0.53**	**0.75**	**1.09**
Dosage (Gy)	**0**	**50**	**150**	**250**	**350**	**0**	**50**	**150**	**250**	**350**
0		0.03	<0.01	<0.01	<0.01		**0.26**	<0.01	<0.01	<0.01
50			<0.01	<0.01	<0.01			0.01	<0.01	<0.01
150				<0.01	<0.01				**0.13**	<0.01
250					<0.01					0.01
	Average number of Micronuclei					Average number of Interphase cells with Incomplete Mitosis				
Average:	**0.00**	**26.25**	**109.36**	**200.36**	**315.67**	**0.00**	**0.12**	**0.24**	**1.22**	**1.64**
0		<0.01	<0.01	<0.01	<0.01		**0.44**	**0.13**	<0.01	<0.01
50			<0.01	<0.01	<0.01			**0.44**	<0.01	<0.01
150				<0.01	<0.01				<0.01	<0.01
250					<0.01					0.01

Nonsignificant differences in bold p = 0.05 significant effect and p < 0.01 highly significant effect.

Table 2.
P values of pairwise comparisons of gamma irradiation dosage for average number of bridges, ring chromosomes, micronuclei and interphase cells with incomplete mitosis in Triticum turgidum ssp. durum *L. cv. Orania.*

50% abnormal cells in two bread wheat cultivars, while a dosage between 300 Gy – 400 Gy was needed to induce the same effect in another cultivar. Silva-Barbosa et al. (2005) [98] obtained small differences in the presence of micronuclei, bridges (dicentrics) and fragments in lymphocyte cultures of five healthy people after exposure to 0.08 Gy gamma irradiation. After exposure of identical lymphocyte cultures to 1.8 Gy large differences were observed in the presence of micronuclei, bridges (dicentrics) and fragments among the five healthy people's lymphocyte cultures.

Correlations between the effects of gamma irradiation on efficiency of energy conversion into growth on the one side and the frequency of cells with different numbers of bridges and the frequency of cells with different numbers of micronuclei on the other (**Table 3**) were done to determine the relatedness between the characteristics. The efficiency of energy conversion displayed highly significant correlations with two to three bridges per cell, with the highest correlation with three bridges per cell. The efficiency of energy conversion into growth had highly significant correlations with one to seven micronuclei, with the best correlation with four micronuclei per cell. These bridges and micronuclei are present at 250 Gy and 350 Gy. This is the irradiation dosage with a highly significant increase in incomplete mitosis in comparison with 50 Gy.

5. Double spike in *Triticum aestivum* L. in M_1 generation

Radiomorphosis, induced by different mutagens such as gamma irradiation, EMS and ^{32}P, are present at certain dosages that lead to infertility. These radiomorphs occur only in the M_1 generation and are not heritable. The mutagens can vary in the

Bridges	**Efficiency of energy conversion into growth**	
	P value	**Pearson's correlation coefficient**
1 Bridge per cell	0.06	−0.44
2 Bridges per cell	<0.01	−0.65
3 Bridges per cell	<0.01	−0.68
4 Bridges per cell	0.89	0.05
5 Bridges per cell	0.22	−0.53
Micronuclei	**Efficiency of energy conversion into growth**	
	P value	**Pearson's correlation coefficient**
1 Micronucleus per cell	<0.01	−0.59
2 Micronuclei per cell	<0.01	−0.77
3 Micronuclei per cell	<0.01	−0.83
4 Micronuclei per cell	<0.01	−0.86
5 Micronuclei per cell	<0.01	−0.84
6 Micronuclei per cell	<0.01	−0.77
7 Micronuclei per cell	<0.01	−0.62

Table 3.
Correlations between the effects of gamma irradiation on efficiency of energy conversion into growth on the one side and the frequency of cells with different numbers of bridges and the frequency of cells with different numbers of micronuclei on the other in Triticum turgidum ssp. durum *L. cv. Orania.*

abundance and types of radiomorphs that they induce [99–101]. The radiomorphs can take different forms, such as double spike, branched spike, branched tiller, double peduncle and double kernel. Plant species react differently to the various mutagenic treatments in the formation of radiomorphs [101]. Double spikes are observed at specific gamma irradiation dosages and absent in others in a specific cultivar [102].

These changes are due to physiological disturbance of cell materials or the rate of production of specific growth hormones due to physiological imbalances [103]. Growth hormone imbalances [101] that lead to the formation of radiomorphs and abnormal growth in plants may occur between auxins and cytokinins. Cell culture studies have shown that different ratios between auxins and cytokinins can lead to altered growth patterns. Auxins and Cytokinins determine the size of the meristematic regions in shoots and roots [104], shoot and root growth and development [105–107] as well as the formation of inflorescence [108–110]). Ahn et al. [111] have shown that an in vitro inflorescence can develop directly from a bud on the base of the first leaf derived from the plumule after hormone-induced formation in *Panax ginseng.*

The effect of gamma irradiation on the presence of double spikes in M_1 was studied in *Triticum aestivum* L. cultivars Ratel and Kwartel. V-Shaped double spikes were observed in Ratel at 200 Gy (**Figure 7**) and 250 Gy gamma irradiation, while only one V-shaped double spike was observed in Kwartel at 200 Gy (**Table 4**). Double spikes were observed at dosages where the shoot/root ratio was at its highest (**Table 4**).

Double spikes in association with sterility were also observed by Gill and Sethi [102] in one variety of *Triticum aestivum* L. at 500 Gy, by Larik [112] in one variety of

Figure 7.
Six V-shaped double spikes in Triticum aestivum *L. cv. Ratel in M_1 generation at 200 Gy gamma irradiation.*

	Ratel			Kwartel		
Dosage (Gy)	**150**	**200**	**250**	**150**	**200**	**250**
Number of double spikes	0	6	4	0	1	0
Shoot/Root ratio	1.19	1.22	1.31	1.12	1.14	1.04

Table 4.
Number of double spikes and shoot/root ratio in Triticum aestivum *L. cultivars Ratel and Kwartel.*

Triticum aestivum L. at 250 Gy, and by Din and Khan [113] in three wheat (*Triticum aestivum* L.) varieties at 350 Gy gamma irradiation treatment.

6. Fertility/sterility in *Triticum aestivum* L. in M_1 generation

Sterility, of which the number of seed set is the most used criterion in quantifying sterility, is another one of the eight parameters used to estimate the degree of plant injury in the M_1 generation. The most common manifestations of reduced fertility when reproductive structures are present in the M_1 are: (1) pollen is not viable or (2) fertilization occurs, but embryos abort before maturity. The sterility in the M_1 plants is caused by: (1) chromosome mutations, (2) gene mutations, (3) cytoplasmic mutations and (4) physiological effects [114].

Sterility/fertility in combination with seedling length, root length and chlorophyll mutations in M_2 plants were mostly used in studies for measuring the effects of the gamma irradiation dosages applied to seed when the emphasis is placed on sterility as measurement. Seedling length reduction and an increase in sterility did not display a good relationship with one another, and this may be the reason why sterility is seldom used as a parameter [114]. In a study of the effect of gamma irradiation on seedling height and fertility in *Triticum monococcum* L. var. *flavescens*a large reduction in

	Ratel				Kwartel			
Dosage (Gy)	**0**	**150**	**200**	**250**	**0**	**150**	**200**	**250**
Fertility (%)	100	99.70	93.38	89.22	100	93.24	84.23	82.50
Seedling height (%)	100	94.82	91.73	84.24	100	94.76	89.40	75.81
Efficiency of energy conversion into growth (%)	100	98.25	91.23	89.47	100	94.83	87.93	81.03

Table 5.
Fertility, seedling height and efficiency of energy conversion into growth as percentages in Triticum aestivum *L. cultivars Ratel and Kwartel.*

seedling height was obtained from 100 Gy (84,9% of control) to 200 Gy (14.4%) gamma irradiation with a reduction from 69–59% fertility respectively for the two dosages [115]. Sasikala and Kalaiyarasi [116] used shoot and root length and fertility for the determination of the effect of gamma irradiation in six rice cultivars. They obtained a decrease of up to 16.2% in shoot length at 100 Gy with a 45,7% decrease at 250 Gy, a decrease of up to 38.8% in root length at 100 Gy with a 63.7% decrease at 250 Gy and a decrease of up to 3.7% in fertility at 100 Gy with a 14% decrease at 250 Gy. Sakin and Sencar [117] used the detection of fertility of M_1 plants and chlorophyll mutations in M_2 plants to determine the efficiency of gamma irradiation treatment in two *Triticum durum* Desf. cultivars. They obtained a mutagenic efficiency of 2.80 with a sterility of 8.39% at 50 Gy gamma irradiation and a mutagenic efficiency of 3.08 with a sterility of 35.01% at 100 Gy in one of the cultivars. In the other cultivar, a mutagenic efficiency of 0.73 with sterility of 19.49% at 50 Gy was obtained.

The effect of gamma irradiation on sterility/fertility in M_1 was studied in *Triticum aestivum* L. cultivars Ratel and Kwartel. The percentage values of seedling height were most affected by the gamma irradiation dosages, followed by fertility and finally by efficiency of energy conversion into growth (**Table 5**). The reduction in fertility was accompanied by almost similar reductions in efficiency of energy conversion into growth.

7. Screening for resistance against wheat stem rust race Ug99 and determination of the ideal dosage range for mutation breeding

M_2 kernels of the two cultivars (Ratel and Kwartel) had been selected to be sent to Kenya to Chepkoilel Campus, University of Eldoret, Eldoret, Kenya for screening against stem rust (*Puccinia graminis* f. sp. *tritici*) race Ug99. A thousand M_2 kernels per entry were planted in rows according to the cultivars and the gamma irradiation dosages. Screening for resistance/susceptibility against Ug99 started at Zadoks growth stage 73 [118], and plants that were moderately susceptible, moderately resistant and resistant were recorded (**Table 6**).

Steps were taken for the determination of the ideal dosage for mutation breeding by making use of the efficiency of energy conversion into growth. In *Triticum monococcum* L. the 100 Gy and higher gamma irradiation treatments were not entangled with the control for the efficiency of energy conversion into growth. The 100 Gy treatment differed from the control with $p = 0.01$ in *Triticum monococcum* [35]. The 100 Gy determination for the optimal dosage for mutation breeding is in line with the suggested dosages (100 Gy – 200 Gy) for practical mutation breeding in

Treatment name	Resistant	Moderately resistant	Moderately susceptible
Ratel			
150 Gy		3	5
200 Gy		1	3
250 Gy		3	7
Kwartel			
150 Gy		4	6
200 Gy			1
250 Gy			1

Table 6.
Resistance/susceptibility scoring against stem rust race Ug99 in Triticum aestivum *L. cultivars Ratel and Kwartel, where the controls were susceptible.*

Triticum monococcum L. by the FAO/IAEA [54]. Firstly, the LSD value at p = 0.01 for *Triticum aestivum* L. cultivars Ratel and Kwartel must be determined. The LSD values were determined as 0.031 and 0.04, respectively. Secondly, the LSD value must be subtracted from the control values to obtain the x-axis value of the graph. Thirdly, a linear equation must be obtained by plotting the efficiency of energy conversion into

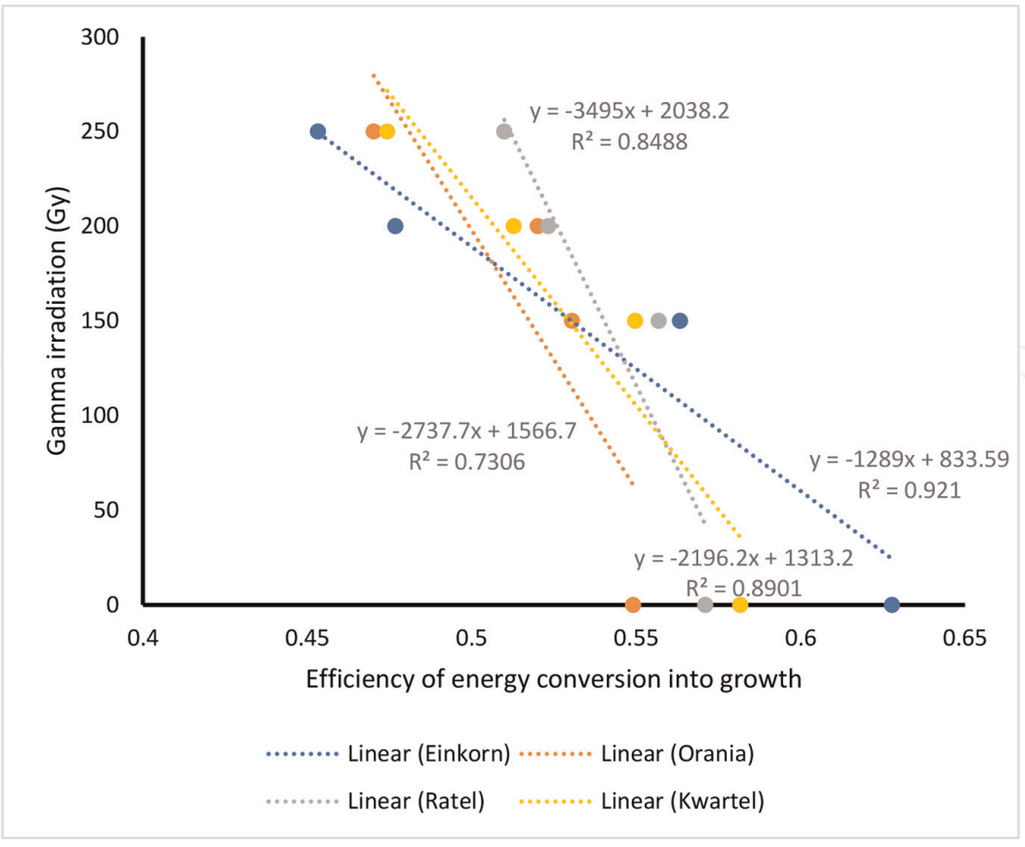

Figure 8.
Determining the ideal gamma irradiation dosage range for Triticum monococcum *L. cultivar Einkorn,* Triticum turgidum *ssp.* durum *L. cultivar Orania and* Triticum aestivum *L. cultivars Ratel and Kwartel.*

Cultivar	Ideal dosage range according to FAO/IAEA [54]	Ideal dosage range for mutation breeding	
		α = 0.01	**α = 0.00001**
Triticum monococcum L.	100–200 Gy		
Einkorn		100.85 Gy	157.30 Gy
Triticum turgidum ssp. *durum* L.	150–300 Gy		
Orania		151.25 Gy	237.45 Gy
Triticum aestivum L.	150–350 Gy		
Ratel		150.80 Gy	230.83 Gy
Kwartel		123.45 Gy	188.24 Gy

Table 7.
Ideal dosage range for mutation breeding in Triticum monococcum *L. cultivar einkorn,* Triticum turgidum *ssp.* durum *L. cultivar Orania and* Triticum aestivum *L. cultivars Ratel and Kwartel.*

growth values on the x-axis and the corresponding gamma irradiation dosage values on the y-axis (**Figure 8**). Fourthly, by making use of the equation, the ideal dosage for mutation breeding will be obtained (**Table 7**).

The ideal dosage range for mutation breeding was obtained by using α = 0.00001 for the determination of the upper limit for Ratel (**Table 7**). The value obtained of 230.83 Gy falls in the range of moderately resistant/susceptible plants obtained for Ratel. The optimal dosage range for *Triticum monococcum* L. cultivar Einkorn, *Triticum turgidum* ssp. *durum* L. cultivar Orania and *Triticum aestivum* L. cultivar Kwartel were determined accordingly (**Table 7**).

Triticum turgidum ssp. *durum* L. cultivar Orania displayed the best resistance against gamma irradiation with the widest ideal dosage range (86.2 Gy), followed by *Triticum aestivum* L. cultivar Ratel with the second widest dosage range (80.03 Gy), followed by Kwartel with the third widest dosage range (64.79 Gy) and finally *Triticum monococcum* L. cultivar Einkorn with the narrowest dosage range (56.45 Gy).

8. Conclusion

The efficiency of energy conversion into growth could be used to determine the optimal gamma irradiation dosage range for mutation breeding. *Triticum turgidum* ssp. *durum* L. cultivar Orania had the highest resistance to gamma irradiation and the widest optimal dosage range. The upper limit for *Triticum turgidum* ssp. *durum* L. cultivar Orania was beneath 250 Gy. This is the dosage where there is a highly significant increase in ring chromosomes and incomplete mitosis. *Triticum aestivum* L. cultivar Ratel had the second highest resistance to gamma irradiation and the second widest optimal dosage range, followed by Kwartel with *Triticum monococcum* L. cultivar Einkorn that had the lowest resistance to gamma irradiation and the narrowest optimal dosage range. The differences between Ratel and Kwartel are supported by the differences in seedling height, fertility and dosages where double spikes were present and the number of double spikes present at these dosages.

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