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The Relationship of the Efficiency of Energy Conversion Into Growth as an Indicator for the Determination of the Optimal Dosage for Mutation Breeding With the Appearance of Chromosomal Bridges, Ring Chromosomes, Micronuclei and Incomplete Mitosis After Gamma Irradiation of Kernels of Triticum Turgidum Ssp. Durum L. Cultivar Orania

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Abstract

Background

A 50% growth reduction of seedling height (GR_{50}) after acute gamma irradiation of dormant kernels is widely used as a measure of irradiation damage to obtain the ideal irradiation dosage for mutation breeding. It became clear in recent years that the GR50 is not sensitive enough to predict the ideal gamma irradiation dosage for mutation breeding and it predicts a value that is higher than ideal. The study aim was to determine whether root, shoot and seedling growth on the one side and the efficiency of energy conversion into growth on the other are measuring different growth retardation effects of gamma irradiation that are the result of DNA damage (bridges, ring chromosomes, micronuclei, incomplete mitosis) in *Triticum turgidum* ssp. *durum*. If the efficiency of energy conversion into growth is measuring different effects, the usefulness of efficiency of energy conversion into growth to predict the optimal dosage for mutation breeding will be investigated.

Results

The kernels were gamma irradiated from 50 - 350 Gy using a ^{60}Cobalt source. The kernels were left to germinate and grow for a period of 132 hours for the shoot and root growth and the efficiency of energy conversion into growth determination and for a period of 47.5 hours for the determination of the number of bridges, ring chromosomes, micronuclei and incomplete mitosis. The control differed highly significantly from 50 Gy and higher dosages and from 250 Gy and higher dosages for root and shoot growth respectively and from 250 Gy for the efficiency of energy conversion into growth. There was a highly significant increase in the number of bridges and micronuclei between 50 Gy and 150 Gy together with the higher irradiation dosages and only from 250 Gy for the ring chromosomes and interphase cells with incomplete mitosis. Root and seedling growth on the one hand and the efficiency of energy conversion into growth on the other were found to be measuring different effects of gamma irradiation on plant growth.

Conclusion

The optimal dosage for mutation breeding was determined by making use of the efficiency of energy conversion into growth.

Background

A 50% growth reduction of seedling height (GR_{50}) after acute gamma irradiation of dormant kernels is widely used as a measure of irradiation damage to obtain the ideal irradiation dosage for mutation breeding. It is used because it is easy to predict the GR_{50}. In cereals, measurements are taken when the shoots of the control seedlings are between 11 and 20 cm, usually after 10 to 14 days, for plants in sowing trays. Seedling height is measured from the soil level to the tip of the primary leaf in cereals. It became clear in recent years that the GR_{50} is not sensitive enough to predict the ideal gamma irradiation

dosage for mutation breeding and it predicts a value that is higher than ideal. The GR_{50} range for *Triticum turgidum* ssp. *durum* is 350 Gy – 500 Gy, while the ideal gamma irradiation dosage range for mutation breeding is 150 Gy – 300 Gy [1].

What factors are then playing a role in growth reduction? Physiological processes, DNA repair as well as cell death [2] influence this reduction in growth. Physiological aspects may increase growth at low dosage levels and decrease at higher dosage levels [3]. Gamma irradiation damages and breaks chromatin in the G1, S and G2 cell stages [4]. It has been reported by several authors that the percentage of abnormal cells increases as the irradiation dosage increases [5]. Various ratios of irradiation-induced aberrations have been used as possible fingerprints of irradiation quality [6, 7].

The effect of gamma irradiation on growth parameters in the *Triticum* polyploid complex. In diploid wheat, *Triticum monococcum* L. (2n=2x=AA=14) a 50 Gy gamma irradiation dosage resulted in a significant reduction in shoot and root growth and mobilisation of food reserves [8]. The *Triticum* polyploid complex also consists of tetraploid (2n=4x=AABB=28) and hexaploid (2n=6x=AABBDD=42) species [9]. Gamma irradiation dosage levels that lead to a decrease in growth and physiological processes in diploid wheat, can lead to an increase in the polyploid species. In *Triticum aestivum* L. low gamma irradiation dosages (10 – 100 Gy) resulted in improved flag leave area and photosynthetic traits as well as a higher nutrient uptake of the roots [10]. Taking into account the irradiation dosage related uniformity in crop performance; the plant response appears to have been realized at the physiological rather than genetic level in polyploid wheat [11]. Increases in Chlorophyll and proline content, shoot and root dry weights after 100 Gy gamma irradiation of seed of hexaploid wheat were found to differ in different genotypes [3]. An increase in root number and length occurred in hard wheat (*Triticum turgidum* ssp. *durum* L.) after low dosage irradiation (20 Gy) of the seed [12, 13].

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 cytokinesis-block micronucleus (CBMN) assay is now commonly used to determine irradiation sensitivity of tumour cells and cytotoxicity in animal and human cell cultures [14; 15]. The CBMN assay can however not be used for the determination of mitotic indexes and the distribution of micronuclei in daughter cells. 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 cell, (8) cellular and (9) nuclear shape deformities [16]. Over time micronuclei can be extruded from the cell, reincorporated in the nucleus, degraded or persist in the cytoplasm of the cell. Ring chromosomes are also carried over separately from one cell cycle to another [17]. These micronucleated cells may survive several cycles of mitosis or are eliminated by means of apoptosis [18].

We created the “efficiency of energy conversion into growth” as a predictor of the ideal dosage for mutation breeding [8]. Respiration rate, calculated as the difference between the initial kernel dry weight and the actual dry weights of the shoot, roots and caryopsis during a specific period of growth [19], is

normally used to predict inefficiency of energy usage for growth in plants due to an external factor. Respiration cannot be used to predict the ideal dosage for mutation breeding, because it is dependent on the metabolic activity of the plant that decreases with an increase in irradiation dosage [8]. The efficiency of energy conversion into growth is calculated as:

$$= \frac{\text{(combined shoot and root dry weight)-(original embryo dry weight)}}{\text{(original caryopsis dry weight)-(actual dry weight of caryopsis at a particular point of time)}} \quad \text{(Eq.1)}$$

By dividing the actual growth rate by the energy used to obtain the specific growth rate, the metabolic activity does not have a decrease effect on the value of the inefficiency of energy conversion into growth with an increase in gamma irradiation. The fact that the whole plant (shoot and root dry weight) are used, makes it more sensitive to gamma irradiation than if only the shoot dry weight had been used, because of the fact that the root growth is more sensitive than shoot growth [8]. In *Triticum monococcum,* the ideal gamma irradiation dosage range for mutation breeding is 100 Gy – 200 Gy (*1*). By making use of the efficiency of energy conversion into growth, 100 Gy was predicted to be the ideal dosage for mutation breeding in Einkorn [8].

The retarded effect on shoot and root growth, efficiency of energy conversion into growth as well as cytogenetic effects of gamma irradiation were studied in tetraploid *Triticum turgidum* ssp. *durum*. The first aim was to determine whether root and seedling growth and the efficiency of energy conversion into growth are measuring different processes that results in growth retardation as measured by bridges, ring chromosomes, micronuclei and incomplete mitosis. The second aim was to determine the ideal dosage for mutation breeding. Complete repair of meristematic cells only take place until a certain degree of damage is obtained by the cells. When the damage to the meristematic cells is above a threshold value, incomplete repair takes place. These damaged cells stop dividing and may even be eliminated. Full repair of meristematic cells leads to full recovery and effective energy conversion into growth. However, as the percentage of repaired meristematic cells that participates in the mitotic cell divisions decreases, so does the efficiency of recovery and thus energy conversion into growth decreases [8]. It is expected therefore that root and seedling growth will have a better correlation with a low number of bridges / micronuclei per cell, while the efficiency of energy conversion into growth will have a better correlation with a large number of bridges / micronuclei per cell.

Methods

The study aim was to determine whether root, shoot and seedling growth on the one side and the efficiency of energy conversion into growth on the other are measuring different growth retardation effects of gamma irradiation that are the result of DNA damage (bridges, ring chromosomes, micronuclei, incomplete mitosis) in *Triticum turgidum* ssp. *durum*. If the efficiency of energy conversion into growth is measuring different effects, the usefulness of efficiency of energy conversion into growth to predict the optimal dosage for mutation breeding will be investigated.

Kernel preparation for gamma irradiation and imbibition

Kernels of *Triticum turgidum* ssp. *durum* L. cv. Orania were obtained from the Small Grain Germplasm Collection at Agricultural Research Council – Small Grain Institute.

Kernel size affects early growth and seedling vigour [40, 41]. The kernels used for this investigation were selected to weigh within 2 mg of the average kernel weight of 46 mg. To ensure repeatability of the experiments, the moisture content of the kernels was adjusted to 14% prior to irradiation [1]. The kernels were irradiated with 50, 150, 250 and 350 Gy using a ^{60}Cobalt source at the South African National Blood Services in Roodepoort, Gauteng, South Africa. These irradiation dosages were used to produce an increase in metabolic activity and growth rate at 50 Gy, while the rest of the irradiation dosages will lead to a decrease in metabolic activity and growth rate. The lower growth rate may also be due to cell cycle arrest in G2 phase, unsuccessful cell division at the highest dosages and cell death as observed in irradiation of cancer cells [35, 36].

The imbibition conditions for the experiments were as follows: the irradiated kernels, as well as kernels that were not irradiated, which acted as the control, were placed in the dark between standard germination papers at a constant temperature of 25°C according to the regulations of ISTA [42]. The kernels were left to germinate and grow for a period of 132 hours for the shoot and root growth determination as well as the determination of the efficiency of energy conversion into growth. The kernels were left to germinate and grow for a period of 47.5 hours for the determination of the number of bridges in anaphase and telophase cells, number of ring chromosomes from prophase to anaphase as well as the number of micronuclei in interphase cells.

Shoot and root growth and determination of the efficiency of energy conversion into growth

The shoot and root growth period was regarded as the time period from 60 hours of growth when the primary roots and shoots had emerged and active growing phase has commenced until 132 hours of growth. The 132 hours growth period was used to give the lower gamma irradiation dosages time to recover and the higher dosages to deteriorate. The 132 hours was long enough for the 50 Gy treatment to recover in *Triticum monococcum* L. [8] and because of the fact that the growth rate of *Triticum turgidum* ssp. *durum* L. is higher than that of the diploid [43], therefore 132 hours was chosen to be the ideal growth period. Kernels were collected every 12 hours, eight kernels at a time, starting at 60 hours after the initiation of imbibition. Kernels with plantlets were then dried for at least 48 hours in an oven at 105°C, after which the dry weights of the shoot, root and remaining caryopsis, were recorded. Shoot and root growth was determined by the increase in shoot and root dry weights [19]. The efficiency of energy conversion into growth was calculated as mentioned in the Introduction [8].

Fixation and slide preparation for analysis of bridges, ring chromosomes and micronuclei

The fixation and slide preparations were according to the combination of two methods [44, 45]. Five roots were collected every two and a half hours from 17.5 - 47.5 hours after onset of imbibition for each

treatment and fixed in La Cour's 2BD for 8 hours. The roots were then bleached in a mixture of one part saturated aqueous solution of ammonium oxalate and one part 20 volumes (6%) hydrogen peroxide for different time periods for each dosage (Table 1). For the squash preparations, the roots were first macerated in 15% pectinase for 20 hours at 30˚C and then hydrolysed in 1N HCl at 60˚C for 15 minutes (non-irradiated, 50 Gy and 150 Gy irradiation) or 12 minutes (250 Gy and 350 Gy irradiation) and stained in Feulgen [44] for two and a half hours. After a 10-minute rinse, squash preparations were prepared by squashing the meristematic root tip (first 1 mm) in 45% acetic acid on a slide. An albumenised coverslip was placed on the material, followed by gentle tapping and squashing between two layers of filter paper. The coverslip was dehydrated by passing it through 70% ethanol and 96% ethanol for two minutes in each solution. The coverslips were then counter stained in 0.25% Fast Green [45] for five minutes and processed in 100% ethanol for two minutes. The ethanol was removed by placing the coverslip in 100% ethanol : xylene (1 : 1) for three minutes and then in xylene for a further three minutes and finally mounted with Entellan.

Cytogenetic analysis

The effect of gamma irradiation on the appearance of bridges, ring chromosomes, micronuclei and incomplete mitosis in meristematic cells were assessed. The determination of the number of bridges was done by scoring the number of bridges in anaphase and telophase cells out of 500 meristematic cells. The number of ring chromosomes were determined by scoring the number of rings in prophase to anaphase cells out of 500 meristematic cells. The number of micronuclei in interphase cells were determined by scoring 500 interphase cells. The number of incomplete mitosis were determined by scoring the number of interphase cells with five and more nucleoli in the nucleus out of 500 interphase cells [22]. The mean number of bridges, ring chromosomes and micronuclei per 500 cells and the frequency of cells with different numbers of bridges, ring chromosomes and micronuclei were determined for the evaluations.

Experimental layout and statistical analysis

The experimental layout for shoot and root growth evaluation and evaluation of the efficiency of energy conversion into growth was a randomized complete block design with eight replicates (blocks). A two factor factorial experimental design was followed with three dependent variables with dosage levels of 0, 50, 150, 250 and 350 Gy and time with levels 60, 72, 84, 96, 108, 120 and 132 hours. The effects of the factors dosage and time and dosage by time interactions were tested using the GLM procedure of SAS software (SAS Institute 2012 North Carolina 27513: SAS Campus Drive, Cary). The least significant means (LSMEANS) of SAS were used to compare the effect of the different dosages on shoot and root growth and efficiency of energy conversion into growth at specific p values.

The experimental layout for bridges, rings and micronuclei evaluations was a randomized complete block design with five replicates (blocks). A two factor factorial experimental design was followed with three dependent variables with dosage levels of 0, 50, 150, 250 and 350 Gy and time with levels 17.5, 20, 22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, 42.5, 45 and 47.5 hours. The effects of the factors dosage and time and

dosage by time interactions were tested using the GLM procedure of SAS software (SAS Institute 2012 North Carolina 27513: SAS Campus Drive, Cary). The least significant means (LSMEANS) of SAS were used to compare the effect of the different dosages on the presence of bridges, rings and micronuclei at specific p values.

Correlations

Pearson's correlation coefficients were determined between root and seedling growth as well as the efficiency of energy conversion into growth on the one hand and the numbers of cells with different numbers of micronuclei / bridges and incomplete mitosis on the other. Root growth was used for the correlations because of the fact that the cytogenetic study was done on root meristematic cells. Seedling growth was used because of the fact that seedling growth and not root growth was used for the determination of efficiency of energy conversion into growth and to see if there is a large difference between the correlations using root dry weight or seedling dry weight. Root / seedling growth represent retardation in growth due to cell death as well as decreased metabolic / physiologic activity. The efficiency of energy conversion into growth decreases due to cell death and energy used for repairing of damaged cells. The numbers of cells with different numbers of micronuclei / bridges and incomplete mitosis were the abnormalities observed in the cytogenetic study and therefor used to explain what happens during root and shoot growth retardation and decrease in efficiency of energy conversion into growth.

Determination of the optimum dosage for mutation breeding

The efficiency of energy conversion into growth will be used to determine the ideal dosage for mutation breeding, if it is found to be measuring different aspects of growth retardation than root growth and seedling growth. In *Triticum monococcum* L. the 100 Gy gamma irradiation treatment was not entangled with the control and differed from the control according to LSMEANS with a p=0.01 value [8]. The p=0.01 value may be used as a guide for predicting the optimal dosage for mutation breeding.

Results

The effect of gamma irradiation on tetraploid wheat was investigated by studying the effect on growth parameters such as shoot and root growth and the efficiency of energy conversion into growth and the effect on chromatin in the form of chromatin bridges (break in one chromosome arm), ring chromosomes (breaks in both chromosome arms), micronuclei and incomplete mitosis.

Shoot growth rates

Shoot growth rate was investigated to investigate metabolic activity as affected by the gamma irradiation that can be observed over time. Shoot growth of the irradiated seed displayed growth retardation when compared to the control group. Retardation also showed an increase with increasing irradiation dosages (Fig. 1a).

Shoot growth by means of the ANOVA tests revealed highly significant effects of dosage, as well as dosage by time interactions (Table 2).

Shoot growth according to the LSMEANS showed highly significant differences for all pairs of shoot growth, except for the treatment pairs of control and 50 Gy, control and 150 Gy, 50 Gy and 150 Gy, which were non-significant (Table 3).

Root growth rates

Root growth rate was investigated to investigate metabolic activity as affected by the gamma irradiation that can be observed over time. Root growth of the irradiated seed also displayed growth retardation when compared to the control group. Retardation also showed an increase with increasing irradiation dosages (Fig. 1b).

Root growth by means of the ANOVA tests revealed highly significant effects of time and dosage, as well as dosage by time interactions (Table 2).

Root growth according to the LSMEANS showed highly significant differences for all pairs of root growth (Table 3).

Efficiency of energy conversion into growth

The efficiency of energy conversion over time was investigated to observe the effect of gamma irradiation on energy expenditure for growth and repairing of damage caused by the gamma irradiation that change over time. The efficiency of energy conversion into growth declined with increasing irradiation dosage. Hundred and fifty Gy displayed a more efficient conversion into growth than the control and 50 Gy. Fifty Gy and 150 Gy were entangled with the control, while 250 Gy and 350 Gy were at lower levels than the control (Fig. 1c).

Efficiency of energy conversion into growth by means of the ANOVA tests showed highly significant effects of dosage and dosage by time interactions. The time effect was slightly significant (Table 2).

Efficiency of energy conversion into growth according to the LSMEANS revealed that most treatment pairs demonstrated highly significantly differences in efficiency of energy conversion into growth except for the treatment pairs, the control with 50 and 150 Gy that were non-significant, the control and 250 Gy which was significant and 50 Gy with 250 Gy which was non-significant. (Table 3).

Presence of bridges

The presence of bridges in anaphase (Fig. 2a) and telophase (Fig. 2b) was assessed over the period from 17.5 to 47.5 hours after the onset of imbibition to visualise the effect of the gamma irradiation on the chromosomes during mitosis (Fig. 3). In the investigation it was not always possible to determine if a bridge was single stranded or double stranded, therefore, all bridges were assessed together without this distinction. However, evidence of the presence of chromatin breaks due to gamma irradiation first

became evident in the form of bridges at 22.5 hours. The number of cells containing different numbers of bridges increased with an increase in irradiation dosage. Fifty Gy and 150 Gy treated cells displayed cells with one to three bridges. The number of cells with more than one bridge became rarer with an increase in number of bridges. Cells with two and three bridges occurred rarely in 50 Gy treated material, while 150 Gy treated material had marginally more of these cells with a substantial increase in cells with a single bridge. 250 Gy and 350 Gy treated material displayed many more cells with chromatin bridges, ranging from cells with one, to cells with seven bridges. Broken bridges were visible in telophase (Fig. 2b) and newly divided cells. Fragments were visible in metaphase (Fig. 2c) and anaphase.

The average number of anaphase and telophase cells containing bridges clearly showed an increase with an increase in gamma irradiation dosage (Fig. 4a). Throughout the assessment period the number of cells displayed periods of an increase and periods with a decrease in the number of cells containing bridges at all dosages.

The extent of DNA damage through gamma irradiation was also assessed by determining the average number of bridges over the investigating period (Fig. 4b). There were large similarities between the average number of anaphase and telophase cells containing bridges and average number of bridges. The similarities decreased with an increase in gamma irradiation. The average number of bridges by means of the ANOVA tests showed highly significant effects of dosage, time and dosage by time interaction (Table 4).

The effect of gamma irradiation on the average number of bridges according to the LSMEANS revealed that the differences among all irradiation treatments were highly significantly different (Table 5).

Presence of ring chromosomes

Ring chromosomes result from chromosomal breaks in both chromosome arms, followed by the fusion of the sticky ends to form a ring chromosome. Ring chromosomes were observable from late prophase (Fig. 2d), through metaphase (Fig. 2e) and in anaphase (Fig.2f). A maximum of two ring chromosomes were observed per cell. The ring chromosomes divide at a later stage in anaphase than the rest of the chromosomes (Fig. 2f).

An investigation of the presence of ring chromosomes in dividing cells revealed that, in general, ring chromosomes appeared earlier in prophase with an increase of gamma irradiation dosage, however, the exception was 50 Gy material, that did reveal ring chromosomes earlier than 150 Gy material (Fig. 5). First appearance of ring chromosomes in metaphase and anaphase followed the same pattern as for prophase, except for 50 Gy material in which ring chromosomes in metaphase and anaphase were observed later than first appearance in prophase. The mean number of ring chromosomes showed a steady increase with an increase in dosage. Most of the ring chromosomes were observed in metaphase cells.

A comparison of the average number of ring chromosomes revealed the average number of ring chromosomes increased with an increase in gamma irradiation dosage (Fig. 6). Fifty Gy material displayed the least number of cells with ring chromosomes and 350 Gy material the greatest number, while 150 and 250 Gy material displayed an intermediate number of cells with ring chromosomes. The presence of ring chromosomes displayed a cyclic pattern with little or no cells with ring chromosomes to peaks of cells with ring chromosomes. The highest peak in 50 Gy material had an average of 0.4 ring chromosomes and the highest peak in 350 Gy material had an average of 3.4 ring chromosomes. It appeared that the number of peaks decreased with an increase of irradiation dosage. Fifty and 150 Gy each had three peaks, while 250 and 350 Gy each had two peaks.

The extent of DNA damage through gamma irradiation was also assessed by determining the total number of ring chromosomes over the investigating period. The total number of ring chromosomes by means of the ANOVA tests showed highly significant effects of dosage, time and dosage by time interaction (Table 5).

The effect of gamma irradiation on the total number of ring chromosomes according to the LSMEANS revealed moderate to highly significant differences among the irradiation dosage treatments except between the 150 Gy and 250 Gy treatments that was non-significant (Table 6).

Presence of micronuclei

Acentric fragments become micronuclei towards the end of telophase when nuclear membranes are formed. Once fragments are formed into micronuclei, they are passed to daughter cells (Fig 2g, h) by chance. They become visible at telophase and are present until prophase. During late prophase, metaphase and anaphase they lose their membranes and are visible as acentric fragments. In this investigation, the greatest number of micronuclei recorded per cell at interphase was seven.

The range and numbers of cells containing different numbers of micronuclei increased with an increase in gamma irradiation dosage (Fig. 7). Fifty Gy material displayed only cells with one or two micronuclei; 150 Gy material displayed cells with one to four micronuclei, while in 250 Gy and 350 Gy material the number of micronuclei ranged from one to seven. In all the treatments, cells with one micronucleus was most frequently, followed by cells with two micronuclei. Cells with three and four micronuclei occurred at lower frequencies, while cells with five to seven micronuclei occurred rarely, less than five cells out of 500.

The total number of micronuclei at various times after the onset of imbibition was determined to obtain an understanding of the effect of gamma irradiation at different dosages (Fig. 8a). The data revealed that there was an increase in the number of micronuclei with an increase in dosage, with a mean number of about 24 micronuclei in 50 Gy material to 310 micronuclei in 350 Gy material.

The total number of micronuclei by means of the ANOVA tests showed highly significant effects of dosage, time and dosage by time interaction (Table 5).

The effect of gamma irradiation on the total number of micronuclei according to the LSMEANS revealed highly significant differences among the dosage treatments (Table 6).

The effect of gamma irradiation on the number of micronuclei was also supported by an increase in the total number of cells containing micronuclei (Fig. 8b) with an increase in gamma irradiation dosage. When the pattern of the total number of micronuclei and the total number of cells containing micronuclei over time were compared, it was found that the developmental patterns were very similar.

Incomplete mitosis

In *Triticum turgidum* ssp. *durum* L. the maximum number of nucleoli in the nucleus is four, formed by NOR's on chromosomes 1B and 6B [20, 21]. Incomplete mitosis was scored as cells with five (Fig. 2i) and more nucleoli in the nucleus resulting from separation of the chromatids during mitosis but still get packed in the same nucleus during interphase, leading to a nucleus in interphase with twice as much DNA [22]. All the irradiation dosages displayed interphase cells with incomplete mitosis from 25 h after the onset of imbibition with an increase of incomplete mitosis with an increase in irradiation dosage. Fifty Gy and 150 Gy had four and three collection times with no incomplete mitosis interphase cells respectively, while 250 Gy and 350 Gy had incomplete mitosis interphase cells at all the collection times from 25h onwards (Fig. 9).

The fact that the first observations of interphase cells with incomplete mitosis was at 25 h, is supported by the fact that many mitotic divisions had completed by that time. It is expected that the changes over time would be different and the effects of the different gamma irradiation dosages would be different. The total number of interphase cells with incomplete mitosis by means of the ANOVA tests showed highly significant effects of dosage, time and dosage by time interaction (Table 5).

The lower irradiation dosages are expected to have little impact on the number of interphase cells with incomplete mitosis, while the higher irradiation dosages will have a larger effect. The effect of gamma irradiation on the total number of interphase cells with incomplete mitosis according to the LSMEANS revealed highly significant differences among the irradiation dosage treatments, except for a non-significant difference between 50 Gy and 150 Gy (Table 6).

Correlations between seedling and root growth and the efficiency of energy conversion on the one side and presence of bridges, micronuclei and interphase cells with incomplete mitosis on the other

Correlations between seedling and root growth and the efficiency of energy conversion into growth on the one side and presence of bridges, micronuclei and interphase cells with incomplete mitosis on the other were determined to evaluate if seedling and root growth are measuring different effects of gamma irradiation than the efficiency of energy conversion into growth does (Table 6).

The relatedness between seedling and root growth with the presence of bridges (Table 6) displayed highly significant correlations between seedling and root growth with the presence of one to three bridges per cell with the highest correlation with two bridges per cell. The efficiency of energy conversion on the other

hand displayed highly significant correlations with two to three bridges per cell with the highest correlation with three bridges per cell.

The relatedness between seedling and root growth with the presence of micronuclei (Table 6) displayed highly significant correlations with one to six micronuclei per cell. Seedling growth had the highest correlation with two micronuclei and root growth with one micronuclei. The efficiency of energy conversion into growth on the other hand had highly significant correlations with one to seven micronuclei, with the best correlation with four micronuclei per cell.

The relatedness between seedling and root growth with the presence of incomplete mitosis (Table 6) displayed highly significant correlations with interphase cells with incomplete mitosis. Root growth had the highest correlation with interphase cells with incomplete mitosis, followed closely by seedling growth. The efficiency of energy conversion into growth also had a highly significant correlation with interphase cells with incomplete mitosis, although it was much less than the other two.

Determination of the optimum dosage for mutation breeding

Due to the differences in the correlations between root and seedling growth on the one hand and efficiency of energy conversion on the other with bridges, micronuclei and interphase cells with incomplete mitosis, it was concluded that the efficiency of energy conversion are measuring other aspects of growth retardation.

In *Triticum monococcum* [8], the 100 Gy and higher gamma irradiation treatments were not entangled with the control like 250 Gy (after 84 h) and 350 Gy gamma irradiation treatments in the present study. The 100 Gy treatment differed from the control with p=0.01 in *Triticum monococcum* [8]. In the present study 250 Gy differed from the control with p=0.02. This makes it ideal for using p=0.01 for the determination of the ideal dosage for mutation breeding.

Steps taken for the determination of the ideal dosage for mutation breeding. Firstly, the LSD value at p=0.01 must be determined. The LSD value was determined as 7.72. Secondly, the LSD value must be subtracted from 100 to obtain the y-axis value of the graph. The y-axis value was determined as: 100 – 7.72 = 92.28. Thirdly, a best-fit equation must be obtained. A quadratic equation, $y=0.0004x^2 + 0.0785x + 97.99$, had the best fit with $R^2=0.9218$. Fourthly, the x and y values must be plotted according to the quadratic equation with the efficiency of energy conversion into growth on the y-axis and the gamma irradiation dosage on the x-axis. Fifly, by using the 92.28 on the y-axis, the ideal dosage for mutation breeding will be obtained on the x-axis (Fig. 10). The ideal gamma irradiation dosage for mutation breeding was determined as 267.6 Gy.

Discussion

Comparisons of the effect of low dosages of gamma irradiation on growth parameters. Hormesis is the concept that biologic systems can respond in a positive way, or be stimulated by, physical or biologic

exposure to low doses of an agent that is toxic at higher doses. Additionally, hormesis is defined as “any physiological effect that occurs at low doses which cannot be anticipated by extrapolating from toxic effects noted at high doses”. Radiation hormesis is the theory that biological systems can respond positively to exposure of low doses of ionizing radiation [23]. Adaptive protection that follow low dosage irradiation causes DNA damage prevention, repair, and immune stimulation in animals [24]. An increase in shoot growth was observed at 50 Gy gamma irradiation in the present study. This is supported by an increase in mitotic index in root apical meristem at 50 Gy gamma irradiation in *Triticum turgidum* ssp. *durum* [22]. Similar observations were made in hexaploid wheat where lower gamma irradiation stimulates, while higher dosages inhibit plant growth and development [25, 26]. All the cultivars responded differently to different gamma ray dosages with respect to some of the characters.

The difference between diploid and polyploid wheat species. The fact that stimulation in shoot growth of *Triticum turgidum* ssp. *durum* took place, while retardation in growth took place in *Triticum monococcum* at 50 Gy gamma irradiation means that specific dosages that are classified as “low dosage irradiation” is depending on and relative to what is being irradiated [27]. The polyploid species are mostly more resistant than the diploid species due to the accumulation of genomes, also known as genetic redundancy, and the combination of different genomes with differences in their irradiation resistance [28, 29, 30].

Comparisons of the effect of high dosages of gamma irradiation on growth parameters. Gamma irradiation has the largest effect on root growth, followed by shoot growth and finally by the efficiency of energy conversion into growth. In root growth, all the irradiation dosages differed highly significantly from the control, while 250 Gy and 350 Gy differed highly significantly from the control in shoot growth and only 350 Gy differs highly significantly from the control in the efficiency of energy conversion into growth. Large differences in shoot and root lengths among Basmati rice cultivars were observed after gamma irradiation of the seed [31]. Root growth were also found to be more sensitive than shoot growth in gamma irradiation of onion seed [32]. The decrease in growth at high gamma irradiation dosages can be attributed to: firstly, reduced mitotic activity partly caused by radiation induced senescence (a condition of permanent cell cycle arrest induced by irradiation) in meristematic tissues and secondly, mitotic catastrophe (when cell death occurs during or as a result of an aberrant mitosis). Senescent cells do not divide but may remain metabolically active. Aberrant mitosis produces an atypical chromosome segregation and cell division and leads to the formation of cells with aberrant nuclear morphology, multiple nuclei, and/or several micronuclei. Giant cells form when cytokinesis does not take place and the cells die over a period. Mitotic catastrophe can occur over a few days [33, 34, 7, 26].

The presence of bridges, micronuclei and ring chromosomes. Gamma irradiation causes chromatid and chromosome breaks, that through joining of sticky chromosome or chromatid ends result in anaphase bridges when centromeres are included, while acentric fragments become micronuclei. These bridges may persist to telophase, which break at the end of mitosis, usually towards the end of telophase and can be observed as broken bridges. Gamma irradiation of chromatin occurred in cells in G1 phase of the cell division cycle, when all chromatin threads are single stranded, as well as cells in S and G2 phase, which

have replicated through the S phase of the cell division cycle. Ring chromosomes originate by breaks in both chromosome arms. The presence and increase of ring chromosomes take therefore place at a slower pace as seen by the fact that the 150 Gy irradiation dosage and above have a highly significant increase in bridges and micronuclei in comparison with 50 Gy, while 250 Gy and above have a highly significant increase in ring chromosomes in comparison with 50 Gy. A highly significant increase in mitotic cells with chromosomal aberrations with an increase in irradiation dosage was also observed in eight winter wheat cultivars by making use of gamma irradiation dosages of 100, 150, 200 and 250 Gy [35].

The relationship between root, shoot and seedling growth and the efficiency of energy conversion into growth with the presence of bridges, ring chromosomes, micronuclei and incomplete mitosis. Physiological processes as well as cell death and cellular senescence influence root, shoot and seedling growth, while the efficiency of energy conversion into growth are mainly influenced by cell death, cellular senescence and cell repair. One and two bridges or micronuclei per cell mostly affected root, shoot and seedling growth. This can be attributed to an increase /decrease in physiological processes as seen in decreased NOR activity [22] as well as G_2 cell cycle arrest for DNA repair at an early checkpoint and a later checkpoint [36, 37, 38]. The efficiency of energy conversion into growth correlates best with three bridges and four micronuclei per cell. Three bridges and four micronuclei per cell only became noteworthy at 250 Gy gamma irradiation. This is the irradiation dosage with a highly significant increase in incomplete mitosis in comparison with 50 Gy. It is also, where the efficiency of energy conversion into growth is not entangled with the control after 84 h. Incomplete mitotic division takes place in all the irradiation dosages with an increase in occurrence with an increase in irradiation dosage and may be the result of a combination of chromosomal abnormalities [16]. Incomplete mitosis is observed by the occurrence of up to six nucleoli per nucleus and up to eleven nucleoli per cell, as well as a decrease in the frequency of cells in telophase [39, 22]. These cells die over a period and together with cellular senescence are responsible for the decrease in the efficiency of energy conversion into growth.

Finding the optimal dosage for mutation breeding. In the present study, 250 Gy gamma irradiation was not entangled with the control after 84 h like 100 Gy in *Triticum monococcum* L. [8]. There was also a highly significant increase in incomplete mitosis between 150 Gy and 250 Gy in *Triticum turgidum* ssp. *durum* L that support the prediction of an optimal gamma irradiation dosage in the region of 250 Gy. According to FAO/IAEA [1], the ideal gamma irradiation dosage range for mutation breeding in *Triticum turgidum* ssp. *durum* L is 150 Gy – 300 Gy. The predicted ideal gamma irradiation dosage of 267.6 Gy falls in this range.

Conclusion

The control differed highly significantly from 50 Gy and higher dosages and from 250 Gy and higher dosages for root and shoot growth respectively and from 350 Gy for the efficiency of energy conversion into growth. There was a highly significant increase in the number of bridges and micronuclei between 50 Gy and 150 Gy together with the higher irradiation dosages and only from 250 Gy for the ring

chromosomes and interphase cells with incomplete mitosis. Root and seedling growth had the highest correlations with two bridges and root growth with one micronucleus, while seedling growth had the highest correlations with two micronuclei. The efficiency of energy conversion into growth had the highest correlations with three bridges and four micronuclei. Root and seedling growth on the one hand and the efficiency of energy conversion into growth on the other are measuring different effects of gamma irradiation on plant growth. Root and seedling growth had significant higher correlations with incomplete mitosis than the efficiency of energy conversion into growth. Physiological processes as well as cell death and cellular senescence influence root, shoot and seedling growth, while the efficiency of energy conversion into growth are mainly influenced by cell death, cellular senescence and cell repair. The predicted ideal gamma irradiation dosage for mutation breeding of 267.6 Gy, falls in the range recommended by the FAO/IAEA.

Abbreviations

ANOVA - Analysis of Variance

CBMN - Cytokinesis-block micronucleus

FAO/IAEA – Food and Agriculture Organization / International Atomic Energy Agency

GLM - Generalized linear model

GR_{50} - 50% growth reduction of seedling height

Gy – Gray

ISTA – International Seed Testing Association

LSD – Least significant difference

LSMEANS - Least-squares means

NOR – Nucleolar Organizer Regions

Ssp. – Subspecies

Declarations

Ethics approval and consent to participate

Not Applicable.

Consent for publication

Not Applicable.

Availability of data and materials

No sequencing data was generated by the study.

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MB - Statistical analysis

AF – Writing of the article and literature study

EvW – Planning of the experiments, conduction of the experiments and writing of the article

All authors read and approved the final manuscript.

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References

1. FAO/IAEA. Chapter 1. Physical Mutagenesis. In: Spencer-Lopes MM, Forster BP, Jankuloski L. Manual on Mutation Breeding. Third Edition. Food and Agriculture Organization of the United Nations. Rome, Italy; 2018.p.5–50.
2. Lagoda PJL. Effects of Radiation on Living Cells and Plants. In: Shu QY, BP Foster and H Nakagawa (Eds). Plant mutation breeding and biotechnology. CABI. Oxfordshire OX 10 8DE, UK; 2012.p.123–134.
3. Borzouei A, Kafi M, Khazaei H, Naseriyan B, Majdabadi A. Effects of gamma radiation on germination and physiological aspects of wheat (*Triticum aestivum* L.) seedlings. Pak J Bot 2010;42(4):2281–2290.
4. Hase Y, Shimono K, Inoue M, Tanaka A, Watanabe H. Biological effects of ion beams in *Nicotiana tabacum* L. Radiat Environ Biophys 1999;38(2):111–5.

5. Viccini LF, de Carvalho CR. Meiotic chromosomal variation resulting from irradiation of pollen in maize. J Appl Genet 2002;43(4):463–469.
6. Ballarini F, Ottolenghi A. Chromosome aberrations as biomarkers of radiation exposure: modeling basic mechanisms. Adv Space Res 2003;31(6):1557–68.
7. Liu Q, Wang Z, Zhou L, Qu Y, Lu D, Yu L, Du Y, Jin W, Li W. Relationship between plant growth and cytological effect in root apical meristem after exposure of wheat dry seeds to carbon ion beams. Nucl Instr Meth Phys Res B 2013;305:9–15.
8. Von Well E, Fossey A, Booyse M. Efficiency of energy conversion and growth of gamma irradiated embryos and young seedlings of Triticum monococcum L. cultivar Einkorn. J Radiat Res Appl Sci 2017; https://doi.org/10.1016/j.jrras.2017.09.004.
9. Dvorak J. *Triticum* Species (Wheat). In: Brenner's Encyclopaedia of Genetics, 2nd edition, Volume 7 2013;p.198–202.
10. Singh B, Datta PS. Effect of low dose gamma irradiation on plant and grain nutrition of wheat. Radiat Phys Chem 2010;79:819–825. https://doi:10.1016/j.radphyschem.2010.03.011.
11. Singh B, Datta PS. Gamma irradiation to improve plant vigour, grain development and yield attributes of wheat. Radiat Phys Chem 2010;79:139–143. https://doi:10.1016/j.radphyschem.2009.05.025.
12. Melki M, Dahmani T. Gamma irradiation effects on durum wheat (*Triticum durum* Desf.) under various conditions. Pak J Biol Sci 2009;12(23):1531–1534.
13. Melki M, Marouani A. Effects of gamma rays irradiation on seed germination and growth of hard wheat. Environ Chem Lett 2010;8:307–310. https://doi:10.1007/s10311-009-0222-1.
14. Fenech M. The Micronucleus Assay Determination of Chromosomal Level DNA Damage. In: C Cristofre Martin (Ed). Methods in Molecular Biology 410: Environmental Genomics. Humana Press, Totowa, NJ, 2008;p.185–216.
15. Mendes ME, Mendonca JCG, Barquinero JF, Higueras M, Gonzalez JE, Andrade AMG. Comparative study of micronucleus assays and dicentric plus ring chromosomes for dose assessment in particular cases of partial-body exposure. Int J Radiat Biol 2019;95(8):1058–1071.
16. Gupta S, Datta AK, Pramanik A, Biswas J, Karmakar R. X-RAY AND GAMMA IRRADIATION INDUCED CHROMOSOMAL ABERRATIONS IN PLANT SPECIES AS THE CONSEQUENCE OF INDUCED MUTAGENESIS – AN OVERVIEW. Plant Arch 2019;19(2):1973–1979.
17. Geard GR. On the continuity of chromosomal subunits: an analysis of induced ring chromosomes in Vicia faba. Chromosoma 1976;55:209–228.
18. Hintzsche H, Hemmann U, Poth A, Utesch D, Lott J, Stopper H, Working Group "In vitro micronucleus test", Gesellschaft für Umwelt-Mutationsforschung (GUM, German-speaking section of the European Environmental Mutagenesis and Genomics Society EEMGS). Mutat Res 2017;771:85–98. http://dx.doi.org/10.1016/j.mrrev.2017.02.002.
19. Bouaziz A, Hicks DR. Consumption of wheat seed reserves during germination and early growth as affected by soil water potential. Plant and Soil 1990;128:161–165.

20. Hutchinson J, Miller TE. The nucleolar organisers of tetraploid and hexaploid wheats revealed by in situ hybridisation. Theor Appl Genet 1982;61:285–288.
21. Carvalho A, Polanco C, Lima-Brito J, Guedes-Pinto H Differential rRNA Genes Expression in Hexaploid Wheat Related to NOR Methylation. Plant Mol Biol Rep 2010;28:403–412. https://doi.10.1007/s11105-009-0165-5.
22. Von Well E, Fossey A, Booyse M. Effect of gamma irradiation on nucleolar activity, an indicator of metabolic activity, in root tip cells of tetraploid *Triticum turgidum* ssp. *durum* L. Unpublished manuscript 2021.
23. Baldwin J, Grantham V. Radiation Hormesis: Historical and Current Perspectives. J Nucl Med Technol 2015;43:242–246. https://doi:10.2967/jnmt.115.166074.
24. Feinendegen LE. Evidence for beneficial low level radiation effects and radiation hormesis. Br J Radiol 2005;78:3–7. https://doi:10.1259/bjr/63353075.
25. Irfaq M, Nawab K. Effect of Gamma Irradiation on Some Morphological Characteristics of Three Wheat (*Triticum aestivum* L.) Cultivars. Online J Biol Sci 2001;1(10):935–937.
26. Grover S, Khan AS. Effect Of Ionizing Radiation On Some Characteristics Of Seeds Of Wheat. IJSTR 2014;3(4):32–39.
27. Eroglu Y, Eroglu HE, Ilbas AI. Gamma Ray Reduces Mitotic Index in Embryonic Roots of *Hordeum vulgare* L. Advan Biol Res 2007;1(1–2): 26–28.
28. Fujii T, Matsumura S Radiosensitivity in Plants III. Experiments with several polyploid plants. Jap J Breeding 1959;9:245–252.
29. Sree Rangsamy SR, Sree Ramulu K. A comparison of mutation induction in diploid and tetraploid rice. Theor Appl Genet 1970;40:312–315.
30. Ukai Y. Polyploidy and radiosensitivity in the genus *Avena*. Jpn J Genet 1981;56:565–579.
31. Ashraf M, Cheema AA, Rashid M, Qamar ZUQ. Effect of gamma rays on M_1 generation in Basmati Rice. Pak J Bot 2003;35(5):791–795.
32. Amjad M, Anjum MA. Effect of gamma radiation on onion seed viability, germination potential, seedling growth and morphology. Pak J Agri Sci 2002; 39(3): 202–206.
33. Vakifahmetoglu H, Olsson M, Zhivotovsky B. Death through a tragedy: mitotic catastrophe. Cell Death Differ 2008;15:1153–1162. https://doi:10.1038/cdd.2008.47.
34. Eriksson D, Stigbrand T. Radiation-induced cell death mechanisms. Tumor Biol 2010;31:363–372.
35. Nazarenko M, Kharytonov M. Characterisation of wheat mutagen depression after gamma-rays irradiated. Agric Forest 2016;62(4):267–276. https://doi:10.17707/AgricultForest.62.4.27.
36. Xu B, Kim S-T, Lim D-S, Kastan MB. Two Molecularly Distinct G2/M Checkpoints Are Induced by Ionizing Irradiation. Mol Cell Biol 2002;22(4):1049–1059. https://doi:10.1128/MCB.22.4.1049–1059.2002.
37. O'Connell MJ, Cimprich KA. G2 damage checkpoints: what is the turn-on? J Cell Sci 2005;118(1):1–6. https://doi:10.1242/jcs.01626.

38. Zhao H, Zhuang Y, Li R, Liu Y, Mei Z, He Z, Zhou F, Zhou Y. Effects of different doses of X–ray irradiation on cell apoptosis, cell cycle, DNA damage repair and glycolysis in HeLa cells. Oncol Lett 2019;17:42–5.
39. Al-Safadi B, Simon PW. The effects of gamma irradiation on the growth and cytology of carrot (Daucus Carota L.) tissue culture. Environ Exp Bot 1990;30(3):361–371.
40. Hampton JG. The extent and significance of seed size variation in New Zealand wheats (*Triticum aestivum* L.). New Zeal J Exp Agr 1981;9:179–184.
41. Lafond GP, Baker RJ. Effects of temperature, Moisture Stress and Seed Size on Germination of Nine Spring Wheat Cultivars. Crop Sci 1986;26:563–567.
42. ISTA. International Rules for Seed Testing 2015; (i-19-8 (276)).
43. Von Well E, Fossey A. Metabolism, seedling growth and nucleolar activity in germinating diploid wheat Triticum monococcum ssp. monococcum cv. Einkorn. Euphytica 2002;124:47–54.
44. Pienaar R deV. Combinations and variations of techniques for improved chromosome studies in the Gramineae. S Afr J Bot 1955;21:1–8.
45. Johansen, DA. Plant Microtechnique. McGraw-Hill, New York, N.Y., 1940.

Table

Table 1 Bleaching times for the different gamma irradiation dosages

Dosage (Gy)	Length of bleaching time (min)
0	15.0
50	14.5
150	13.0
250	11.5
350	9.5

Table 2 Two-way ANOVA showing sources of variation for shoot and root growth and efficiency of energy conversion into growth in *T. turgidum* ssp. *durum* L. cv. Orania.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Shoot growth					
Block	7	0.381	0.054	1.95	0.06
Dosage	4	12.903	3.226	115.73	<0.01
Time	6	0.303	0.050	1.81	0.10
Dosage × Time	24	1.353	0.056	2.02	<0.01
Root growth					
Block	7	0.085	0.012	0.74	0.64
Dosage	4	30.765	7.691	465.06	<0.01
Time	6	0.824	0.137	8.30	<0.01
Dosage × Time	24	1.066	0.044	2.69	<0.01
Efficiency of energy conversion into growth					
Block	7	0.146	0.021	1.14	0.34
Dosage	4	3.502	0.875	48.07	<0.01
Time	6	0.244	0.041	2.23	0.04
Dosage × Time	24	1.212	0.051	2.77	<0.01

Table 3 P values of pairwise comparisons of gamma irradiation dosage for shoot and root growth and efficiency of energy conversion into growth

P values of least squares means comparison										
T. turgidum ssp. *durum* L. cv. Orania										
Shoot growth (mg)							Root growth (mg)			
Average:	4.47	4.63	4.08	3.53	3.09	3.98	3.55	2.92	2.28	1.85
Dosage (Gy)	0	50	150	250	350	0	50	150	250	350
0		**0.82**	**0.13**	<0.01	<0.01		<0.01	<0.01	<0.01	<0.01
50			**0.08**	<0.01	<0.01			<0.01	<0.01	<0.01
150				<0.01	<0.01				<0.01	<0.01
250					<0.01					<0.01
Efficiency of energy conversion into growth (%)										
Average:	100	97.16	104.07	93.44	79.94					
0		**0.25**	0.06	0.02	<0.01					
50			<0.01	**0.23**	<0.01					
150				<0.01	<0.01					
250					<0.01					

Non-significant differences in bold

Table 4 Two-way ANOVA showing sources of variation for total number of bridges, total number of ring chromosomes, total number of micronuclei and incomplete mitosis in *T. turgidum* ssp. *durum* L. cv. Orania.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Bridges					
Block	4	10.785	2.696	0.26	0.90
Dosage	4	3222.095	805.524	77.41	<0.01
Time	10	536.502	53.650	5.16	<0.01
Dosage × Time	40	998.225	24.956	2.40	<0.01
Ring chromosomes					
Block	4	2.996	0.749	1.31	0.27
Dosage	4	42.524	10.631	18.61	<0.01
Time	10	50.102	5.010	8.77	<0.01
Dosage × Time	40	69.716	1.743	3.05	<0.01
Micronuclei					
Block	4	1137.287	284.322	0.21	0.94
Dosage	4	3713260.887	928315.222	672.20	<0.01
Time	10	1209263.287	120926.329	87.56	<0.01
Dosage × Time	40	1907249.513	47681.238	34.53	<0.01
Incomplete mitosis					
Block	4	2.776	0.694	1.13	0.34
Dosage	9	27.636	3.071	5.00	<0.01
Time	4	108.816	27.204	44.28	<0.01
Dosage × Time	36	75.664	2.102	3.42	<0.01

Table 5 P values of pairwise comparisons of gamma irradiation dosage for shoot and root growth and efficiency of energy conversion into growth

P values of least squares means comparison										
T. turgidum ssp. *durum* L. cv. Orania										
Total Bridges						**Total 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
Total Micronuclei						**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

Non-significant differences in bold

Table 6 Correlations between the effects of gamma irradiation on seedling and root growth and efficiency of energy conversion into growth on the one side and the frequency of cells with different numbers of bridges, the frequency of cells with different numbers of micronuclei and the frequency of cells with incomplete mitosis on the other.

Bridges	Seedling growth		Root growth		Efficiency of energy conversion into growth	
	P value	Pearson's correlation coefficient	P value	Pearson's correlation coefficient	P value	Pearson's correlation coefficient
1 Bridge per cell	<0.01	-0.81	<0.01	-0.86	0.06	-0.44
2 Bridges per cell	<0.01	-0.90	<0.01	-0.90	<0.01	-0.65
3 Bridges per cell	<0.01	-0.79	<0.01	-0.74	<0.01	-0.68
4 Bridges per cell	0.67	-0.16	0.59	-0.20	0.89	0.05
5 Bridges per cell	0.33	-0.43	0.36	-0.41	0.22	-0.53
Micronuclei	Seedling growth		Root growth		Efficiency of energy conversion into growth	
	P value	Pearson's correlation coefficient	P value	Pearson's correlation coefficient	P value	Pearson's correlation coefficient
1 Micronucleus per cell	<0.01	-0.91	<0.01	-0.96	<0.01	-0.59
2 Micronuclei per cell	<0.01	-0.94	<0.01	-0.92	<0.01	-0.77
3 Micronuclei per cell	<0.01	-0.91	<0.01	-0.86	<0.01	-0.83
4 Micronuclei per cell	<0.01	-0.89	<0.01	-0.84	<0.01	-0.86
5 Micronuclei per cell	<0.01	-0.83	<0.01	-0.78	<0.01	-0.84
6 Micronuclei per cell	<0.01	-0.77	<0.01	-0.70	<0.01	-0.77
7 Micronuclei per cell	0.03	-0.43	0.06	-0.37	<0.01	-0.62
Incomplete Mitosis	Seedling growth		Root growth		Efficiency of energy conversion into growth	
	P value	Pearson's correlation coefficient	P value	Pearson's correlation coefficient	P value	Pearson's correlation coefficient
Interphase cells with Incomplete Mitosis	<0.01	-0.83	<0.01	-0.86	<0.01	-0.72

Figures

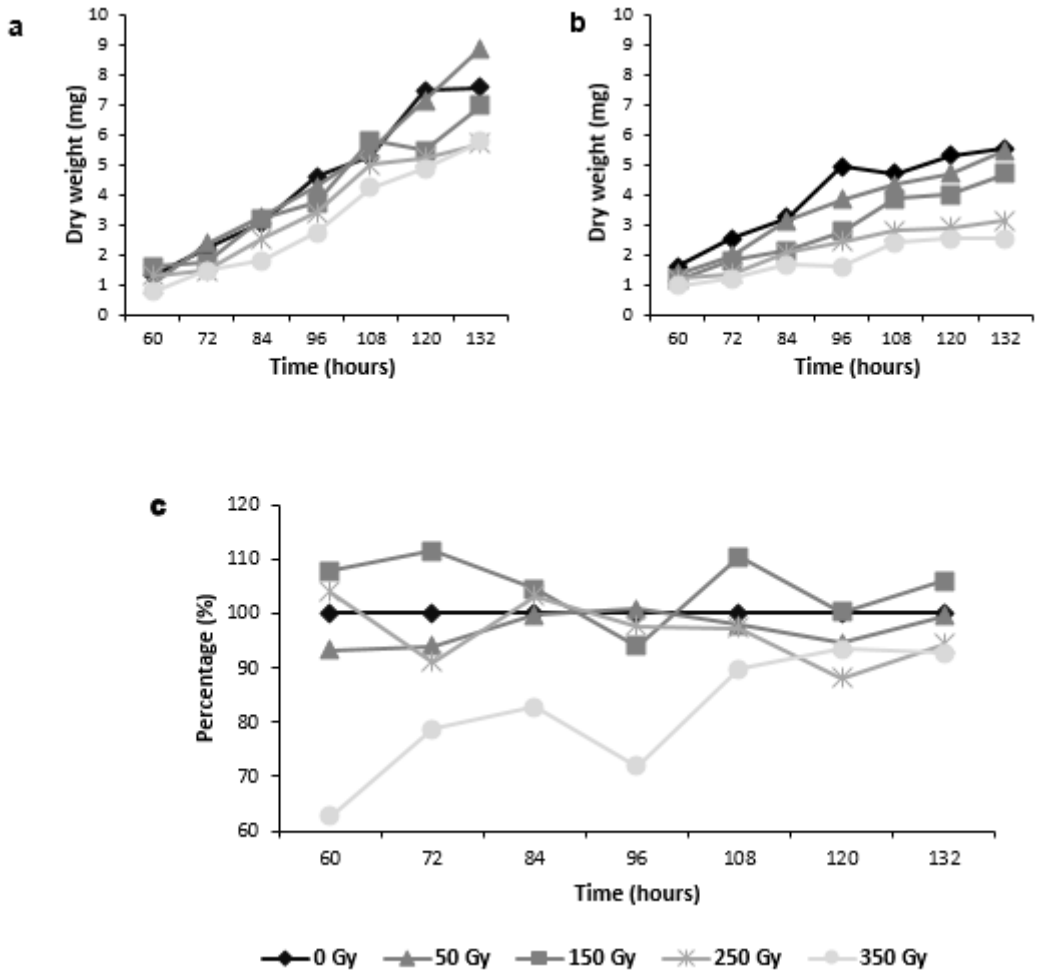


Figure 1

Effect of gamma irradiation dosages of 0, 50, 150, 250 and 350 Gy on (a) shoot growth, (b) root growth, (c) efficiency of energy conversion into growth.

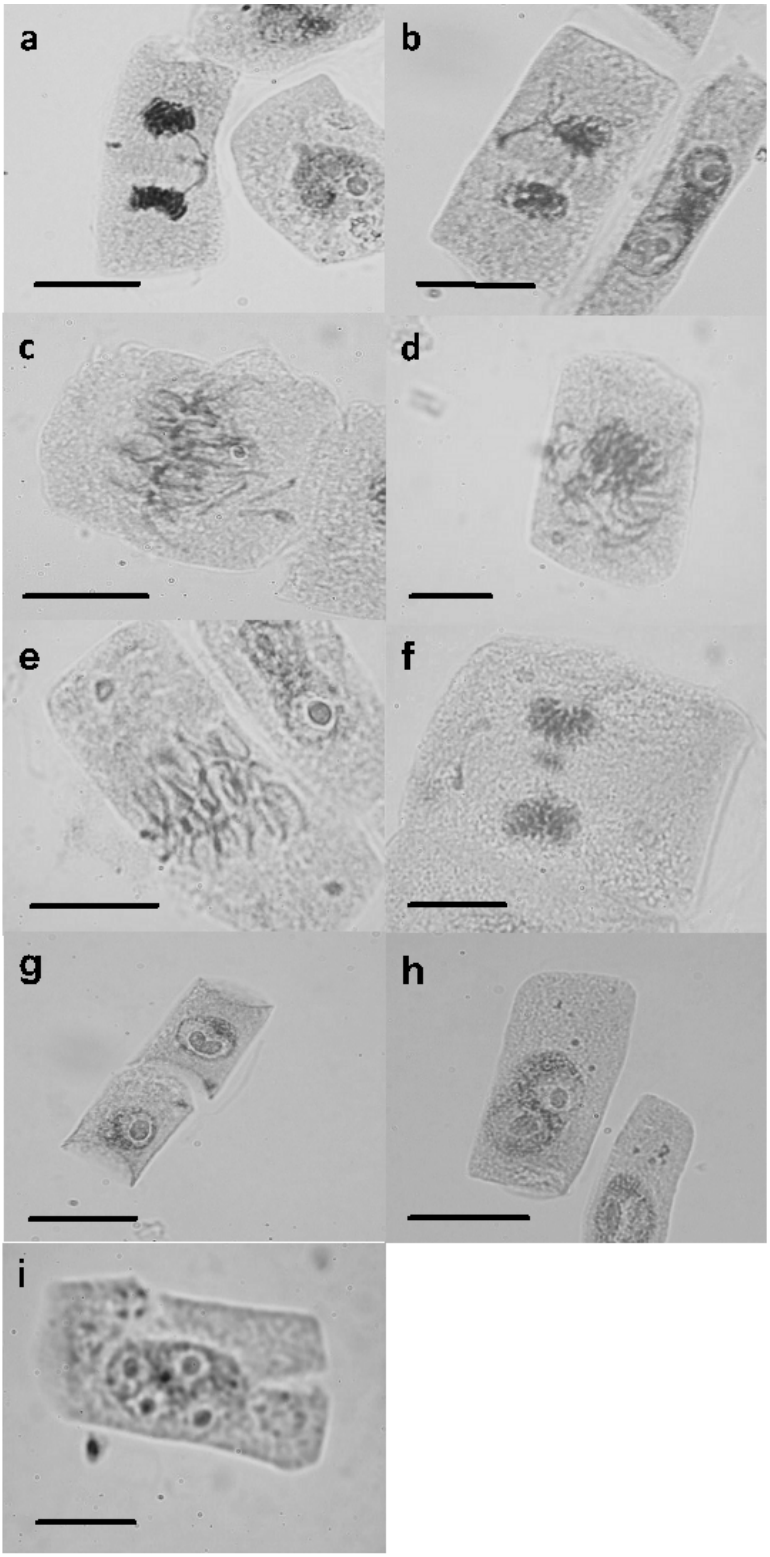


Figure 2

Chromosomal abnormalities. (a) A side-arm bridge due to half chromatid breakage. (b) Early telophase with one intact bridge and two broken bridges. (c) Metaphase with three fragments. (d) Late prophase with two fragments and a ring chromosome. (e) Metaphase with two ring chromosomes of different sizes. (f) Dividing ring chromosome in late anaphase. (g) Two daughter cells, each containing a

micronucleus. (h) Interphase cell containing three micronuclei. (i) Five nucleoli in the nucleus as well as small nucleoli in micronuclei in interphase cell with incomplete mitosis. Bar = 10 μm.

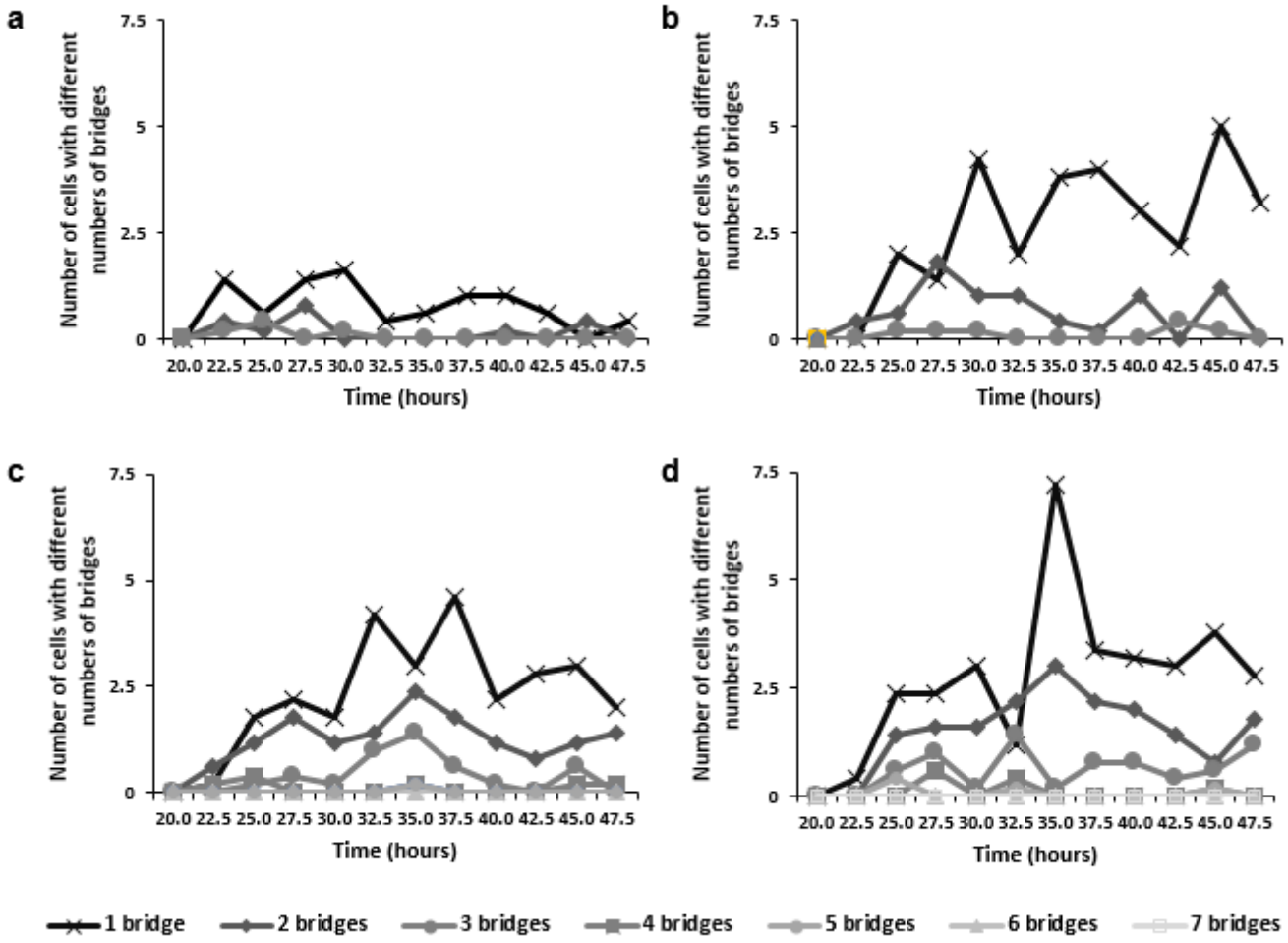


Figure 3

Number of cells with different numbers of bridges at different irradiation dosages.

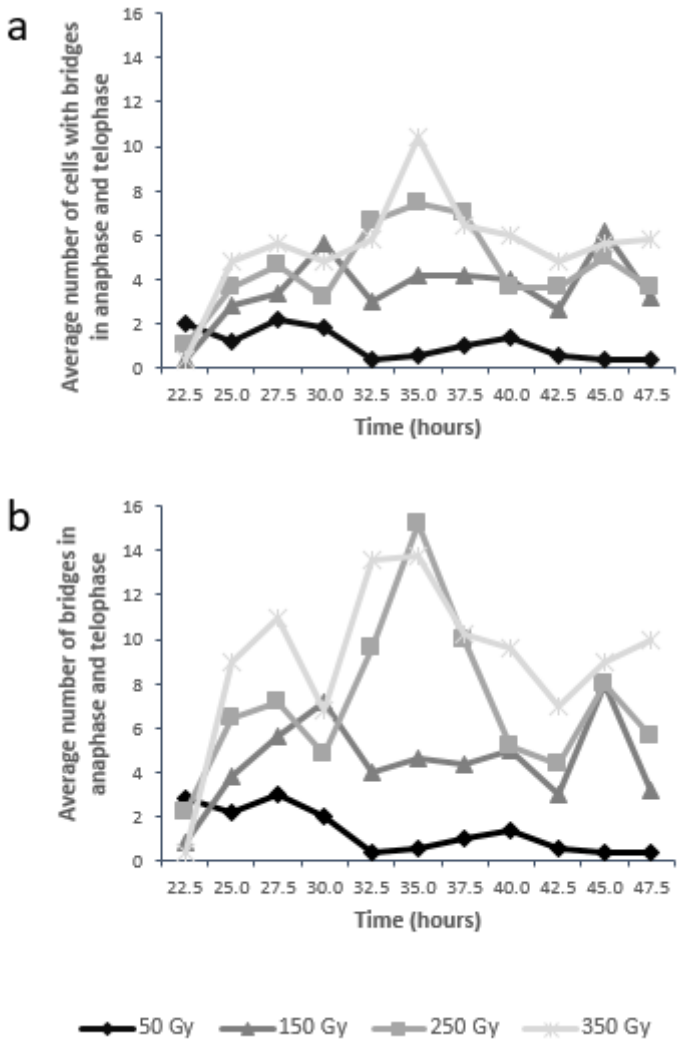


Figure 4

Bridges in anaphase and telophase cells. (a) Number of cells with bridges in anaphase and telophase. (b) Number of bridges in anaphase and telophase.

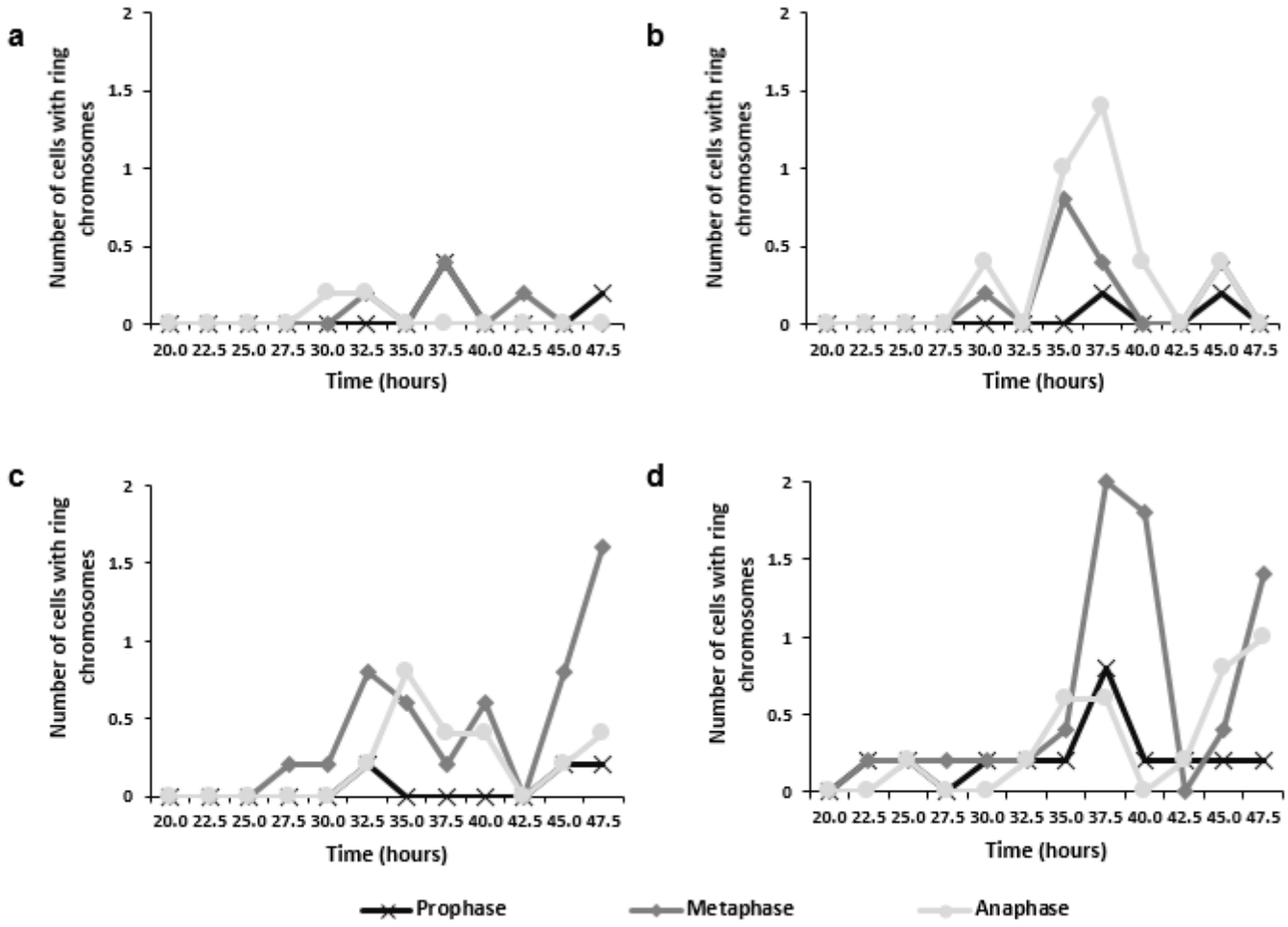


Figure 5

Number of prophase, metaphase and anaphase cells with ring chromosomes at different gamma irradiation dosages.

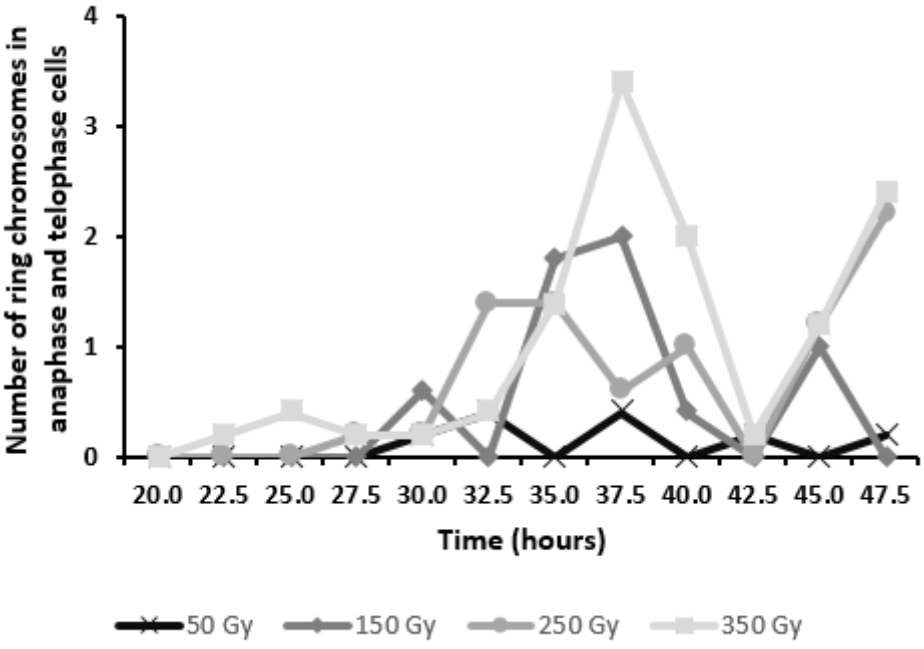


Figure 6

Number of ring chromosomes in prophase, metaphase and anaphase cells at different gamma irradiation dosages.

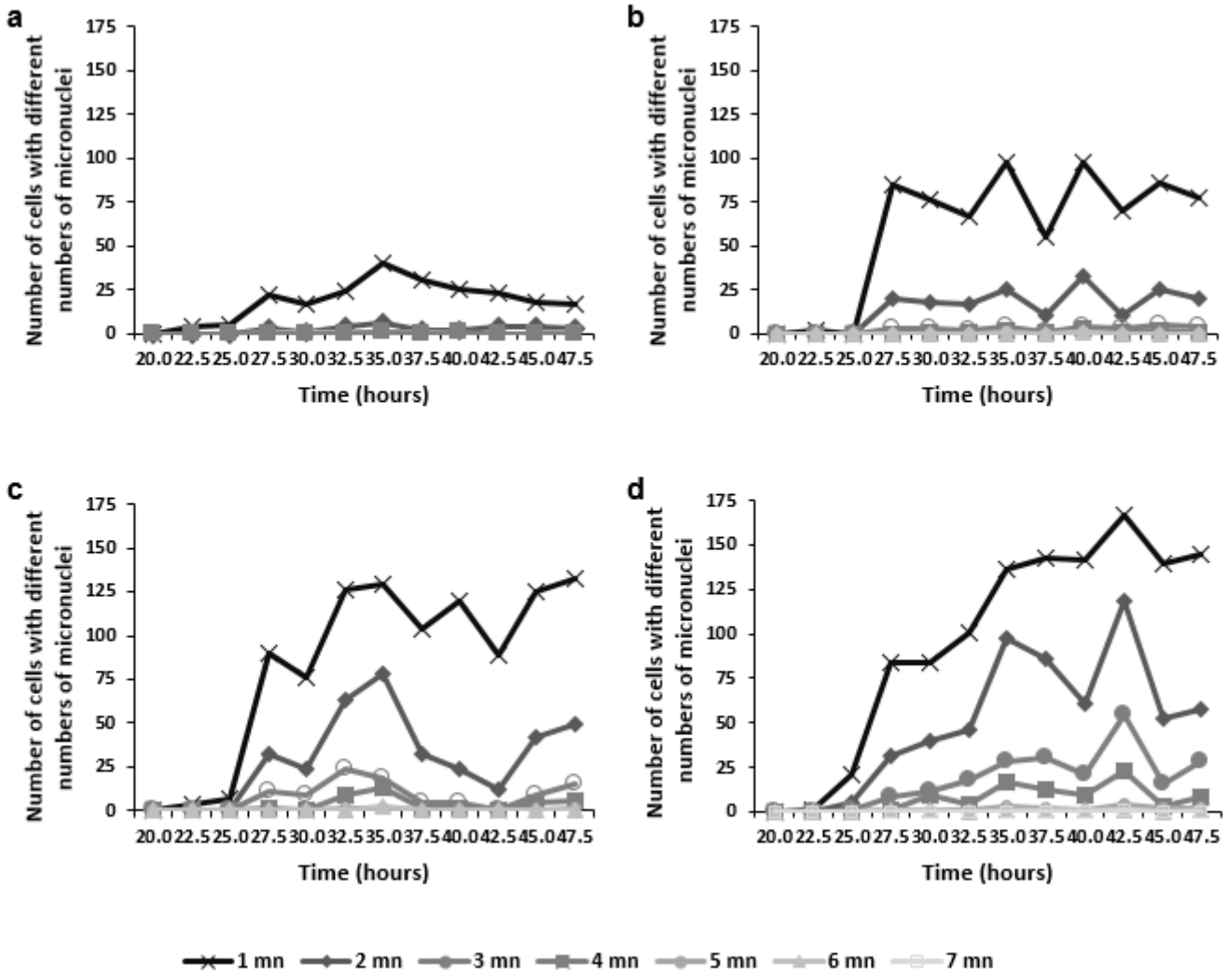


Figure 7

Number of cells with different numbers of micronuclei at different irradiation dosages. (a) 50 Gy, (b) 150 Gy, (c) 250 Gy and (d) 350 Gy.

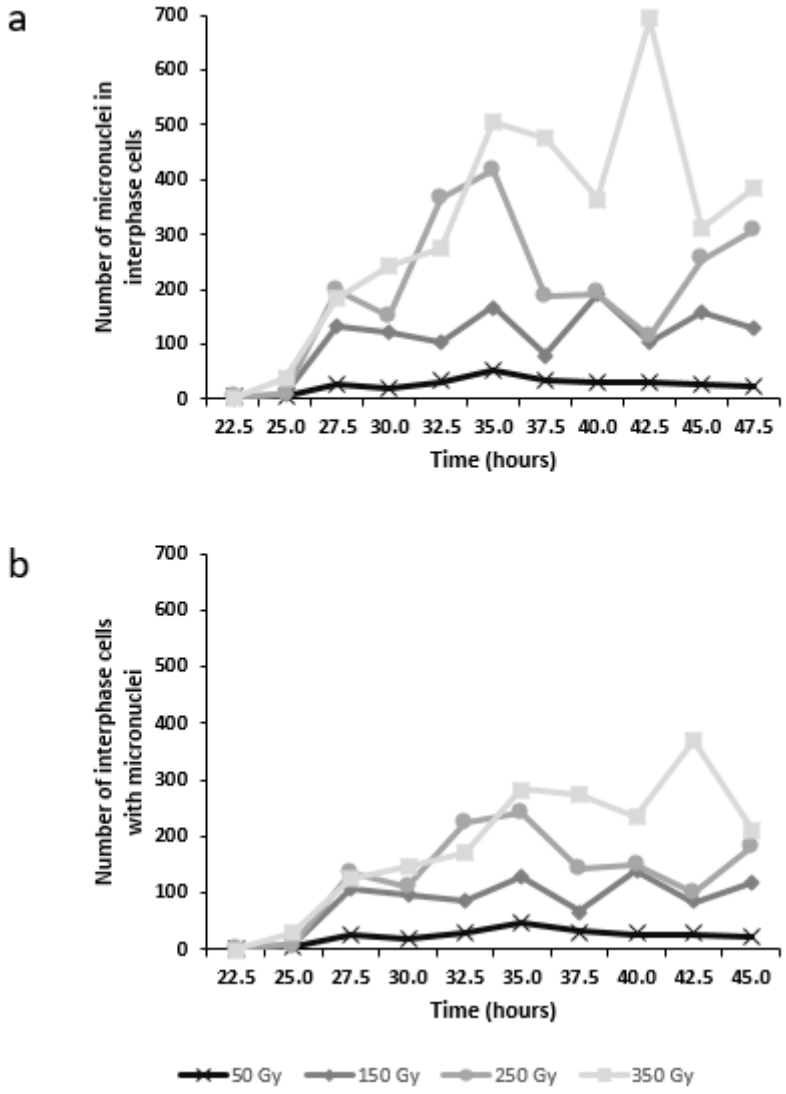


Figure 8

Number of micronuclei and number of cells containing micronuclei. (a) Number of micronuclei in 500 interphase cells (b) Number of interphase cells containing micronuclei.

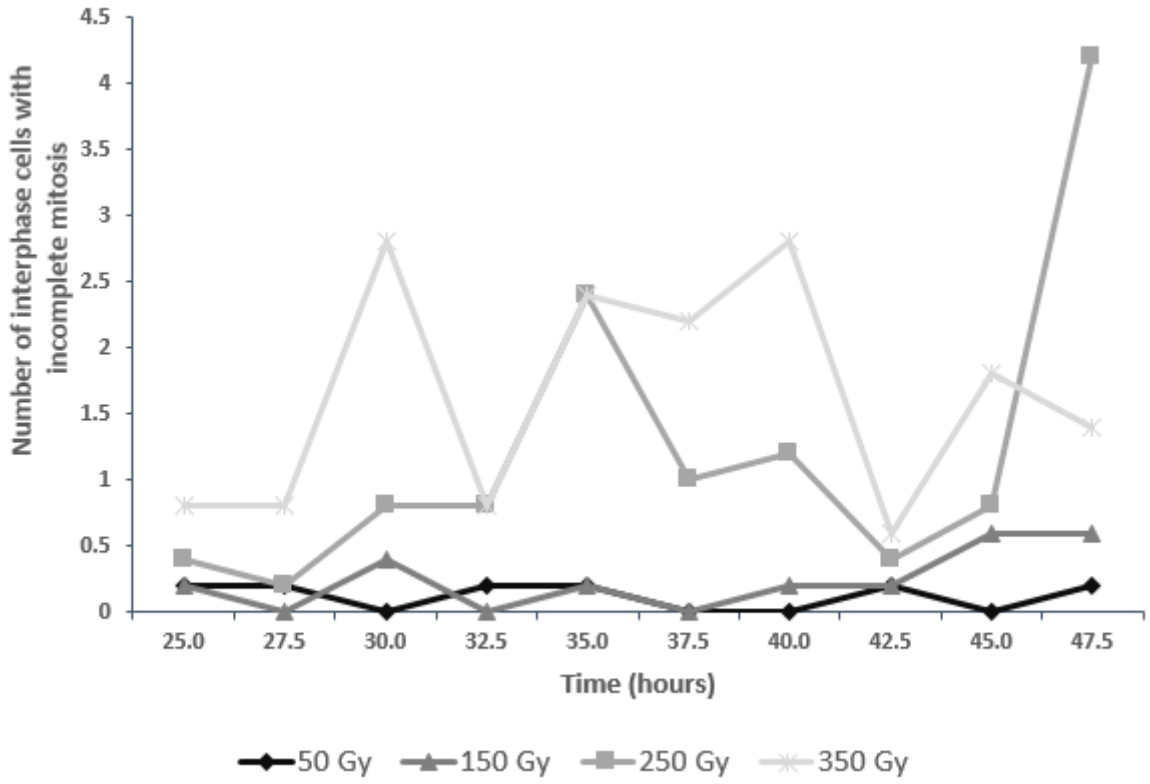


Figure 9

Number of interphase cells with incomplete mitosis out of 500 interphase cells.

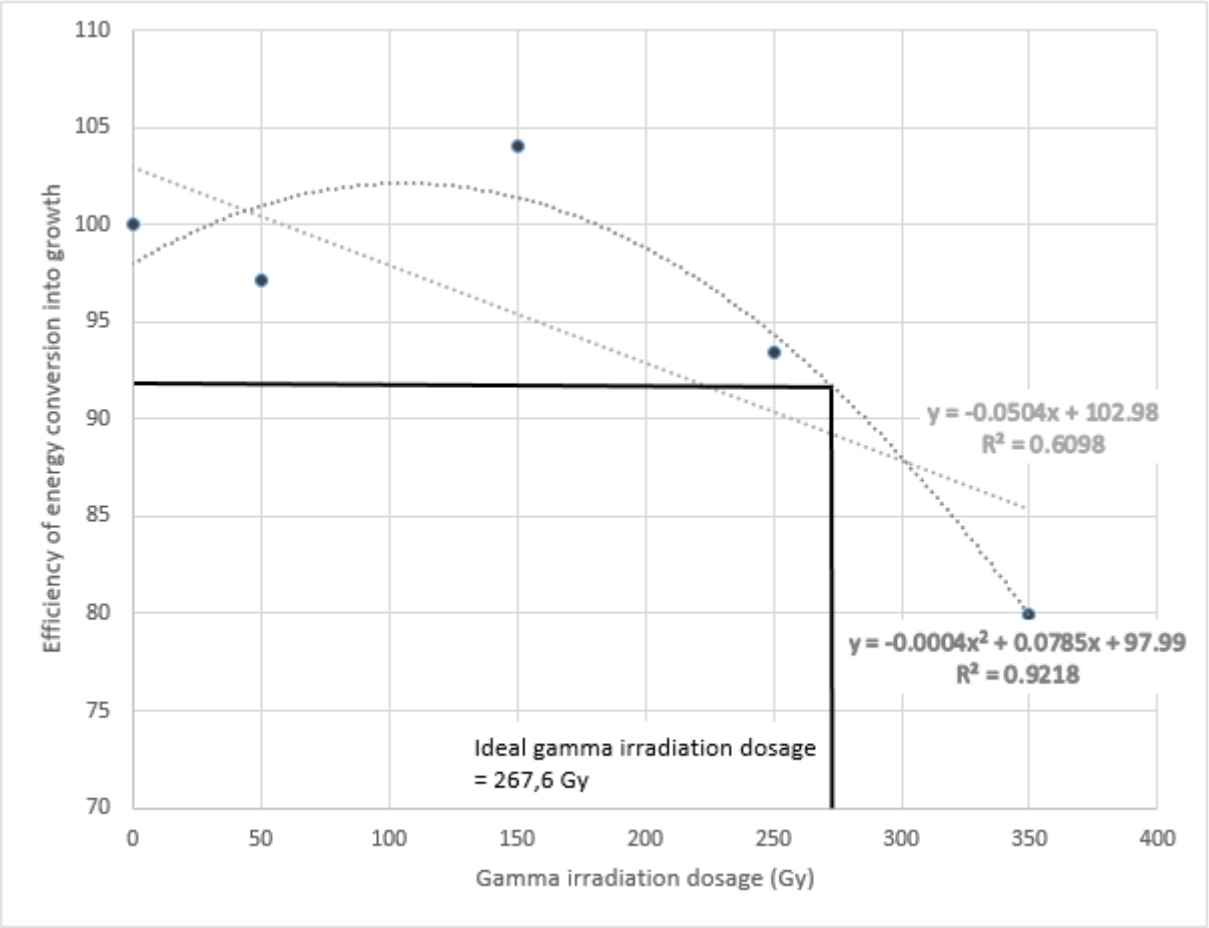


Figure 10

Determination of the ideal gamma irradiation dosage for mutation breeding.