Cytogenetic Impact of Gamma Irradiation and Its Effects on Growth and Yield of Three Soybean Cultivars

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Healthy seeds of three soybean cultivars; Crawford, Giza 111 and Giza 35 were irradiated with γ-irradiation at doses ranging from 100Gy to 600Gy with 100Gy interval. The cytogenetic impact of the applied doses was assessed in M1 and M2 plants, on mitotic activity and chromosomes in root tip meristems and vegetative growth, expressed as shoot and root length and fresh and dry weight of shoot and root as well as leaf area. The low doses of 100 and 200Gy increased mitotic activity, expressed as mitotic index MI and enhanced growth rate. In contrary, higher doses significantly retarded mitotic activity, increased the frequency of chromosomal abnormalities and reduced vegetative growth and yield, expressed as number of pods and seeds weight. The frequency of chromosomal abnormalities was dose dependent and its percentage varied among cultivars. Chromosomal stickiness and non-congression were the most common abnormalities at metaphase and chromosomal bridges and free chromosomes were the most common aberrations at ana-telophase whereas C-metaphase and c lagging chromosomes were only occasionally observed. No abnormalities were reported in the interphase of the M1 plants, but in the M2 plants, micronuclei and vacuolation of nuclei were frequently observed. The abnormalities reported here are mainly due to a clastogenic action that may have cause breakage and reunion of chromosomes. These findings emphasise the importance of assessing mitotic activity and chromosomes behaviour in plants used for mutation breeding to predict changes in vegetative traits and yield of target genotypes.

Keywords: Gamma radiation, Soybean, Mitosis, Chromosomes, Growth, Yield.
detrimental by reducing germination, growth rate, vigour or pollen and ovule fertility as well as yield (Singh, 2005). At low doses, γ-radiation has been reported to induce both useful and harmful effects on crops, so there is a need to estimate the most beneficial dose for improving specific trait(s) of the crop plant of interest (Badr et al., 2014a). Low doses of γ-radiation were used for conventional breeding of agriculturally and economically important legume crops including soybean to increase their genetic variability. Examples include Gobinath et al. (2015), El-Gazzar et al. (2016) and Gaafar et al. (2017).

Cytogenetic analyses are important in assessing genetic impact due to chemical and physical mutagens (Grant, 1999). The most common influence of γ-irradiation is the chromosomal aberrations induction and affecting the mitotic activity and yield (Melki & Salami, 2008 and Badr et al., 2014b). The most common chromosomal changes recorded in response to γ-irradiation were stickiness at metaphase, lagging chromosomes and bridges at anaphase and telophase (Dhanavel et al., 2012). However, the only recoverable chromosomal rearrangements are those that are able to produce and replicate DNA molecules and hence can stably inherit to the next generations (Tan et al., 2015). These changes provide the basis for introducing genetic variability in many plant traits (Auger & Sheridan, 2011).

In the present investigation, γ-irradiation was applied, at different doses, to explore the possibilities of inducing genetic variability in the three different cultivars of soybean. The induced cytogenetic changes were assessed in two successive generations (M₁ and M₂) following the parent’s seeds exposure to different doses of γ-radiation. The impact on some vegetative parameters and the yield components were also evaluated in M₁ and M₂ plants.

Materials and Methods

Plant materials

Three soybean cultivars; Giza 111, Giza 35, and Crawford were used in this study. Seeds were obtained from the Legumes and Field Crops Research Department, Agriculture Research Centre (ARC), Giza, Egypt. The three cultivars differ in seed characters and seed weight. Dry seeds of the three soybean cultivars were exposed to six doses of gamma irradiation at the National Centre for Radiation Research and Technology (NCRRT), Nuclear Research Centre, Inshas, Egypt, using Co⁶⁰ as a source. The applied doses were 100, 200, 300, 400, 500 and 600Gray (Gy); seeds of control samples were not exposed to irradiation.

Cytological procedures

For recording the effects of γ-irradiation on cell division and chromosomes, seeds of M₁ and M₂ soybean cultivars were surface sterilized for two minutes in 1% sodium hypochlorite followed by several washes in distilled water then grown inside a filter paper moistened with distilled water for seven days. Roots of at least 10 seedlings from each treatment were then fixed in a freshly prepared fixative composed of absolute ethanol and glacial acetic acid (3:1) for 24h and kept in 70% ethanol at 4°C until use. The Feulgen’s squash technique was used for making permanent preparations as described in Darlington & La Cour (1976) with some modifications. The Feulgen stained tips were squashed in a drop of 1% Aceto-Orcein (La Cour, 1941).

The slides were soaked in 70% ethanol for coverslips separation then, the preparations were fixed by mounting in (D.P.X). Approximately, 6000 cells for each treatment and the control were examined under the 100X oil objective lens of (JENALAB) light microscope. Mitotic activity was estimated as mitotic index (MI), which is calculated as the ratio of the number of dividing cells to the total number of cells examined. Mitotic stage index (MSI), which is calculated as the ratio of the number of dividing cells at a stage to the total number of dividing cells examined. Chromosomal abnormalities (CA) were scored at all mitotic stages and at interphase and the percentage of cells showing chromosomal abnormalities to the total number of cells at the corresponding stage.

Morphological measurements and yield evaluation

Exposed and control seeds of the three soybean cultivars were grown to maturity under the recommended conditions for growing soybean in field. Plants were irrigated every ten days from sowing until maturity and natural organic fertilizer was applied at the flowering stage. Morphological measurements were made on plants after eight weeks of sowing. The measured traits were length of shoot and root and their fresh and dry weights as well as leaf area and leaves number. At maturity, yield was evaluated by measuring the number of
of pods per plant and weight of 100 seeds. The morphological data were statistically analysed using the one-way analysis of variance (ANOVA) to determine the significance of the variations between treatments. The least significant differences (LSD) were used to determine the level of significance of differences between treatments as compared to their control at 0.05 and 0.01 levels of significance. These statistical methods were performed using the Microsoft office-Excel 2007 and the SPSS version 21 software.

Results

Impact of γ-irradiation on mitotic index (MI)

The mitotic activity in root tip cells of the three *Glycine max* cultivars used in this study was scored as MI values (Table 1). For the M₁ plants the Crawford cultivar showed the highest mitotic activity (MI = 11.17±0.44) and lower MIs (10.45±0.42) and (10.61±0.38) were scored for cv. Giza 111 and cv. Giza 35 cultivars, respectively. The low γ-radiation doses of 100Gy and 200Gy caused a significant increase in the MI values in the three cultivars compared to their controls. High values of 12.25±0.30 and 12.27±0.31 were scored for cv. Crawford. In contrast, the 500Gy and 600Gy doses significantly decrease the MI values for all cultivars. Lower values of 8.21±0.24 and 7.82±0.19, respectively were scored for cv. Giza 35 and cv. Giza 35 cultivars, respectively. The applied γ-radiation doses of 400Gy and 500Gy doses showed a highly significant reduction in the MI values for both cv. Giza 35 and cv. Giza 111 while a non-significant reduction was observed for cv. Crawford.

Interestingly, the mitotic activities in the M₂ plants displayed similar pattern to M₁ plants (Table 2). The cv. Crawford scored the highest MI value of 11.17±0.48, while cv. Giza 111 scored the lowest value of MI (10.21±0.43). The effect of applied γ-radiation was doses dependent regardless of the cultivar type. Generally, the low doses of 100Gy and 200Gy significantly enhanced mitotic activity for all cultivars; MI reached to 12.76±0.32, 12.97±0.49 and 12.37±0.47 for cv. Crawford, cv. Giza 35 and cv. Giza 111, respectively at the dose of 200Gy. On other hand, the 400Gy and 500Gy showed a significant to highly significant reduction in MI in the three cultivars. While the 300Gy dose shows a non-significant reduction in all cultivars.

Frequency and types of chromosomal abnormalities

All doses of γ-radiation induced highly significant elevations in the proportion of cells showing chromosomal abnormalities. This elevation was proportional to an increment of γ-radiation dose in both M₁ and M₂ generations. In M₁ plants, both cv. Giza 111 and cv. Giza 35 showed high percentage of chromosomal abnormalities at the dose of 600Gy (23.68±1.93 and 22.91±2.93) respectively, compared to 16.78±2.49 in cv. Crawford at the same dose. Meanwhile, the lower doses of 100Gy and 200Gy induced abnormalities but at lower levels. In cv. Giza 35, much lower proportions of abnormalities (4.31±0.64) were recorded compared to the other cultivars at a dose of 100Gy (Table 1).

Interestingly, in M₁ plants no chromosomal abnormalities were observed for the cells at interphase and prophase stages. The highest proportional of chromosomal abnormalities was observed at metaphase and ana-telophase in all cultivars (Table 1). Non-congregation and stickiness were the most predominant types of abnormalities at metaphase stage. While Chromosome bridge and free chromosome were the most common abnormalities observed for all cultivars at ana-telophase stage. In M₂ plants the highest significant percent of chromosomal abnormalities was observed for Crawford cultivar (45.20±1.66) at γ-irradiation dose of 400Gy. While the lower value of (7.69±0.66) was observed for Giza 111 at 100Gy of γ-irradiation dose.

Interestingly, in M₂ plants chromosomal abnormalities were observed for the cells at interphase stage of M₂ plants however to low extent. All γ-irradiation doses showed greatly varied proportional values of vacuolated nuclei, irregular shaped nucleus and micronucleus at interphase stage for the three cultivars. Like M₁ plants the highest proportional of chromosomal abnormalities for M₂ plants was observed at metaphase, anaphase and telophase in all cultivars. Non-congregation and stickiness were the most predominant types of abnormalities at metaphase stage. While chromosome bridge and free chromosome were the most common abnormalities observed for all cultivars at anaphase and telophase stage in addition to lagging chromosomes (Table 2 and Fig. 1).
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* and ** LSD test at 0.05 and 0.01 significance levels, respectively.
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* and ** LSD test at 0.05 and 0.01 significance levels, respectively.
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The impact of γ-radiation on growth and yield

All vegetative growth parameters measured, in the current study, clearly indicate that the low doses of 100Gy and 200Gy were highly significantly effective in enhancing vegetative growth of the shoot and root lengths, fresh and dry weights also, caused a similar influence on leaf measurements at early stages of growth of 8 weeks after sowing. In contrary, all high doses of 300Gy to 500Gy were highly significantly reducing all growth parameter measurements. Figures illustrating variations in shoot and root length, dry weight, leaf number per plant and leaf area of M1 plants of the three soybean cultivars following parent seed exposure to different doses of γ-radiation are shown in Fig. 2 (A-I), respectively. Moreover, an increased sensitivity of cv. Crawford to the high dose of 500Gy was noticed and causes a detrimental effect on its growth leading to complete death of the M2 plants. The same morphological observations at the low doses of 100Gy and 200Gy and high doses of 300Gy to 500Gy were generally maintained in M2 plants (data not shown).

Egypt. J. Bot. 58, No.3 (2018)
As observed for the vegetative traits measurements, the low doses of 100Gy and 200Gy resulted in a highly significant increase in the measured yield parameters, i.e., the number of pods per plant, number of seeds per plant and weight of 100 seeds in the three soybean cultivars. In contrary, a significant retardation in all yield parameters that were measured was observed following exposure to 300Gy, 400Gy and 500Gy doses in both M₁ (Fig. 3 A-C) and M₂ plants (data not shown).

Fig. 2. Histograms illustrating changes in some vegetative parameters of M₁ plants of three soybean cultivars following seed exposure to different doses of γ-radiation. (A) Shoot length, (B) Root length, (C) Shoot fresh weight, (D) Root fresh weight, (E) Shoot dry weight, (F) Root dry weight, (G) leaves number/plant, (H) Leaf area.

Discussion

The positive and negative consequences of γ-radiation were evaluated by comparison to non-irradiated plants for two successive generation of soybean. Mitotic index was used as an indicator to describe the cell activity and proliferation (Simon et al., 2014); it was significantly increased, in both $M_1$ and $M_2$, in the three soybean cultivars when the parent’s seeds were exposed to low doses of 100Gy and 200Gy of γ-radiation. In the contrary, higher doses of 500Gy and 600Gy negatively affect the MI values in all cultivars. Similar inhibitory effect on the mitotic activity following exposures to high doses of γ-radiation was observed in $M_1$ and $M_2$ plants of cowpea (Badr et al., 2014b) and faba bean (El-Gazzar et al., 2016). Low doses of gamma irradiation could stimulate the production of few reactive oxygen species (ROS) (Smith et al., 2012) that mediate the acceleration of cell cycle entry to G0/G1 leading to a positive effect on the plant cell cycle machinery (Fehér et al., 2008).

Although, normal cell cycle segregation was displayed in the $M_1$ plants; a significant increase in the mitotic chromosomal abnormalities was observed following exposure to γ-irradiations, most likely is a result of increased sensitivity of chromatin in mitotic cells to radiation than the dispersed chromatin of interphase cells (Stobbe et al., 2009). However, in $M_2$ plants, considerable proportions of interphase cells showed nuclear abnormalities. The level of chromosomal rearrangements observed was proportional to the γ-irradiations dose. The chromosomal abnormalities were stickiness and non-congression at metaphase and free chromosome, chromosome laggards and bridges at ana-telophase stages. Similar observations were observed in other legumes following exposure to γ-irradiations such as cowpea (Dhanavel et al., 2012 and Badr et al., 2014b) and faba bean (El-Gazzar et al., 2016 and Nurmansyah et al., 2017). High doses of ionized radiation induce DNA double-strand breaks which trigger genetic instability if persisted without repair and lead to gross chromosomal rearrangements to alleviate the destabilizing effect of the radiation. This allows cell survival with a delay in the passage of cells through the G2/M phase cell-cycle checkpoint (De Veylder et al., 2003 and De Simone et al., 2017). Low doses of γ-irradiations, on the other hand stimulate the production of few reactive oxygen species (ROS) (Smith et al., 2012) that mediate the acceleration of cell cycle entry to G0/G1 leading to enhanced plant cell cycle machinery (Fehér et al., 2008).

Fig. 3. Histograms illustrating changes in some yield parameters of M1 plants of three soybean cultivars following seed exposure to different doses of γ-radiation. (A) Pod number/plant, (B) Seed number/plant, (C) weight of 100 seeds.
Chromosome stickiness might be due to changes in specific non-histone proteins, histone proteins and DNA breaks induced during chromosome condensation (Gaulden, 1987 and Piskadlo et al., 2017). Chromosome non-congression represent an expelled chromosomes at metaphase due to improper balance between opposing pulling action of kinetochore and/or pushing ejection forces of the poles along chromosome arms that fails to reach an equilibrium near the spindle equator (Maiaota et al., 2017). A mitosis-specific and R loop–driven ATR pathway promotes faithful chromosome segregation, it stimulates Aurora B through Chk1, preventing formation of lagging chromosomes (Kabeche et al., 2018). The free and the lagging chromosomes at ana-telophase might be formed due to the failure of spindle fibres to push the respective chromosomes to the poles due to failure in the ATR pathway due to exposure to γ-irradiations.

The behavior of laggards is characteristic in that they generally lead to micronuclei formation (Badr, 1987 and Kumar & Rai, 2006). At telophase the segregated sister chromatids de-condense and the nuclear envelope re-forms around them, the same happens for the spatially expelled free chromosomes or chromosome fragments leading to the formation of the micronucleus (Potapova & Gorbsky, 2017). Micronuclei also arise if laggards or non-oriented free chromosomes that fail to reach the poles in time to be in the main telophase nucleus (Utsunomiya et al., 2002). Micronuclei derived from a whole chromosome, due to lagging, have a higher probability to survive and undergo condensation in synchrony with the main nuclei than micronuclei derived from a chromosome fragment (Gustavino et al., 1987). Micronuclei often serve as a marker of chromosomal instability, so it used as a tool to assess the genotoxicity of various environmental chemicals and other hazardous substances. However, Luzhna et al. (2013) proposed that micronucleus formation may precisely reflect individual sensitivity due to single gene polymorphisms.

Chromosome bridges were commonly observed during anaphase and telophase, in M₁ and M₂ plants, indicating a clastogenic effect caused by breakage and fusion of chromatids or sub-chromatids (Badr, 1987 and Grant, 1999) indicating stable structural aberrations that are transmissible such as inversions, translocations and some small deletions. Bridges reported here like bridges produced by other mutagenic agents might have arisen through breaks followed by reunion of chromosomes (Kumar et al., 2003 and Kumar & Rai, 2006) or due to stickiness of chromosome at metaphase and their failure to separate at anaphase (Grant, 1999 and Dhanavel et al., 2012). Most likely, the γ-irradiation induces chromosomal breaks in two chromosomes that tend to reunite forming a chromosomal connection between the two poles (Pampalona et al., 2016).

Low radiation levels of 100Gy and 200Gy highly promoted plant growth and yield of M₁ plants that constantly maintained in the M₂ plants. While high levels of 300Gy to 500Gy of γ-irradiation negatively affected both plant growth and yield causing deleterious damage, particularly on the M₂ generation of cv. Crawford, which failed to grow to flowering and hence seed production. The positive effect on vegetative and yield in plants grown from seeds exposed to 100Gy and 200Gy is associated with a similar positive effect on mitotic activity in the root meristems. Meanwhile, the reduction in growth and yield at high doses of γ-irradiation are associated with reduced mitotic activity in the three soybean cultivars compared to their controls. Similar correlation, i.e “low dose-high growth and yield” was also observed in other legumes including cowpea (Badr et al., 2014a; b), lens (Kumar et al., 2003), common bean (El-Gazzar et al., 2016) and in soybean (Gobinath et al., 2015 and Gaafar et al., 2017).

The enhanced effect of low doses of irradiation may be the result of a “radiation hormesis” due to transfer of energy to cellular atoms practically, hydrogen (H), carbon (C), oxygen (O), nitrogen (N) and phosphorus (P) that may lead to stimulating effect on the physiological reactions in living cells including, cell division and growth (Yadav, 2016). On other hand, higher doses of γ-irradiation impair physiological processes leading to cytotoxic effects. These effects may be produced as a response to elevated levels of oxidative stress that exceeds the capacity of cellular antioxidant defences to remove stress (Taguchi & Kojima, 2005). In association, DNA damage repair mechanisms may alleviate the encountered damage and enable the plants to survive (Datta et al., 2011 and De Simone et al., 2017).

The measured yield parameters, i.e., number of pods per plant and the 100-seed weight, increased
following exposure to the 100Gy dose. While the 300Gy, 400Gy and 500Gy doses significantly reduced all yield parameter in the three used cultivars in M₁ and M₂ plants. However, a deleterious effect was observed for the Crawford cultivar at the 500Gy dose of γ-irradiation in M₁ plants. This finding is proportionally correlated with the measurements of vegetative parameters. Improvement of agronomic traits by using γ-irradiation has been reported in other legumes. In cowpea, low γ-irradiation doses of 100 and 200Gy enhanced yield; interestingly, this improvement was cultivar dependent (Badr et al., 2014b). Similar findings were found in faba bean (El-Gazzar et al., 2016). Low doses of γ-radiation were also used to increase the genetic variability in soybean (Gobinath et al., 2015) and cowpea (Badr et al., 2014a; b and Gaafar et al., 2017).

Conclusions and Recommendations

Low doses of γ-irradiation (100Gy and 200Gy) enhanced mitotic activity in the root tip meristems of three soybean cultivars (Crawford, Giza 35, and Giza 111) that has been reflected as increased vegetative growth and improved yield. However, high doses (300Gy to 600Gy) reduced M₁ vegetative growth and yield; the later dose was lethal to cv. Crawford. The frequency of chromosomal abnormalities at mitosis was dose dependent and its percentage varied among cultivars, but nuclear abnormalities were only observed in the M₂ generation plants. The selection of individual plants in the M₂ generation can be studied to observe the spectrum of variation for traits and observation of mutants, synchronous maturation in M₃ and M₄ generations that can be used as donors for restructuring soybean genotypes. When transmitted to the next generations, mutations could boost adaptive genome evolution and generate new beneficial traits.

References


Gaafar, R.M., Elshanshory, A.R., Hamouda, M. and


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Stobbe, C.C., Park, S.J. and Chapman, J.D. (2009) The radiation hypersensitivity of cells at mitosis, (Received 24 / 4 / 2018; accepted 14 / 5 / 2018)