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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-irradiationat doses ranging from 100Gy to 600Gy with 100Gy interval. The cytogenetic impact of the applied doses was assessed in M_1 and M_2 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 M_1 plants, but in the M_2 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 invegetative traits and yield of target genotypes.

Keywords: Gamma radiation, Soybean, Mitosis, Chromosomes, Growth, Yield.

Introduction

Soybean (*Glycine max* (L) Merr; 2n=40) is an annual economically important grain legume crop. It is a commercial crop in many countries as a source of food, feed, as well as use in pharmaceutical and agricultural industry. In addition, soybean is an excellent crop for agriculture cropping systems due to its ability to fix atmospheric N_2 symbiotically with sufficient populations of effective rhizobia, a process that leads to increased soil fertility (Keyser & Li, 1992; Muller et al., 1998 and Tairo & Ndakidemi, 2013). Mature seeds of soybean contain, approximately 35% protein, 31% carbohydrate, 17% fats, 5% mineral and 12% moisture (Messina, 2008). In addition, soybean protein is rich in the valuable amino acid lysine (5%) in which most of the cereals is deficient. Moreover, it contains a good quantity of minerals, salts and vitamins. Increased demand of soybean as a multipurpose legume has encouraged using

breeding new varieties to increase crop yield and improve the traits of interest (Mudibu et al., 2012; Gobinath et al., 2015 and Kusmiyati et al., 2018).

In plants, breeding is often based on induction of genetic and morphological variations using induced mutations, which has been one of the most efficient methods of breeding in the last five decades. Radiation is an excellent means of stimulating the expression of recessive genes, thus inducing new genetic variation (Song & Kang, 2003). Since 40 decades ago, gamma rays were found to be more effective than other mutagens in producing viable mutants (Micke, 1984). However, γ-radiation is ionizing radiation,with low linear energy transfer (LET) that may lead to inherited genetic variation and complicated chromosomal alterations (Sachs et al., 2000). It generates free radicals when interact with a living cell. These radicals may affect all biological activities of the cell (Reisz et al., 2014). High doses can also be

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 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 (M_1 and M_2) 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 M_1 and M_2 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 Co^{60} 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 M_1 and M_2 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 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 M_1 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 Giza 111. Also, the 300Gy and 400Gy 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 M_2 plants displayed similar pattern to M_1 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 reductionsin 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 M_1 and M_2 generations. In M_1 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 (Table1).

Interestingly, in M_1 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-congression 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 M_2 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.

In contrast to the M_1 plants, chromosomal abnormalities were observed for the cells at interphase stage of M_2 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 M_1 plants the highest proportional of chromosomal abnormalities for M_2 plants was observed at metaphase, anaphase and telophase in all cultivars. Non-congression 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).

TABLE 1. Number of total cells examined, interphase percentage, the value of MI and the percentages of abnormal cells at mitotic stages in the root tips of M1 seedlings of the three soybean cultivars following parent's seeds exposure to the applied doses of γ-irradiation.

Cultivar	γ-Dose	Total cells examined	Mitotic index	Interphase %	Metaphase				Ana-telophase		Total Abnormalities %
					Stickiness	Non-congression	C-metaphase	Chromosomal bridge	Lagging chromosome	Free chromosome	
Crawford	Control	5079	11.17±0.44	88.83	41.67	58.33	0.00	00.0	0.00	0.00	1.95±0.83
	100Gy	6374	12.25±0.30*	88.75	26.31	63.16	10.53	71.43	0.00	0.00	9.34±3.54*
	200Gy	4056	12.27±0.31*	87.73	28.57	28.57	28.57	0.00	0.00	33.33	8.87±0.53*
	300Gy	5204	10.78±0.39	89.22	38.71	61.29	0.00	34.16	0.00	53.76	8.30±1.72*
	400Gy	4816	10.61±0.40	89.39	53.85	46.15	0.00	33.33	0.00	58.33	17.98±0.90 **
	500Gy	5186	9.23±0.48**	90.77	26.09	65.22	8.70	20.08	26.32	66.75	13.46±1.68**
	600Gy	5007	7.63±0.62**	92.37	18.42	76.32	5.26	70.00	0.00	30.0	16.78±2.49**
Giza 35	Control	7139	10.61±0.38	89.39	15.00	85.00	0.00	0.00	0.00	0.00	3.11±0.54
	100Gy	6236	11.32±0.27*	88.68	18.18	81.82	0.00	87.5	0.00	0.00	4.31±0.64
	200Gy	6178	12.24±0.13**	87.76	35.71	64.28	0.00	42.08	9.16	34.16	7.11±0.67
	300Gy	6120	9.48±0.13**	90.52	41.18	58.82	0.00	50.00	0.00	12.5	9.33±1.25*
	400Gy	6484	9.51±0.19**	90.48	5.55	94.44	0.00	38.70	0.00	40.92	14.72±1.90**
	500Gy	5920	9.38±0.27**	90.62	50.00	50.00	0.00	71.43	0.00	4.76	17.46±2.98**
	600Gy	6402	9.36±0.12**	90.64	21.74	78.26	0.00	49.46	0.00	48.92	22.91±2.93**
Giza 111	Control	4383	10.45±0.42	89.55	25.00	43.75	31.25	0.00	0.00	0.00	3.86±0.98
	100Gy	4936	11.02±0.32*	88.98	35.29	64.71	0.00	36.90	2.78	43.65	7.27±1.42
	200Gy	6495	11.1±0.35*	88.89	9.09	90.91	0.00	16.66	0.00	33.33	8.89±2.14
	300Gy	6182	8.96±0.25**	91.03	41.67	58.33	0.00	33.33	0.00	36.66	15.90±1.97**
	400Gy	5064	8.79±0.32**	91.20	41.18	58.82	0.00	33.33	16.67	30.20	19.42±2.24**
	500Gy	3755	8.21±0.24**	91.79	35.29	64.71	0.00	44.44	0.00	50.00	17.08±3.29**
	600Gy	4814	7.82±0.19**	92.18	10.53	89.47	0.00	33.65	2.78	44.99	23.68±1.93**

* and ** LSD test at 0.05 and 0.01 significance levels, respectively.

TABLE 2. Number of total cells examined, the interphase percentage, the values of M_1 and the percentages of abnormal cell at each mitotic and interphase stage in the root tips of M2 seedlings of the three soybean cultivars following soybean seeds exposure to the applied doses of γ-irradiation.

Cultivar	γ-Dose	Total cells examined	Mitotic index	Interphase %				Metaphase			Ana-telophase			Total Abnormalities percent
				Total percent	Vacuolation	Irregular nucleus	Micro nucleus	Stickiness	Non-congression	C-metaphase	Chromosomal bridge	Lagging chromosome	Free chromosome	
Crawford	Control	6025	11.17±0.48	88.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	100Gy	6058	12.58±0.64*	87.42	50.00	42.10	7.90	58.80	41.20	0.00	38.06	24.56	45.21	9.40±1.30**
	200Gy	5869	12.76±0.32*	87.23	54.20	27.10	18.70	70.00	30.00	0.00	40.00	22.63	37.25	14.30±0.55**
	300Gy	6021	10.26±0.54	89.73	80.80	14.16	5.00	64.00	36.00	0.00	38.15	45.8	38.95	29.70±1.7**
	400Gy	5664	8.73±0.27**	91.26	61.60	26.02	12.30	59.30	40.60	0.00	34.15	25.3	36.6	45.20±1.66**
Giza 35	Control	6005	11.16±0.43	88.84	0.00	0.00	0.00	0.0	0.00	0.00	0.00	0.00	0.00	0.30±0.18
	100Gy	5979	12.76±0.66	87.23	66.60	25.00	8.30	80.00	20.00	0.00	26.35	30.75	42.85	10.50±0.96**
	200Gy	5851	12.97±0.49*	87.03	63.90	27.80	8.20	62.50	37.50	0.00	20.8	29.4	49.2	13.90±0.81**
	300Gy	5876	10.24±0.37	89.75	63.20	25.00	11.80	63.00	27.30	9.10	27.21	29.99	42.85	19.40±1.03**
	400Gy	5728	8.94±0.57**	91.06	50.60	29.90	19.40	47.10	52.90	0.00	32.12	45.29	22.58	29.96±2.2**
	500Gy	6092	8.80±0.31**	91.20	49.30	35.20	15.40	35.70	64.30	0.00	19.71	40.26	40.03	30.05±1.25**
Giza 111	Control	6019	10.21±0.43	89.78	0.00	0.00	0.00	0.0	0.00	0.00	0.00	0.00	0.00	0.14±0.14
	100Gy	5950	12.23±0.73*	87.76	60.90	20.33	18.69	63.50	36.50	0.00	32.54	5.83	61.60	7.69±0.66**
	200Gy	5980	12.37±0.47*	87.60	53.20	29.90	33.16	38.00	48.50	12.50	44.9	4.16	53.00	14.00±1.0**
	300Gy	6008	8.92±0.27	91.10	56.79	25.18	18.03	70.30	25.90	3.70	20.36	18.46	61.16	19.17±0.75**
	400Gy	5948	8.33±0.51*	91.66	41.66	22.90	35.40	62.06	37.90	0.00	36.56	15.69	50.14	19.40±1.3**
	500Gy	5631	8.58±0.67*	91.42	35.70	30.30	33.90	69.20	30.70	32.90	31.20	26.65	42.05	25.90±2.1**

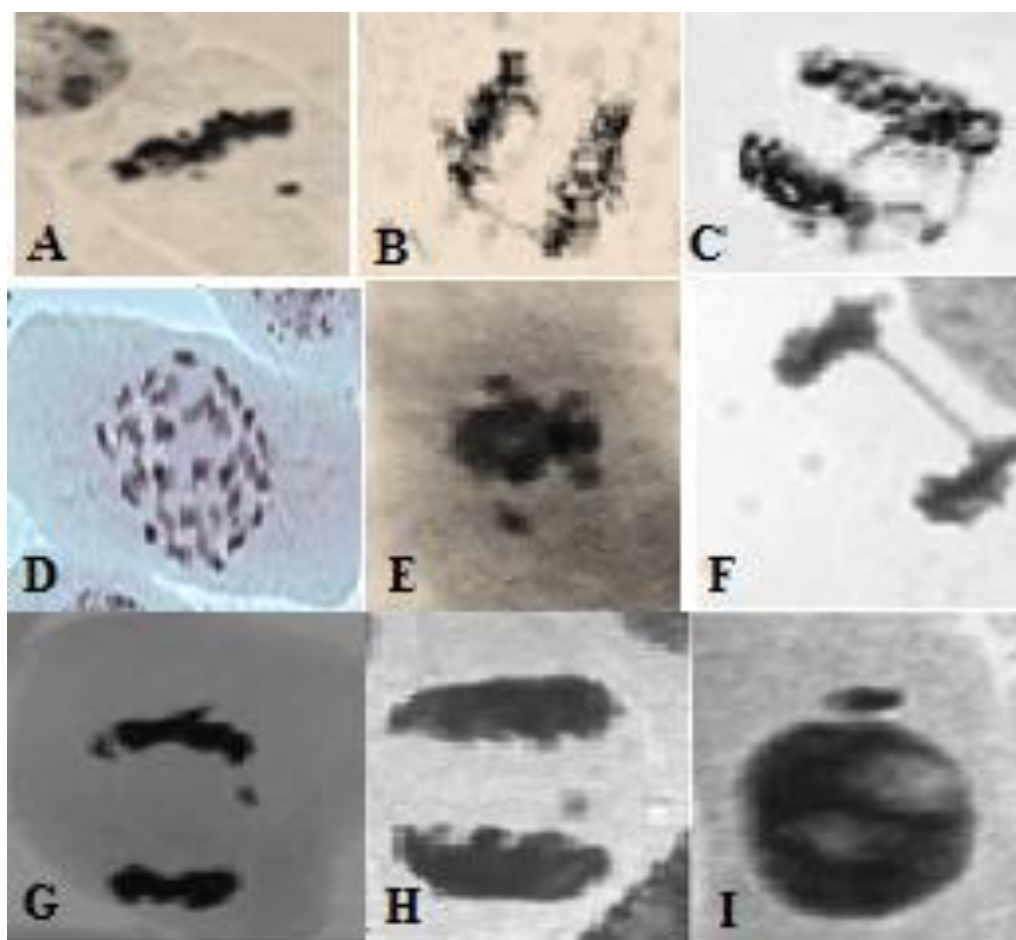* and ** LSD test at 0.05 and 0.01significance levels, respectively.

Fig. 1. Types of chromosomal abnormalities in the root tips of M1 seedlings of the three soybean cultivars following seeds exposure to the applied γ-irradiation doses. (A) Non congression chromosome at sticky metaphase induced by 200Gy in M1 seedlings of cv. Giza 111. (B) Chromatid bridge at anaphase induced by 100Gy in M1 seedlings of cv. Giza 111. (C) Two chromatid bridges at telophase induced by 600Gy in M1 seedlings of cv. Giza 35. (D) C- metaphase induced by 500 Gy in M2 seedlings of cv. Giza 111. (E) Un-oriented chromosome at metaphase induced by 500Gy in M2 seedlings of cv. Giza 35. (F) Chromatid bridge at anaphase induced by 400Gy in M2 seedlings of cv. Giza 35. (G) Free chromosome and lagging chromosome at telophase induced by 400Gy in M2 seedlings of cv. Giza 35. (H) Lagging chromosome at telophase induced by 300Gy in M2 seedlings of cv. Crawford. (I) Micro-nucleus at interphase induced by 200Gy in M2 seedlings of cv. Giza 111.

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 M_1 plants of the three soybean cultivars following parent seed exposure to different doses of γ-radiation are shown in Fig. 2 (A-H), 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 M_2 plants. The same morphological observations at the low doses of 100Gy and 200Gy and high doses of 300Gy to 500Gy were generally maintained in M_2 plants (data not shown).

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 M_1 (Fig. 3 A-C) and M_2 plants (data not shown).
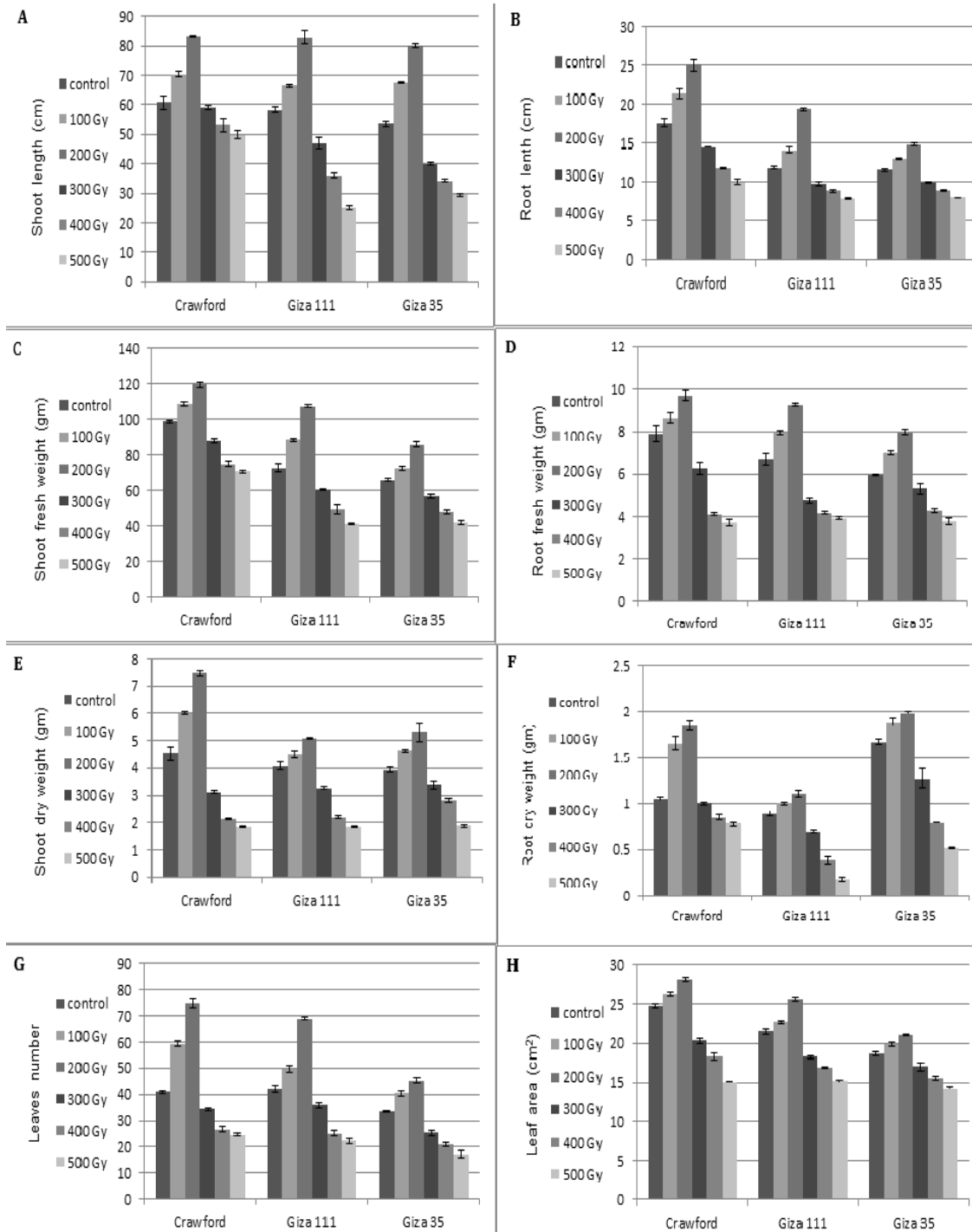

Fig. 2. Histograms illustrating changes in some vegetative parameters of M1 plants of three soybean cultivars following seed exposure to different doses of γ-radiation. (A) Shoot length, (B) Root length, (C) Shoot fresh weight, (D) Root fresh weight, (E) Shoot dry weight, (F) Root dry weight, (G) leaves number/plant, (H) Leaf area.
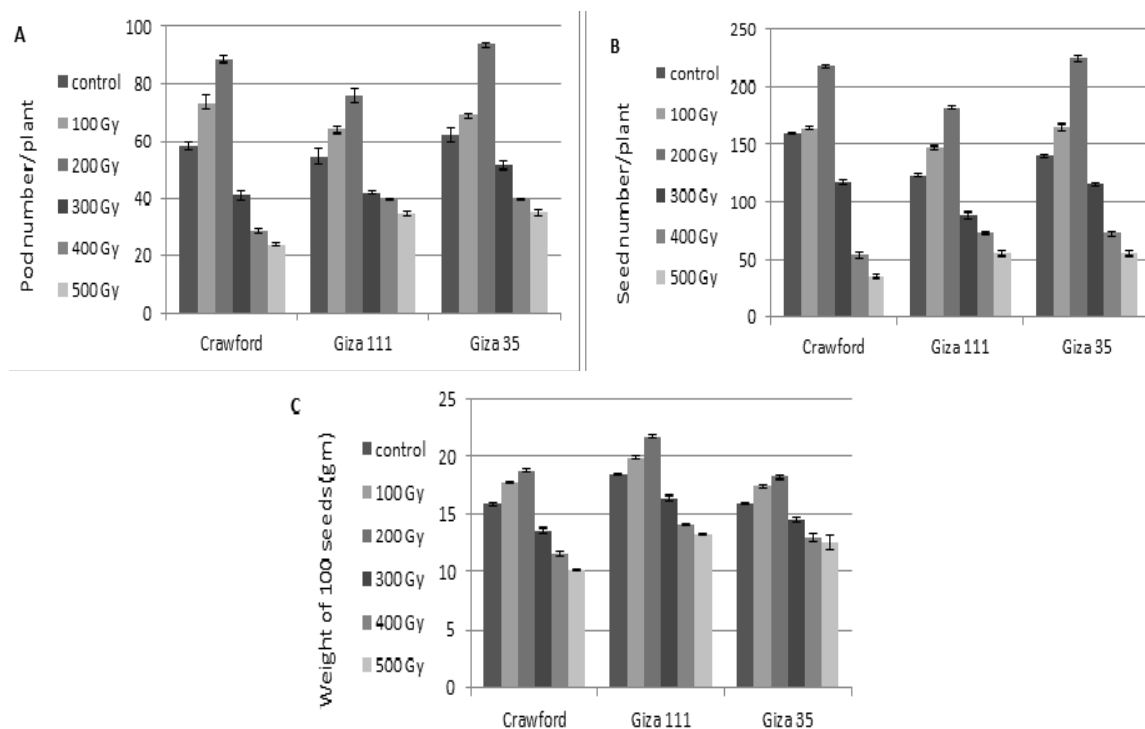
Fig. 3. Histograms illustrating changes in some yield parameters of M1 plants of three soybean cultivars following seed exposure to different doses of γ-radiation. (A) Pod number/plant, (B) Seed number/plant, (C) weight of 100 seeds.

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'sseeds 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).

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 (Maiato 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 M_1 and M_2 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 M_1 plants that constantly maintained in the M_2 plants. While high levels of 300Gy to 500Gy of γ-irradiation negatively affected both plant growth and yield causing deleterious damage, particularly on the M_2 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 M_1 and M_2 plants. However, a deleterious effect was observed for the Crawford cultivar at the 500Gy dose of γ-irradiation in M_2 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 M_1 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 M_2 generation plants. The selection of individual plants in the M_2 generation can be studied to observe the spectrum of variation for traits and observation of mutants, synchronous maturation in M_3 and M_4 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.

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التأثيرات الوراثية الخلوية لأشعة جاما وتأثيرها على نمو وإنتاجية ثلاثة أصناف من فول الصويا

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يتناول هذا البحث دراسة تأثير تشعيع بذور ثلاثة أصناف من فول الصويا هى: كروفورد وجيزة 111 وجيزة 53 بجرعات أشعة جاما تتراوح من 100جراي إلى 600 جراي، مع زيادة 100 جراي بين الجرعات، على نشاط الإنقسام الخلوى والكروموسومات فى الخلايا المرستيمية بالقمة النامية للجذور وعلى النمو الخضرى والإنتاجية المحصولية. وقد أدت الجرعات المنخفضة (100 و 200جراى) إلى زيادة معدل الانقسام الخلوى وزيادة معدل النمو، على العكس من ذلك، أدت الجرعات المرتفعة إلى إعاقة معدل انقسام الخلية بشكل ملحوظ، كما أدت إلى زيادة معدل التشوهات الكروموسومية، وخفض النمو الخضري والإنتاجية المحصولية. أشارت النتائج أيضا إلى أن تواتر التشوهات الكروموسومية يعتمد على الجرعة المستخدمة من أشعة جاما وصنف فول الصويا. كان الإلتصاق الكروموسومي والكروموسومات الشاردة أكثر التشوهات شيوعًا عند الطور الاستوائى كما كانت الجسور الكروموسومية والكروموسومات الحرة أكثر التشوهات شيوعًا في الطورين الإنفصالى والنهائى، في حين لم يلاحظ الطور الإستوائى الكولشيسينى والكروموسومات المتأخرة في مرحلة الطورين الإنفصالى والنهائى، كما لم تلاحظ تغيرات فى خلايا الطور البينى لنباتات الجيل الأول، أما فى جذور نباتات الجيل الثانى فقد لوحظت الأنوية الصغيرة والفجوات النووية وأنوية غير منتظمة فى كثير من الخلايا البينية، ومن المرجح أن التشوهات الكروموسومية المذكورة تعود إلى تأثير كلاستوجينى نتيجة كسر وإعادة التحام الكروموسومات، مما قد يسفر عن طفرات كروموسومية، وتؤكد نتائج هذه الدراسة على أهمية تقييم معدل انقسام الخلية وسلوك الكروموسومات عند استخدام المطفرات للحصول على أنماط وراثية جديدة للتنبؤ بالتغيرات في نمو وانتاجية الأنماط الوراثية المستهدفة.