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SOYBEAN GENERATIONS UNDER GAMMA RAYS AND EFFECTS ON SEED QUALITY

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ABSTRACT

Mutation induction is used to improve existing or generate new varieties. The objective of this study was to evaluate the effect of gamma radiation doses under successive generations of cultivation on seed quality of two soybean lines (VX04-6828 and VX04-5692). Different gamma-ray doses (^{60}Co) were evaluated (0; 50; 150 and 250 Gray) in the M0 (first sowing) generation, and analyzed for water content, germination, vigor, abnormal seedlings, dead seeds, and hilum color. Then, sowing (M0) was carried out to obtain plants and seeds of the first production cycle (M1 generation), and then those of the subsequent cycle (M2 generation). The same analyses of M0 were performed for M2 and M3 generations, with the addition of germination rate and emergence speed index in the field. The data were submitted to analysis of variance in a factorial arrangement. The generations were compared using Tukey's test, while doses were evaluated by regression analysis. Seeds of the two soybean lines showed significant interaction for the different generations and gamma-ray doses in all the variables analyzed, except for water content. The application of gamma rays in soybean seeds showed effects on successive generations of cultivation, with better physiological quality up to the dose of 150 Gy (Gray) and greater sensitivity to gamma radiation for the VX04-5692 line.

Keywords: genetic variability, germination, *Glycine max*, ionizing radiation, radiosensitivity.

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INTRODUCTION

Soybean (*Glycine max* L.) production is expected to increase, seeking to meet the demand for the production of grains, their derivatives and by-products. Therefore, research on new technologies aiming to increase productivity and quality of grains and seeds is constantly expanding. In this context, the use of physical methods to increase production, such as ionizing radiation, with the use of gamma rays, can significantly improve the economic return of the crop without side and undesirable effects. Depending on radiation dose level, organisms may or may not have apparent changes, such as stimulation, death or inhibition. However, lethal or inhibitory doses, which are generally very high, have been extensively studied (Araújo et al., 2018; Gudkov et al., 2019).

Gamma radiation can be used as a modifying agent for certain plant characteristics. When applied at adequate doses, it can provide stimulating effects on the physiological quality of seeds, disinfection of germination inhibiting agents, inactivation of microorganisms, and increases in plant growth and agricultural production; however, high doses (greater than 300 Gy) may inhibit germination (Miranda et al., 2009; Santos et al., 2010; Yun et al., 2013; Nepal et al., 2014; Franco et al., 2019). Before any selection by induced mutagenesis, a radiosensitivity test is thus recommended for measuring mutagenic effects and determining the optimal radiation dose for mutation induction (Ernest et al., 2020).

Abu et al. (2020) has indicated that mutagenesis induced by doses of gamma radiation from 50 to 200 Gy can be exploited to create genetic variability and favor morphological and yield characteristics in plants. However, information about the physiological quality of irradiated seeds from different cultivation cycles is still scarce.

As an alternative to conventional breeding, mutation induction is often used to improve existing and/or generate new varieties. In addition, gamma radiation can expand the spectrum of genetic variability in plants, making them more productive and resistant (Alikamanoglu et al., 2011; Prasad et al., 2021). In this sense, studies of the interaction between doses of gamma rays x generations of cultivation after the initial irradiation are necessary to identify the effects on physiological seed quality. Therefore, the objective of this study was to evaluate different doses of gamma rays under successive generations of cultivation, and their effects on the physiological quality of seeds of two soybean lines.

MATERIALS AND METHODS

The experiment was conducted in the 'Diogo Alves de Mello' Experimental Field and the Soybean Breeding Laboratory of the Federal University of Viçosa (UFV), Viçosa, Minas Gerias state, Brazil.

Soybean (*Glycine max* L.) seeds of two lines, VX04-6828 and VX04-5692, with an average water content of 12.0%, were irradiated at the Center for Nuclear Energy in Agriculture (CENA / USP), Piracicaba, São Paulo state, Brazil. The evaluated doses were 0 (control), 50, 150 and 250 Gy of gamma radiation in a ^{60}Co source (Gammacell-220, at a rate of 0.312 kGy h^{-1}). Once irradiated, seeds were separated into two lots, one for propagation in the field and another for analysis in the laboratory. Both activities were carried out at UFV.

Non-irradiated (M0 seeds) became M1 seeds once irradiated. When sowing M1 seeds (M1 generation), this generation produced M2 seeds, while M2 seeds (M2 generation) produced M3 seeds (M3 generation), and so forth. The M0 seeds were sown in the Experimental Field in a clayey Red-Yellow Argisol, using a randomized block design, with three replications. Cultural management was carried out according to the requirements of the crop. At the end of the production cycle, once the seeds were harvested from the M1 generation (originated from the M0 seeds), two lots were separated again, for new propagation and evaluation of the quality of the seeds. M1 sowing was repeated to obtain M2 generation plants and seeds. Cycles M1 and M2 were conducted in the Experimental Field.

After obtaining the seeds of all generations, M0, M1 and M2 were sent to the Soybean breeding Lab of the UFV for physiological quality analysis and water content determination. A completely randomized design was used, with four replications of 50 seeds, except for water content, in which four replicates of 25 seeds were used for each treatment. Water content was determined by the standard method of drying ovens at 105 ± 3 ºC for 24 hours, while the results were expressed as percentage (Brasil, 2009).

The physiological quality of soybean seeds was initially analyzed by germination test on a germitest® paper roll at a constant temperature of 25 ºC. The evaluations were carried out on the fifth and eighth day after the beginning of the test, with the results of the number of normal seedlings that presented complete essential structures (developed, proportional and healthy) expressed as percentage, and the number of abnormal seedlings and dead seeds. In addition, the first count of germination test was performed, characterized in terms of vigor, which was determined by the number of normal seedlings evaluated on the fifth day after the test was set up; the results were also expressed as percentage (Brasil, 2009). Emergence speed index was determined in the field for the M1 and M2 generations by daily counts of the number of normal seedlings emerged (cotyledons at an angle of 90° above the soil) until the 21st day after sowing (Maguire, 1962). The initially irradiated soybean seeds (M0) and those from the last generation of cultivation (M3), were evaluated for hilum color (Brasil, 2011), totaling 180 seeds per treatment in each generation.

For each soybean line, the data collected were subjected to individual analysis of variance, in a 3 x 4 factorial design (three generations x four doses of gamma radiation). When the analysis of variance was significant ($P < 0.05$), the generations (M0, M1, M2 and M3) were compared using Tukey's test at 5% significance level, while doses of gamma radiation (0, 50, 150 and 250 Gy) were analyzed by regression ("t" test at 5%).

RESULTS

The water content of soybean seeds did not differ between generations (M0, M1 and M2) or doses of gamma radiation evaluated ($P > 0.05$). Average values for the VX04-6828 and VX04-5692 lines were 11.8% and 12.5%, respectively.

Seeds of the two soybean lines evaluated showed significant interaction for the generations and doses of gamma radiation, in the different variables evaluated by the physiological quality (Table 1). Comparison of the different generations under gamma radiation doses is provided in Fig. 1 for germination, vigor, abnormal seedlings, and dead seeds of the two lines studied.

The germination of seeds of line VX04-6828 (Fig. 1A) presented lower mean values in the M0 generation at doses of 0, 50 and 100 Gy, while no significant effect was observed between generations for the 250 Gy dose. For line VX04-5692 (Fig. 1B), only M2 generation showed reduced averages to seed germination at doses of 0, 50 and 100 Gy. However, same as for line VX04-6828, no significant effect was observed between generations for the 250 Gy dose.

Regarding vigor (assessed by the first count of germination), the results for line VX04-6828 were similar to those found in germination, except for the 250 Gy dose, in which generations differed (Fig. 1C). In line VX04-5692, the M2 generation showed the best vigor (Fig. 1D) for all doses of gamma rays evaluated.

Abnormal seedlings recorded a lower incidence in the M1 generation from line VX04-6828. However, an increase in abnormal seedlings was observed when the highest radiation doses (250 Gy) were applied (Fig. 1E). For line VX04-5692, the M2 generation recorded the highest values (%) of abnormal seedlings for all the doses of gamma radiation evaluated (Fig. 1F). However, the highest incidence of abnormal seedlings was observed in the control treatment (no radiation dose).

Regarding dead seeds, the highest rate was observed in the M2 generation from line VX04-6828 for all irradiation doses (Fig. 1G). The same was observed for line VX04-5692, except for the 250 Gy dose, which showed no differences between generations (Fig. 1H).

Table 2 shows the regression equation for the variables analyzed in soybean lines VX04-6828 and VX04-5692, while their behaviors are shown in Fig. 2. When comparing the doses of gamma rays in the evaluated generations for the two lines, there were increases in the average germination of soybean seeds for line VX04-6828, with an increase in the doses of gamma rays in the M0 generation (Fig. 2A). A similar behavior was observed for seeds from VX04-5692 but only in the M2 generation (Fig. 2B).

There was a decrease in vigor from the 100 Gy dose in line VX04-6828 (Fig. 2C). Conversely,

Table 1. Statistical data (*P*-value) for the analyzed variables of the two soybean lines.

	VX04-6828			VX04-5692		
	Generations	**Doses**	**Gen*Doses**	**Generations**	**Doses**	**Gen*Doses**
Germ.	0.0001	0.0001	0.0003*	0.0001	0.0021	0.0001*
Vigor	0.0001	0.0001	0.0001*	0.0001	0.0035	0.0001*
A.Seed	0.0001	0.0001	0.7026	0.0001	0.0001	0.0001*
D.Seed	0.0051	0.0005	0.0001*	0.0001	0.1029	0.0001*
ESI	0.0013	0.0001*	0.2056	0.4596	0.0001*	0.2895

VX04-6828 and VX04-5692: soybean lines evaluated; Gen*Doses: statistical interaction between the factors "generations" and "doses"; Germ.: Germination; A. Seed.: Abnormal Seedlings; D. Seeds: Dead Seeds; ESI: Emergence speed index. * significant at the level of 5% probability.

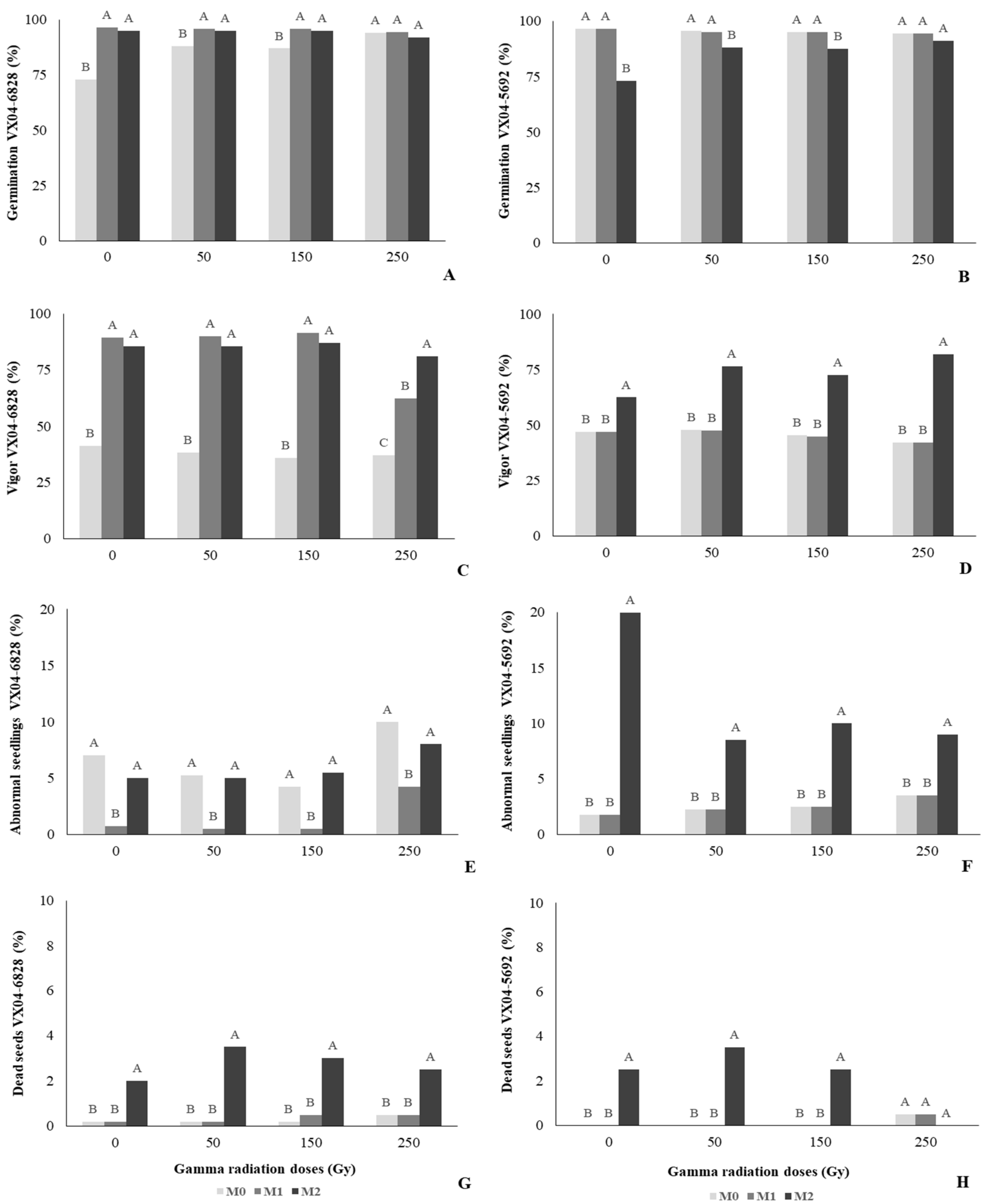

Fig. 1. Germination (A and B), vigor (C and D), abnormal seedlings (E and F) and dead seeds (G and H) from two soybean lines (VX04-6828 and VX04-5692), evaluated in the M0, M1 and M2 generations under different doses of gamma radiation (Gy).

there was an increase in vigor with increasing doses of gamma radiation in the M2 generation from line VX04-5692 (Fig. 2D).

Reduced levels of abnormal seedlings were observed at doses 50 and 150 Gy for M0 and M1 generations from line VX04-6828 (Fig. 2E), with an increase at 250 Gy dose. In the M2 generation, increasing doses resulted in higher rates of abnormal seedlings. In addition, the M0 and M1 generations from line VX04-5692 showed an increasing increment in abnormal seedlings with the doses of gamma rays, while the M2 generation

Table 2. Regression equations for the variables analyzed in soybean lines VX04-6828 and VX04-5692, treated with different doses of gamma radiation (0, 50, 150 and 250 Gy) and evaluated in different generations (M0, M1 and M2).

	VX04-6828		VX04-5692	
	Equation	R^2	Equation	R^2
Germination (%)				
M0	$y = -0.0071x + 96.5510$	0.83	$y = -0.0004x^2 + 0.1670x + 75.6930$	0.77
M1	$y = -0.0010x^2 + 0.0138x + 94.8190$	0.97	$y = -0.0064x + 95.9750$	0.70
M2	$y = 0.0610x + 77.9490$	0.70	$y = -0.0073x + 96.1950$	0.90
Vigor (%)				
M0	$y = -0.0002x^2 + 0.0449x + 96.5510$	0.89	$y = 0.0592x + 66.7200$	0.65
M1	$y = -0.0011x^2 + 0.1720x + 87.6210$	0.96	$y = -0.0207x + 47.8340$	0.90
M2	$y = 0.0002x^2 – 0.0671x + 41.4480$	0.99	$y = -0.0208x + 47.9630$	0.86
Abnormal seedlings (%)				
M0	$y = 0.0117x + 4.5593$	0.81	$y = 0.0004x^2 - 0.1270x + 17.8790$	0.68
M1	$y = 0.0001x^2 - 0.0229x + 0.9359$	0.97	$y = 0.0064x + 1.7754$	0.94
M2	$y = 0.0003x^2 - 0.0613x + 0.2060$	0.98	$y = 0.0064x + 1.7754$	0.94
Dead seeds (%)				
M0	$y = -7e-05x^2 + 0.0172x + 2.2810$	0.90	$y = -0.0001x^2 + 0.0169x + 2.6508$	0.98
M1	$y = 0.0014x + 0.1898$	0.83	$y = 0.0019x - 0.0847$	0.96
M2	$y = 0.0011x + 0.1492$	0.92	$y = 0.0019x - 0.0847$	0.97
Emergence speed index				
M0	-	-	-	-
M1	$y = -1.5275x^2 + 5.7165x + 30.6630$	0.86	$y = -0.9610x + 26.2550$	0.95
M2	$y = -0.0114x + 25.1310$	0.98	$y = 0.9800x + 28.5400$	0.45

R^2: coefficient of determination of the regression equation.

showed a reduction with the application of the doses (Fig. 2F).

When studying the effects of gamma radiation on dead seeds for both lines, the most expressive values were found in the M2 generation, with higher average values for the 50 and 150 Gy doses (Fig. 2G and 2H).

For line VX04-6828 (Fig. 3A), emergence speed index (ESI) had higher average values in the M1 generation for all treatments evaluated. For line VX04-5692, ESI recorded the lowest average values in the M2 generation for doses of 150 and 250 Gy (Fig. 3B), while differences between doses of gamma radiation (Gy) in both generations resulted in no significant effect (Fig. 3B).

After gamma irradiation and two cultivation cycles (M3 seeds), hilum color classes of seeds from the two soybean lines increased compared to the initially evaluated lines (M0), which showed color uniformity (light brown) (Fig. 4).

For line VX04-6828, seeds with three different colors of hilum were observed, being 150 Gy the dose that exhibited the lowest number of seeds with hilum color similar to the initial line (Fig. 4A). In addition, line VX04-5692 showed greater variation in hilum color with the application of different doses of gamma radiation (Fig. 4B) compared to the control.

DISCUSSION

The literature has described that gamma radiation applied to seeds of different species is influenced by water level, with responses in germination and vigor (Santos et al., 2010; Garcia et al., 2016). The lower the water content in the seed, the less the radiation effect on germination (Viccini et al. 1997). However, high water levels increase metabolic activity and, therefore, intensify the vulnerability of seeds to radiation, since water acts as a means of diffusing physical and chemical mutagens, as well as free radicals from the radiation process (Miranda et al., 2009). In the present study, water content did not interfere with the results. Thus, it can be ensured that the effects are produced by gamma radiation, where

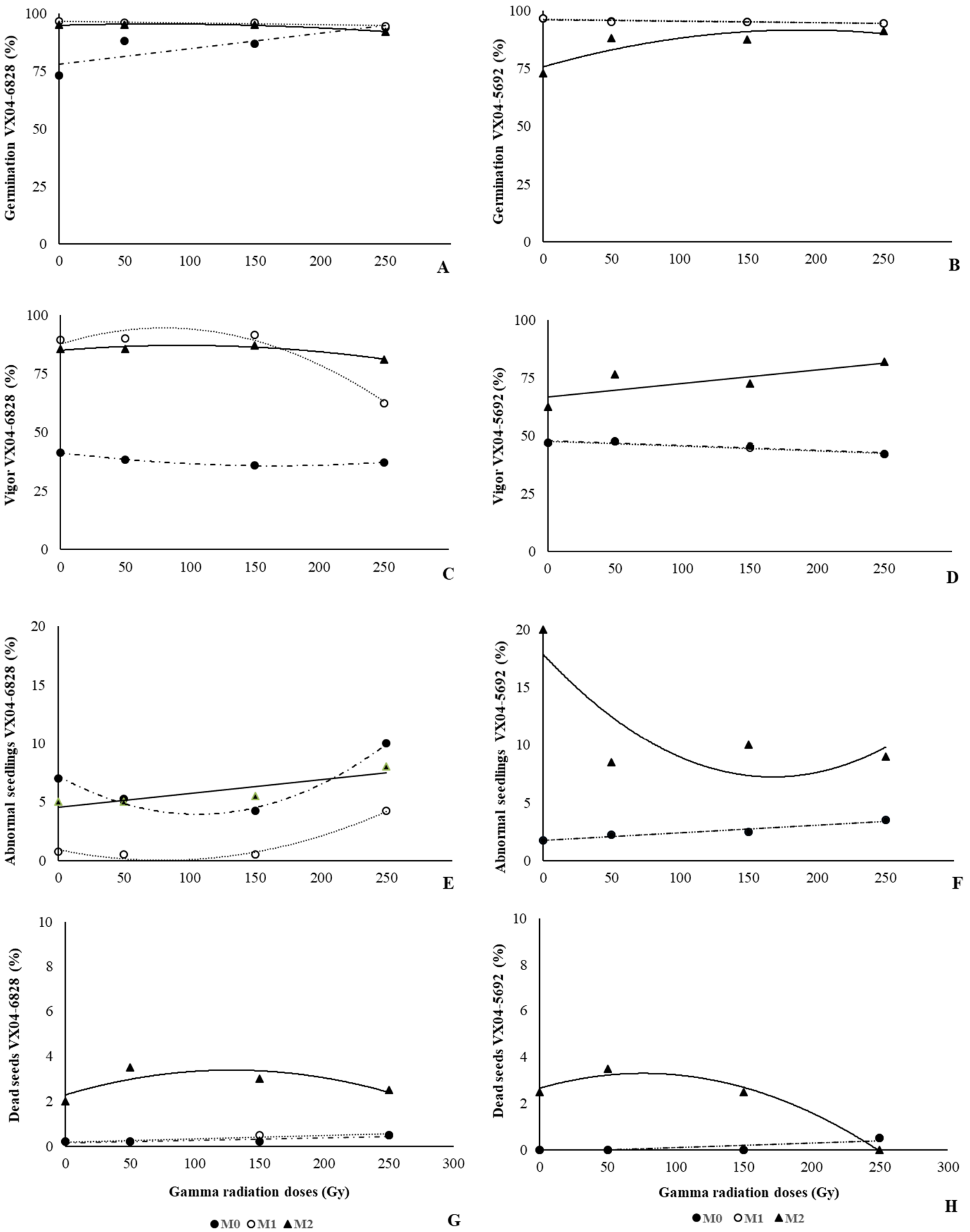

Fig. 2. Germination (A and B), vigor (C and D), abnormal seedlings (E and F) and dead seeds (G and H) from two soybean lines (VX04-6828 and VX04-5692), evaluated at different doses of gamma radiation (Gy) in the M0, M1 and M2 generations.

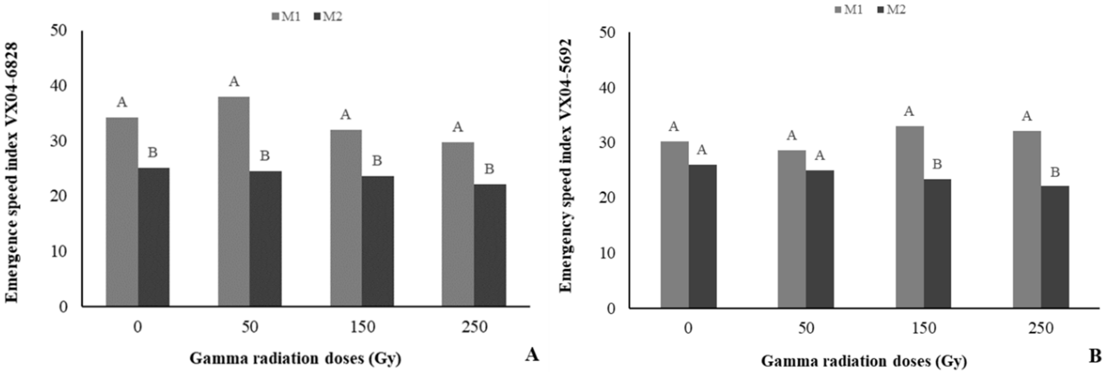

Fig. 3. Emergence speed index from the soybean lines VX04-6828 (A) and VX04-5692 (B), evaluated at different doses of gamma radiation (Gy) in the M1 and M2 generations.

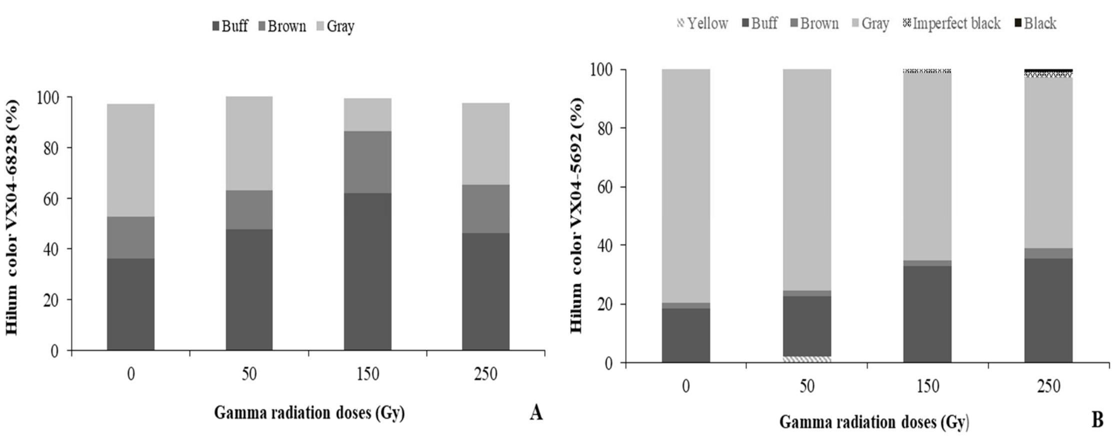

Fig. 4. Hilum color from seeds of two soybean lines (VX04-6828 and VX04-5692), in different doses of gamma radiation (Gy) in the M3 generation.

M1 and M2 generations from line VX04-6828 and M0 and M1 from line VX04-5692 showed positive responses in terms of germination, showing that the exposure of seeds to gamma radiation brings benefits to subsequent generations.

The different germination responses to the increasing doses of gamma radiation exhibited by the soybean lines may be related to a genotype-dependent effect (Vargas et al., 2008). However, studies show that radiation at lower doses promotes germination, while negative effects have been reported with doses above 150 Gy (Chaudhuri, 2002; Beyaz et al., 2016). Araújo et al. (2018) evaluated the effect of gamma radiation at low doses (0 to 100 Gy) on cotton seeds and found that doses up to 50 Gy induced greater cotton production. Similarly, Beyaz et al. (2016) evaluated the effect of different doses of gamma rays (0 to 250 Gy) on seed germination of the species *Lathyrus chrysanthus*, which is an ornamental plant with low germination rate, being widely spread in countries with temperate climates, and reported positive effects using doses up to 150 Gy. In addition, a study conducted by Nepal et al. (2014) revealed that higher doses of gamma radiation affected germination rate to a lower extent in two maize cultivars. Garcia et al. (2016) also studied the effects of gamma rays (0 to 900 Gy) on chamomile seeds, reporting increases in genetic variability but no differences in gemination response with respect to the control. In the present study, the highest dose of gamma rays evaluated (250 Gy) had no influence on the germination process of soybean seeds. Abdel-Hady et al. (2008) and Beyaz et al. (2016) found that the stimulating effect of low doses of gamma irradiation on seed germination can be attributed to the activation of the synthesis of

RNA or a certain protein.

According to Gudkov et al. (2019), several short-term reactions and the fixation of long-term ionizing radiation effects are largely mediated by genetic regulation. At the same time, the signaling pathways in this regulation must be studied in more detail, particularly focusing on proteomes and the activity of physiological processes. Therefore, the results obtained can be associated with the time between seed irradiation and sowing since peroxidases and repair enzymes can play a fundamental role in the recovery of cells and in the decomposition of radiation products, when this time is greater (Rodrigues and Ando, 2003; Vargas et al., 2008; Garcia et al., 2016).

Vigor of soybean seeds, presented by the first count germination test, was greater for the generations after cultivation, especially for the M2 generation. However, the opposite effect was observed in ESI in the field. In this sense, it is worth mentioning that the seeds evaluated in the field were subjected to bad weather conditions during the germination process, which may account for the obtained results. In fact, they can be explained by mutations due to stability of genetic characteristics after generations of cultivation. In this sense, Udensi et al. (2015) have indicated that characteristics stabilize after mutagenic treatment.

Nurmansyahi et al. (2020) carried out a study with fava beans submitted to low doses of gamma radiation and evaluated generations, reporting that the effects of gamma radiation persisted in the following generations. The authors associated these results with DNA repair mechanisms and indicated that evaluation of subsequent generations is required to obtain the true behavior of the mutant plants.

For abnormality of seedlings and dead seeds, responses of possible mutations observed by the soybean lines were evident and when they presented similar behaviors in the M2 generation for the different doses evaluated. These results can be elucidated by the radiosensitivity of soybean seeds to plant segregation after cultivation and at doses greater than 150 Gy, which results in modified plants that produce seeds of low physiological quality (Nobre et al., 2016). In the present study, however, the characteristics set for the M2 generation showed low percentages of abnormal seedlings and dead seeds. However, all parameters must be analyzed together so that the best dose of gamma radiation is used in order to increase the physiological quality of seeds regardless of the generation.

After successive generations, the soybean lines showed variations in hilum color in all treatments, which can be explained by plant segregation after cultivation. For the line VX04-5692, changes in hilum colors with respect to the control treatment show the effects of gamma radiation.

Characteristic hilum color, which is an important morphological marker in soybean seeds, has been a valuable aid in the process of cultivar breeding because the eventual occurrence of natural crosses, mixing of genotypes and mutation of plants, can be confirmed by the presence of different hilum colors within segregating generations of progenies (Vernetti and Vernetti Junior, 2017). As soybean seed hilum is maternal tissue, if no mutations have occurred, all seeds of a plant should have identical hila (Taylor and Caviness, 1982). According to Garcia et al. (2016), mutation induction through radiosensitization has a relevant use in plant breeding programs since variability increase is extremely important due to the introduction of alleles presenting resistance and/or tolerance to biotic and abiotic stress. In addition, the use of genetic variability may be associated with the increase in characters of agronomic interest for soybean, such as productivity and oil or protein yield, as well as identification of potential parents for breeding programs. Accordingly, the use of techniques that induce increased variability through mutations, with the generation of progenies that may indicate favorable changes and fixation of these in subsequent crops, is of great relevance for agribusiness.

CONCLUSION

The application of gamma-ray doses in soybean seeds showed effects on successive generations of cultivation and greater sensitivity to gamma radiation for line VX04-5692.

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