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# High gamma irradiation doses and long storage times reduce soybean seed quality

## Dose elevada de irradiação gama e longo período de armazenamento reduzem a qualidade da semente da soja

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### Abstract

High quality seeds are required for soybean production. This study evaluated the effect of gamma irradiation and storage time on seed quality in soybean lines VX04-6828 and VX04-5692. Seeds were gamma irradiated (<sup>60</sup>Co) with 0, 50, 150, and 250 Gy. After the first seed production cycle (M1), the harvested seeds were stored in the laboratory for 0, 2, 4, and 6 months. Moisture content, seed quality (germination rate, dead seeds, and normal and abnormal seedlings), and seed vigor (first germination count, germination index, and seedling length) were determined. Data were submitted to analysis of variance for each soybean line using a 4 x 4 factorial design (four storage times x four gamma irradiation doses). Response surfaces were constructed based on the F test significance ( $p \leq 0.05$ ). VX04-5692 seeds were more sensitive to gamma radiation than were VX04-6828 seeds. Soybean seed quality was highest in M2 seeds derived from seeds irradiated with less than 100 Gy and stored for up to two months. High gamma irradiation doses and long storage times reduced soybean seed quality.

**Key words:** Germination. *Glycine max*. Gamma radiation. Vigor.

### Resumo

Sementes de elevada qualidade fisiológica são necessárias à produção e à comercialização da soja. Portanto, avaliaram-se os efeitos da radiação gama e do armazenamento na qualidade fisiológica de sementes das linhagens de soja VX04-6828 e VX04-5692. As sementes dessas linhagens foram irradiadas com 0, 50, 150 e 250 Gy de radiação gama (<sup>60</sup>Co). Após o primeiro ciclo de produção (M1), as sementes foram armazenadas em condições laboratoriais e avaliadas aos 0, 2, 4 e 6 meses, quanto ao teor de água das sementes e à qualidade fisiológica, por meio dos testes de germinação (plântulas normais, anormais e sementes mortas) e de vigor (primeira contagem de germinação, índice de velocidade de germinação e comprimento das plântulas). Os resultados foram submetidos à análise de variância para cada linhagem, utilizando-se o modelo fatorial 4 x 4 (quatro tempos de armazenamento x quatro doses de radiação gama). Com base no teste F ( $p \leq 0,05$ ), foi realizado o ajuste de superfícies de resposta. As sementes da linhagem VX04-5692 são mais sensíveis à radiação gama que da linhagem Vx04-6828. Melhor qualidade fisiológica das sementes M2 de soja é expressa em doses inferiores a 100 Gy e armazenadas até 2 meses. Doses elevadas de radiação gama e longo período de armazenamento reduzem a qualidade fisiológica das sementes de soja.

**Palavras-chave:** Germinação. *Glycine max*. Radiação gama. Vigor.

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## Introduction

Successful soybean production depends on the use of suitable varieties with high potential productivity and high-quality seeds. Seed quality is genetically inherited, and different cultivars have seeds with different levels of vigor, germination rate, and seedling emergence (FRANÇA NETO et al., 2007; MERTZ et al., 2009; SANTOS et al., 2012).

Mutations induced by physical mutagenic agents such as gamma radiation have been used to cause modifications in DNA structure to increase genetic variability and create new genes of interest (BORÉM; MIRANDA, 2013). This technique is an attractive alternative for improving soybean yields (TULMANN NETO et al., 1995). The use of gamma radiation in soybean has enabled the identification of mutant lines with several favorable characteristics for use as food such as low trypsin inhibitor activity and low or no lipoxygenase content, i.e., lines that have a reduced content of anti-nutritional factors and better sensory quality (DIXIT et al., 2011; LEE et al., 2011). Moreover, gamma radiation has been used in foods for increasing shelf life, disinfection, and inactivation of organisms that cause seed degradation or that inhibit germination (SANTOS et al., 2010). However, germination rate and soybean shoot length may be negatively affected by the use of gamma radiation in seeds (YUN et al., 2013).

Little is known about the physiological quality of gamma-irradiated ( $^{60}\text{Co}$ ) seeds. Miranda et al. (2009) reported no effect on seed quality in gamma-irradiated rice seeds, whereas Santos et al. (2010) observed negative effects on peanut seed germination, with no germination at all above 3.0 kGy. Irradiation dose and storage time are the main factors affecting seed quality in irradiated seeds (MIRANDA et al., 2009; ALVES et al., 2011). Thus, studies that investigate the interaction of gamma irradiation dose and seed storage time are crucial, because each factor may affect seed quality differently when alone or in combination. In the present study, we evaluated the effect of gamma

irradiation dose and storage time on seed quality in two soybean lines.

## Material and Methods

Seeds from two soybean lines (VX04-6828 and VX04-5692) with a moisture content of approximately 12.0% were gamma irradiated with 0 (control), 50, 150, and 250 Gy using a Gammacell 220 irradiation unit (Nordion, Ottawa, ON, Canada) at a dose rate of  $0.312 \text{ kGy h}^{-1}$  ( $^{60}\text{Co}$ ), at the Center for Nuclear Energy in Agriculture (CENA/USP), Piracicaba, São Paulo, Brazil. Seeds were sown at the Diogo Alves de Mello experimental field of the Federal University of Viçosa (UFV), Viçosa, Minas Gerais, Brazil.

Seeds harvested from M1 generation plants were stored for 0, 2, 4, and 6 months at  $\pm 25^\circ\text{C}$  and  $\pm 50\%$  RH in the Soybean Seed Improvement Laboratory at UFV, Viçosa. The experiment was conducted in a completely randomized design with four replicates of 50 seeds each for each soybean line. Moisture content was determined from four replicates with 25 seeds for each soybean line using a standard oven-drying method at  $105 \pm 3^\circ\text{C}$  for 24 h (BRASIL, 2009), and results were expressed in percentage.

Soybean seed quality was initially determined by a germination test using Gernitest<sup>®</sup> paper rolls moistened with distilled water at 2.5 times the weight of the dry paper. The rolls were placed in a germination chamber at a constant temperature of  $25^\circ\text{C}$  for eight days. Seedlings were evaluated on the fifth and eighth day after the start of the test (BRASIL, 2009), and results were expressed as percentage of first germination count and germination rate, respectively. For each replicate, 10 seedlings from the middle third of the paper roll were measured with a ruler to determine seedling length. In addition, the number of seeds showing radicle protrusion was recorded daily at the same time of day during the eight-day trial to calculate the germination index (GI) using the formula proposed by Maguire (1962).

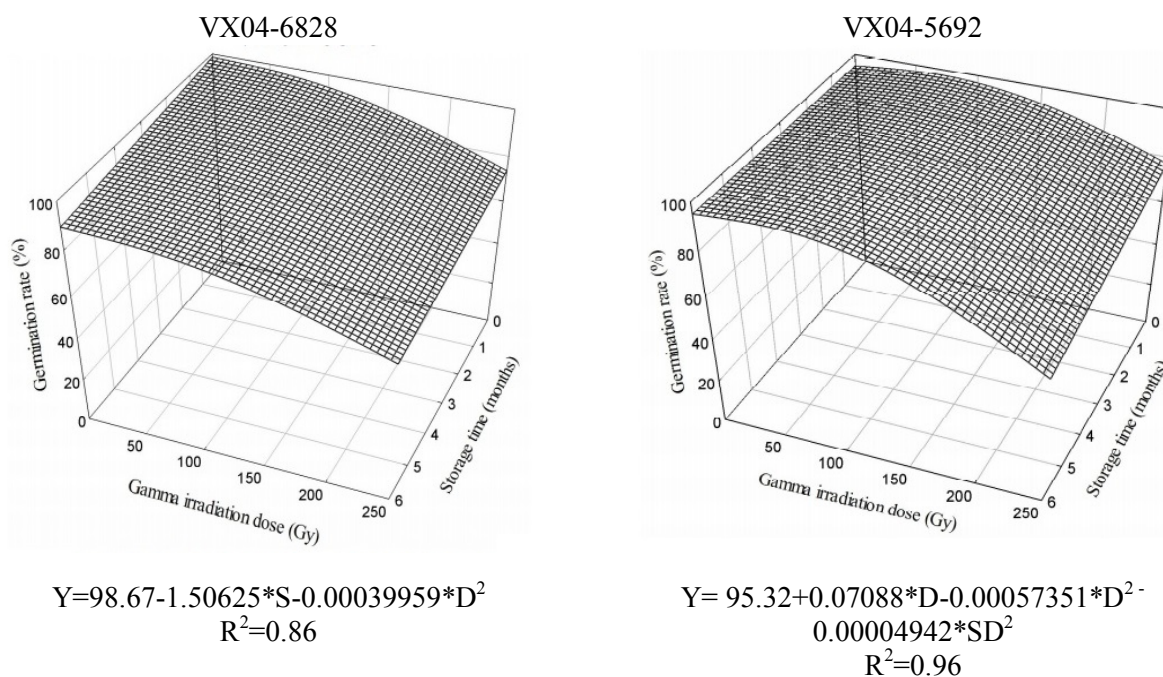
Data were analyzed using analysis of variance (ANOVA) for each soybean line following a 4 x 4 factorial design (four storage times x four gamma irradiation doses). Response surfaces were constructed using the stepwise procedure based on the F test significance ( $p \leq 0.05$ ), and variables with a  $p \leq 0.15$  were included in the multiple regression model. All analyses were performed using SAS software (SAS INSTITUTE, 2004).

## Results and Discussion

In this study, irradiation dose and storage time and the interaction between irradiation dose and storage time significantly affected germination rate, first germination count, abnormal growth, seed mortality, and germination index in both soybean lines. In addition, the irradiation dose x storage time interaction had a significant effect on seedling length in the VX04-5692 line.

Figure 1 shows the germination response to radiation dose and storage time for the VX04-6828 and VX04-5692 soybean lines. Germination rates decreased with increasing radiation dose and storage time. Maity et al. (2009) investigated the effect of gamma irradiation dose (1-6 kGy) on long-term rice seed germination and reported similar results. However, the authors in that study examined seed germination following irradiation, whereas in the present study, seed germination was evaluated in seeds that had been harvested from plants that were grown from irradiated seeds, and therefore the detected effects are a result of changes to the physiology or genetic makeup of seeds caused by radiation that affected the performance of mature plants and their seeds.

**Figure 1.** Effect of gamma irradiation dose and storage time on overall germination rate in soybean lines VX04-6828 and VX04-5692.



Seed germination and seedling emergence are determined by seed quality (VASCONCELOS et al., 2009). High vigor seeds are expected to emerge faster and start photosynthesis earlier, favoring shoot and root development. Thus, seeds from more vigorous plants are expected to be normal and to produce a greater number of pods and seeds than less vigorous ones (KRZYZANOWSKI; FRANÇA NETO, 1999).

Maize seeds treated with gamma radiation at radiosensitive doses showed reduced germination rate, lower photosynthetic pigment content, and higher reactive oxygen species (ROS) content (i.e., free radicals) than did non-irradiated seeds (MARCUS et al., 2013). Çelik et al. (2014) investigated the response of soybean plants to high gamma radiation doses (300 Gy) and found a reduction in leaf size and concentration of photosynthetic pigments and proteins in plants grown from irradiated seeds compared to plants derived from non-irradiated seeds. Radiation caused oxidative stress, resulting in higher ascorbate peroxidase activity, reduced number and density of stomata, and higher trichome density on the abaxial surface of leaves 14 and 21 days after irradiation (ÇELİK et al., 2014). Because different tissues respond differently to gamma radiation, irradiation doses should be adjusted accordingly in order to prevent major performance losses in irradiated individuals (GAZZANELO et al., 2007). Even though gamma radiation has been successfully used to stimulate seed germination (ALVES et al., 2011; SANTOS et al., 2010), the effects of irradiation dose and intensity are unique to each culture. In the current study, irradiation doses higher than 100 Gy combined with longer storage times negatively affected seed germination rates in the two soybean lines (Fig. 1).

Moisture content is another factor affecting seed quality, with high moisture content increasing seed deterioration (ALENCAR et al., 2008). However,

moisture content in the present study ranged from 10.96 to 12.52%, and, according to Alencar et al. (2009), seeds with this moisture content do not significantly deteriorate during up to six months of storage at temperatures below 30 °C. This indicates that the reduction in seed quality we observed can be explained by the interaction of irradiation dose and storage time.

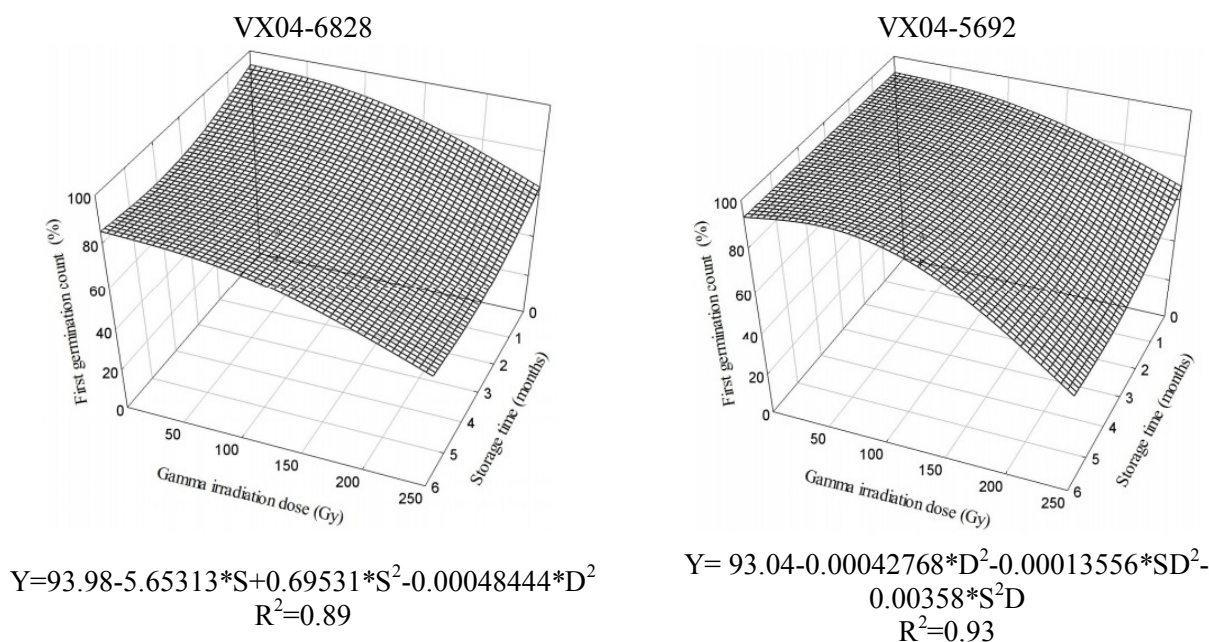
The seed vigor tests, represented by the first germination count (Fig. 2) and the germination index (GI; Fig. 3), showed results similar to those of the germination test (Fig. 1). Both vigor tests showed a reduction in seed vigor with increasing gamma irradiation dose and storage time in both soybean lines.

Loss of seed viability is caused by seed deterioration during storage and may be aggravated by high temperatures and moisture content (SMANIOTTO et al., 2014). In the present study, the observed reductions in viability may have been intensified by gamma radiation, because high irradiation doses have been shown to negatively affect seed germination (ALVES et al., 2011; PRADO et al., 2006).

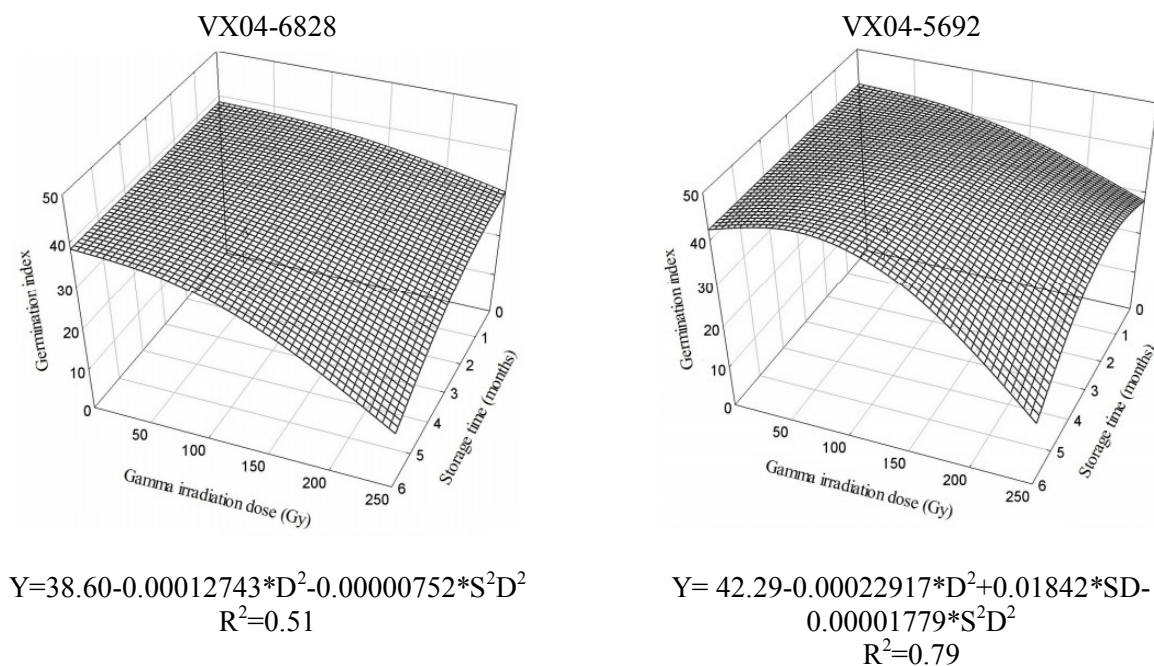
The reduction in germination rate and seed vigor (Figures 1, 2, and 3) with higher irradiation doses and longer storage times may be explained by changes in the biochemical structure of the cells. Gamma radiation ( $^{60}\text{Co}$ ) reduced seed germination and vigor and increased ROS production in maize seeds irradiated with doses higher than 0.1 kGy (MARCUS et al., 2013). Free radicals readily react with almost all structural and functional organic molecules, causing metabolic disorders at the cellular level. Cell membranes may lose their stability and permeability, resulting in structural damage to the cell and physiological disorders (AL-RUMAIH; AL-RUMAIH, 2008; MOGHADDAM et al., 2011).



**Figure 2.** Effect of gamma irradiation dose and storage time on first germination count in soybean lines VX04-6828 and VX04-5692.



**Figure 3.** Effect of gamma irradiation dose and storage time on germination speed (GI = germination index) in soybean lines VX04-6828 and VX04-5692.



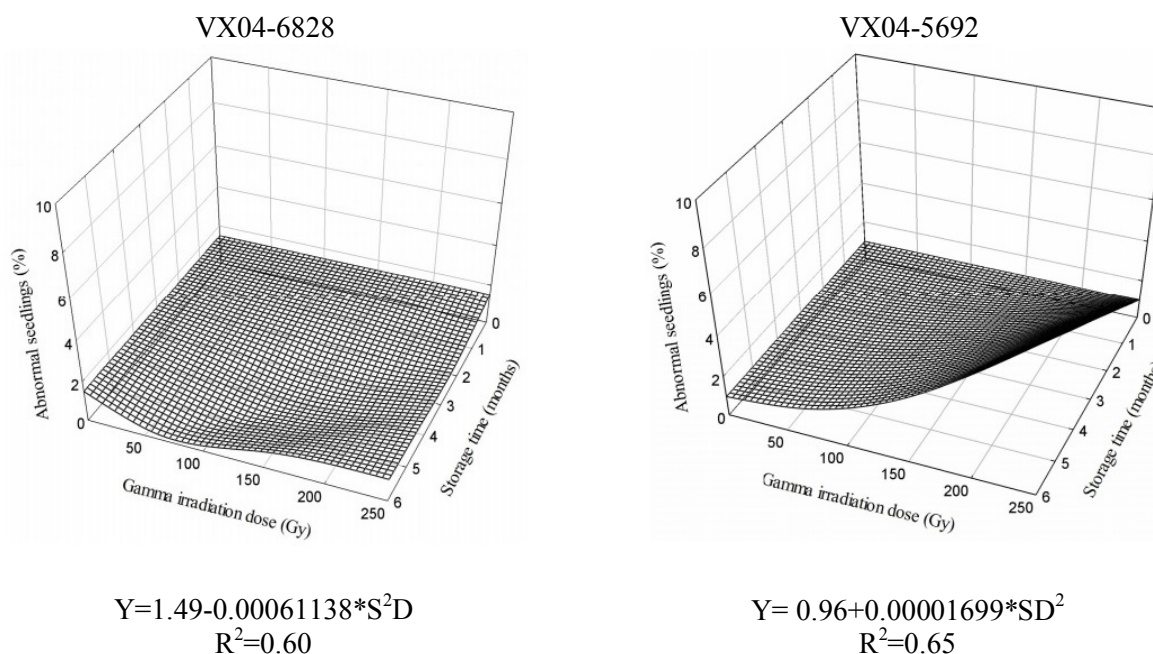
The percentage of abnormal VX04-6828 seedlings was low at 50 and 100 Gy and gradually increased at higher doses, whereas the frequency of abnormal VX04-5692 seedlings was higher than that of VX04-6828 at low radiation doses and increased with increasing radiation dose and storage time (Figure 4).

Irradiation dose and storage time increased the frequency of dead seeds in the two soybean lines (Figure 5). The high frequency of abnormal seedlings and dead seeds that was observed may be explained by the radiosensitivity of soybean seeds to doses greater than 100 Gy, resulting in modified plants that produced seeds of low physiological quality. Nevertheless, gamma radiation has been shown to significantly improve seed storage capacity.

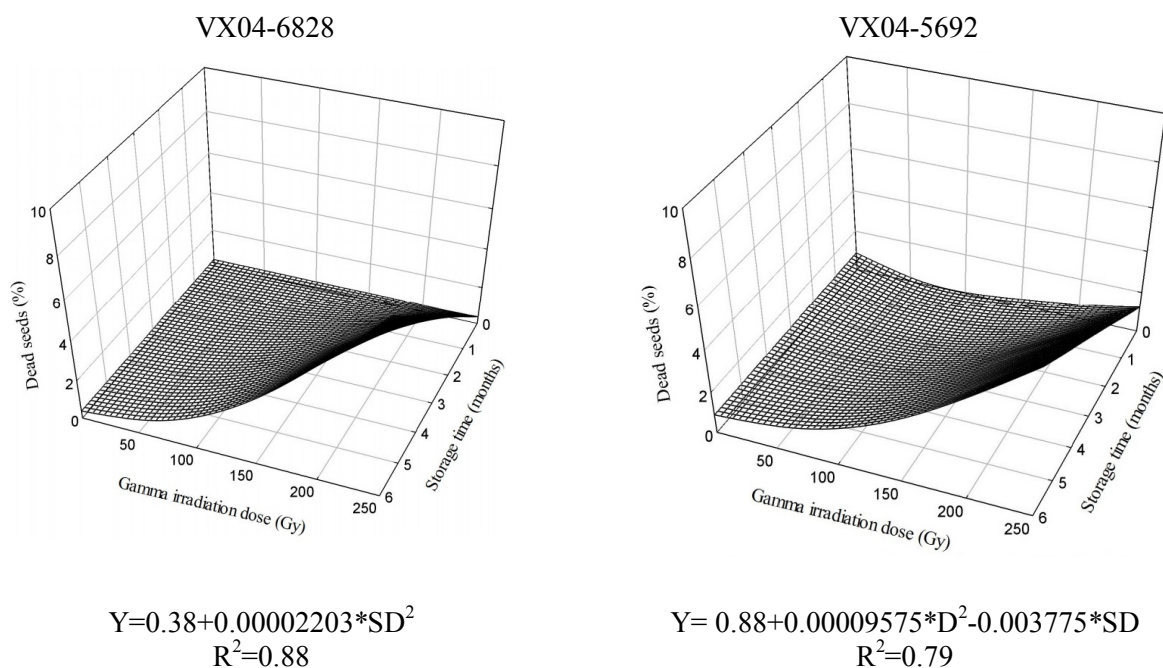
In peanuts, doses of 10–30 kGy were capable of eliminating fungal contamination in seeds stored for up to six months at room temperature (PRADO et al., 2006), whereas in another study, 4.0 kGy were sufficient to completely control the mycoflora (ALVES et al., 2011), and Costa et al. (2013) reported that doses of 9, 12, and 15 kGy effectively prevented infestation by aflatoxigenic *Aspergillus flavus*.

Figure 6 shows the response of seedling length to irradiation dose and storage time in the VX04-5692 line. Seedling length (i.e., seedling vigor) decreased with increasing irradiation dose and storage time, but only in the VX04-5692 line, suggesting that this line is more sensitive to gamma radiation than is the VX04-6828 line.

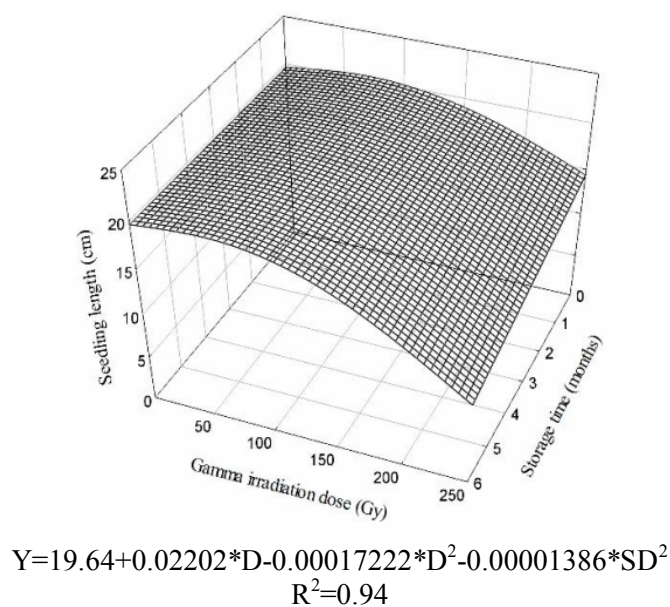
**Figure 4.** Effect of gamma irradiation dose and storage time on abnormal seed germination in soybean lines VX04-6828 and VX04-5692.



**Figure 5.** Effect of gamma irradiation dose and storage time on seed mortality in soybean lines VX04-6828 and VX04-5692.



**Figure 6.** Effect of gamma irradiation dose and storage time on seedling length in the VX04-5692 soybean line.





Barros and Arthur (2005) observed a significant reduction in plant height with increasing irradiation dose in gamma-irradiated soybean plants after 30 days. In the current study, significant differences in seedling length were detected in VX04-5692 seedlings only five days after the start of the germination test (first germination count). Similar reductions in germination index and seedling length caused by high gamma irradiation doses have also been reported in other species (HELL; SILVEIRA, 1976; SILVA et al., 2011).

In conclusion, for both soybean lines, seed quality was highest at irradiation doses lower than 100 Gy and in seeds stored for up to two months. Seed viability decreased with increasing irradiation dose and storage time. Doses between 50 and 100 Gy showed little to no effect on seed viability and vigor compared to control seeds (0 Gy). Seedling length was negatively affected by irradiation dose and storage time in the VX04-5692 line, whereas the frequency of abnormal seedlings in the VX04-6828 line was low at 50 and 100 Gy and gradually increased at higher doses.

## Conclusions

VX04-5692 seeds are more sensitive to gamma radiation than are VX04-6828 seeds. Soybean seed quality was highest in M2 seeds grown from seeds irradiated with less than 100 Gy and stored for up to two months. High gamma irradiation doses and long storage times reduced soybean seed quality.

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