Rapid Communication

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Gamma radiosensitivity study on wheat (*Triticum turgidum* ssp. *durum*)

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Abstract: Fifty wheat seed variety (var.) CIRNO C2008 were gamma irradiated with 100, 200, 300, 400, 500, or 600 Gy by using a Cobalt-60 source at 907.39 Gy/h, and the nonirradiated seeds were considered the control (M_0). A photoperiod of 13 h of darkness (14°C) and 11 h of light (2 h at 18°C, 7 h at 25°C, and 2 h at 18°C) was used for 30 days, in a growth chamber (BJPX-A450; BIOBASE) for quantifying the morphometric traits of the irradiated vs nonirradiated seeds. The germination percentage (from 88.89% in M_0 to 77.78% in 600 Gy) did not show significant differences among treatments; while the survival percentage (from 100% in M_0 to 6.66% in 600 Gy) and plant height (from 19.21 cm in M_0 to 1.16 cm in 600 Gy) showed negative significant ($p < 0.05$) difference among nonirradiated seeds (M_0) and the rest of the treatments. Finally, based on the survival percentage, the calculated lethal dose was 290.6 Gy, which will allow obtaining promising wheat mutants. Gamma irradiation is a promising alternative to obtain a greater wheat variability for advanced mutant lines.

Keywords: CIRNO C2008, gamma rays, LD₅₀

1 Introduction

Wheat is the most used cereal for human consumption worldwide; in Mexico, this cereal occupies the second place, after maize (Figueroa-López et al. 2010). Wheat production in Mexico is about 31,48,000 tons year⁻¹ (SAGARPA 2017), where Yaqui and Mayo Valleys (Sonora State) and Del Fuerte and Carrizo Valleys (Sinaloa State) contribute 63.5% to this national production (SIAP 2019). The Yaqui Valley, the birthplace of the Green Revolution in 1960, is one of the most intensive agricultural regions in the world, which using irrigation water, fertilizers, and constantly improving cultivars produced some of the highest yields of wheat (Matson and Falcon 2012). At present, the average wheat yield in this region is about 6,300 kg ha⁻¹, which is higher than those obtained in 1960 (1,800 kg ha⁻¹; Argente-Martinez et al. 2018; Liu et al. 2020).

At present, wheat production is negatively affected by several biotic (pests, pathogens, and soil degradation) and abiotic (drought, salinity, and increment of temperature) stress conditions (Verma et al. 2013; Villar-Rodriguez et al. 2016; INIFAP 2017). Thus, the generation of new varieties tolerant of or resistant to those conditions is determined to achieve food security. Currently, mutation induction is one of the most used techniques to obtain improved varieties (Villaseñor 2015). This technique allowed in obtaining 264 wheat mutant varieties and also the official release of 3,332 mutant varieties of more than 240 plant species (FAO/OIEA 2020). Mutation induction consists of increasing the plant genetic variability to obtain desired traits that cannot be found naturally (Muñoz and Hewstone 1995; van Harten 1998; Horn and Shimelis 2013). This genetic variability is obtained by using chemical (ethyl methane-sulfonate, sodium azide, colchicine, and *N*-ethyl-*N*-nitrosourea) and/or physical (X-rays, ultraviolet irradiation, alpha, beta, and gamma rays) agents, which alter genes and/or cause changes in chromosomal structures (Mickey

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et al. 1985; Novak and Brunner 1992; Spencer-López et al. 2018).

The first step involved in carrying out effective mutation induction is to identify the mutagen and the dose to be used, which is determined by dosimetry or radiosensitivity tests. This assay allows quantifying the radiation absorbed by a plant material and its physical interpretations (effects on plant morphology; Spencer-López et al. 2018). The effect of a mutagen on plants is fundamentally determined by their survival percentage and the level of regeneration and/or multiplication (Pardo 2015). In addition, the mean lethal dose (LD_{50}) needs to be calculated, which is a widely used parameter to determine the optimal mutation frequency with the least possible involuntary damage (Cubero 2003; Owoseni et al. 2007). At present, gamma rays are one of the most used mutagens due to the lower cost (easy availability) and increased effectivity (higher penetration into matter) compared to other mutagens (Moussa 2006; Spencer-López et al. 2018). The aim of this work was to quantify the effect of gamma rays on wheat (*Triticum turgidum* ssp. *durum* var. CIRNO C2008) seeds and seedling morphology as well as to identify the LD_{50} for further mutation studies.

2 Methods

Fifty wheat seed (M_0) varieties (var.) CIRNO C2008 were irradiated with 100, 200, 300, 400, 500, or 600 Gy (Brunner 1985), while the nonirradiated seeds (M_0) were considered as the control. Gamma rays were generated from a Cobalt-60 source (*Transelektro* LGI-01), at a dose rate of 907.39 Gy/h at the Instituto Nacional de Investigaciones Nucleares (ININ, Mexico). Thus, the irradiated M_0 seeds resulted in M_1 seeds, which were used to perform the radiosensitivity test.

2.1 Radiosensitivity test

This assay was carried out in Instituto Tecnológico de Sonora (ITSON, Mexico), using germination trays containing a substrate (70:30, peat moss–soil) in a growth chamber (BJPX-A450, BIOBASE) with 13 h of darkness at 14°C and 11 h of light (2 h at 18°C, 7 h at 25°C, and 2 h at 18°C) for 30 days (Valenzuela-Aragon et al. 2019). The germination percentage ($n = 6$, by three independent biological replicates) of M_0 and M_1 wheat seeds was

determined by counting the number of seedlings at 10 days after sowing by following the protocol described by Warham et al. (1996). The survival percentage was determined by counting the viable seedlings at 30 days after sowing (Songsri et al. 2011). Plant height was estimated by measuring the aerial portion (from the base of the plant to the first leaf tip) following the protocol described by Borzouei et al. (2010). The LD_{50} of gamma irradiation on wheat seeds was determined with a Probit analysis based on the seedling's survival percentage in order to determine the optimal irradiation dose (Postelnicu 2011).

2.2 Statistical analysis

Experimental data were statistically analyzed by analysis of variance and a significant difference (Tukey test significant difference [HSD] at $p = 0.05$) in Statgraphics Centurion XVI. II software.

3 Results and discussion

3.1 Germination percentage

The wheat germination percentage did not show significant differences as the doses increased (Figure 1). This finding has been previously observed in wheat, where the irradiated seeds maintained their germination

Figure 1: Germination percentage of irradiated wheat var. CIRNO C2008 seeds. Different letters indicate a significant difference (Tukey HSD $p < 0.05$).

capacity similar to the nonirradiated seeds (Melki and Marouani 2009; Borzouei *et al.* 2010). This biological behavior suggests positive effects of mutagens on genes that control this trait, by efficient DNA repair mechanisms, acceleration of cell divisions in meristematic tissues, and the stimulation of hormones and enzymes involved in germination and growth processes (Dhakshnamoorthy *et al.* 2011).

3.2 Survival percentage

The survival percentage decreased as the irradiation doses increased, showing significant differences among them (Figure 2). It has been found that lower doses of irradiation (100 and 200 Gy) promote an early emergence and a higher percentage of germination and survival of seedlings (Horn and Shimelis 2013; Gómez *et al.* 2017). Treatment using 300 Gy showed a survival percentage of 46.6% (Figures 2 and 3). Thus, the LD₅₀ obtained by a Probit analysis was 290.6 Gy; similar findings have been reported by Brunner (1985) in durum wheat, obtaining an LD₅₀ from 150 to 300 Gy. Survival percentages between 300 and 500 Gy were negatively affected as the dose increased, due to the persistence of damaged DNA having a significant growth-inhibitory influence (Manova and Gruszka 2015). In addition, higher irradiation doses (500 and 600 Gy) are often lethal because they destroy genes or metabolites involved in seed germination and/or plant growth and induce undesirable modifications of chromosomal structures inhibiting the expression of genes involved in the cell cycle (Preuss and Britt 2003; Saraswathi *et al.* 2016).

Figure 2: Survival percentage of irradiated wheat var. CIRNO C2008 seeds. Different letters indicate a significant difference (Tukey HSD $p < 0.05$).

Figure 3: Survival percentage and plant height of irradiated wheat var. CIRNO C2008 seeds, under growth chamber conditions.

3.3 Plant height

Significant differences were observed in plant height, which decreased as the dose increased (Figure 4). The lowest height (1.16 cm) was obtained with 600 Gy, while the highest height (21.57 cm) was obtained with 100 Gy, which was even higher than the plant height recorded in nonirradiated seeds (19.21 cm). Irfaq and Nawab (2001) and Borzouei *et al.* (2010) reported that irradiated wheat seeds (100 Gy) showed the highest height compared to the rest of treatments (0, 200, 300 and 400 Gy), because lower irradiation doses cause hormetic effects, which are mainly attributed to changes in hormonal signaling in plant cells or an increase in its antioxidant capacity to face the stress caused by irradiation (Hernández-Muñoz *et al.* 2017). On the other hand, high irradiation doses

Figure 4: Plant height of irradiated wheat var. CIRNO C2008 seeds. Different letters indicate a significant difference (Tukey HSD $p < 0.05$).

cause greater damages to chromosomes and an early activation of protein synthesis, which reduces the plant height (Jaipo et al. 2019).

4 Conclusions

Lower doses of gamma irradiation (100 Gy) induce positive morphological changes in wheat growth, while higher doses (300, 400, 500, and 600 Gy) negatively affect plant growth and survival due to deleterious effects that reduce the stability of the plant genome, such as chromosomal damages. The calculated LD₅₀ (290.6 Gy) was obtained based on the survival percentage of irradiated vs nonirradiated wheat seedlings, in which a greater genetic variability is expected to be obtained in advanced mutant lines.

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