RESEARCH

Effect of the physical mutagenesis with ^{60}Co on jalapeño pepper seed quality

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ABSTRACT

Physical mutagenesis with ^{60}Co is a technique used in plant breeding to induce desired phenotypic characteristics. This research objective was to evaluate physical and physiological quality and protein content of jalapeño pepper (*Capsicum annuum* L.) seeds from plants that were selected as tolerant to water stress, these plants were derived from seeds irradiated with ^{60}Co at 100 Gy dose (T1P2 and T1P5 both from tolerant plants to water stress). Control seeds were significantly superior to the mutants in the variable standard germination (90.66%), standard germination with accelerated aging (57.3%), normal seedlings (95.6%), 1000 seeds weight (6.349 g), hectoliter weight (51.97 kg hL^{-1}) and radicle dry weight (18 mg). Mutant seeds were significantly superior to control seeds in seed length and width (4.356 mm in T1P2 and 4.256 mm in T1P5 for length; 3.544 mm in T1P2 and 3.632 mm in T1P5 for width). In seed thickness tests, germinated seeds aerial part fresh and dry weight, radicle fresh weight, the two mutants seed types and the control were significantly equal. In seed storage proteins content, T1P2 treatment had a higher globulins concentration. In the prolamin fraction, the two mutant seed types were superior to control. T1P5 treatment seeds had the highest glutelins concentration. Mutant seeds showed different characteristics as compared to control in physical, physiological and plantlet parts weight components.

Key words: *Capsicum annuum*, endosperm, mutant, physical mutagenesis, seed storage proteins.

INTRODUCTION

Mexico is the country with the highest number of *Capsicum* genus species, in addition to being an important center for domestication and diversification. According to 2019 production data, Mexico was the world's second largest peppers and green peppers producer, with 3 238 245 t, after China with 19 007 248 t (FAOSTAT, 2021). The 2019 green pepper planted area (irrigation and rain fed conditions) was 152 772 ha, but harvested area was 149 577 ha; mean yield was 21.65 t ha^{-1} (SIAP, 2021).

The most cultivated pepper species in Latin America and worldwide is *C. annuum*, followed by *C. chinense*. Most of this crop hybrid seed production comes from China, India and Thailand (Hernández-Pérez et al., 2020). There are genotypes so expensive that market seeds price ranges from 500 to 1000 USD per thousand seeds (Aguirre-Mancilla et al., 2017).

Today's agriculture challenge is to find alternatives that ensure increased crop yields and reduced input use that may cause high environmental pollution risk, besides crops must be more tolerant to biotic and abiotic stress (Raza et al., 2019). Mexico is a country vulnerable to drought because a large part of the territory (52%) is classified as arid or semi-arid, together with climate change it has caused changes in the rainy periods, which generates water stress in various crops, especially in vegetables such as pepper (Pontifes et al., 2018).

Physical mutagenesis is a technique that uses a source of ionizing radiation to cause random changes at the chromosomal level in the DNA of somatic and gametic cells. In addition, it is considered a simple and relatively cheap technique compared to other biotechnological breeding techniques (Shu et al., 2011). Mutation breeding is an alternative methodology used to generate genetic variability and to create characteristics that do not exist in the nature (Oladosu et al., 2016), generating new genetic combinations (desirable traits) or increasing variability in a population or to get genotypes that can be used as parents in breeding programs (Canul-Ku et al., 2012).

Gamma rays are mostly used to generate variability in agricultural crops. Application of this methodology which has high tissue penetration, reproducibility and high mutation frequency is advantageous and, in this way, crops can obtain improved characteristics (Piri et al., 2011).

Gamma rays are a type of ionizing radiation obtained by radioisotopes, usually using cobalt 60 (^{60}Co) and cesium 137 (^{137}Cs) as a source of radiation. The units used to measure the amount of radiation exposure are Gray (Gy) or their equivalents rad (1 rads = 0.01 Gy) (IAEA, 2006).

Some of the variables that are important to analyze after obtaining mutant plants through radiation are germination and vigor of the seeds obtained from these plants, to identify inherited modifications compared to the non-mutant control. Seed vigor and seed quality are determined by genetic, physiological, morphological and phytosanitary factors (Mazvimbakupa et al., 2015). It also depends on mother plant constitution and the biotic and abiotic environment that surrounds plant, from germination and later seedling development, until harvest. The prevailing environmental conditions during the post-harvest stage and in storage also influence seed vigor. Determining seed quality is a very important parameter because a good seed quality will give rise to vigorous plants, with high yields (Marcos-Filho, 2015).

The objective of this study was to evaluate the physical, physiological quality and protein content of M1 jalapeño pepper (*Capsicum annuum* L.) mutant seed that was derived from plants obtained from irradiated seeds; the plants obtained from irradiated seeds subjected to hydric stress as a selection criterion for the M1 seeds.

MATERIALS AND METHODS

Irradiation and seeding of 'jalapeño' pepper seeds

Physical mutagenesis was carried out at Instituto Nacional de Investigaciones Nucleares (ININ), Mexico. A 5 g sample of jalapeño pepper (*Capsicum annuum* L.) seeds were subjected to radiation with research irradiator (LGI-01, Transelektro, Budapest, Hungary); ^{60}Co was used as a radiation source; seeds were exposed from 100 to 400 Gy doses (Díaz-López et al., 2016).

The plants derived from the seeds irradiated with the dose of 100 Gy showed the favorable characteristics of development for the subsequent stage of this investigation. The plants derived from the highest doses were discarded due to high mortality and abnormal development.

Seeds were placed in germination trays with vermiculite substrate. When plantlets reached 15 cm height, they were transplanted into plastic bags of 30 × 30 cm using tezontle as substrate (4 L bag^{-1}) under greenhouse, when plants were 110 d-old after sowing, they were subjected to 7 d without irrigation; the plants that survived were selected and maintained until fruit production; fruits were cut and left to dry, after reached physiological maturity then seeds were obtained (T1P2 and T1P5); these mutant seeds both, came from plants that survived to water deficit by 7 d without symptoms of wilting, unlike control plants derived from non-irradiated seeds.

Two jalapeño pepper mutant seeds of M1 (T1P2 and T1P5) and one from non-irradiated seed (control) were evaluated for further testing. Radiation causes different effects in individuals of the same species because DNA aberrations are induced randomly, even with the same dose of radiation, therefore, mutations do not occur in the same place in the genome of two individuals.

Physical seed quality

For 1000 seeds weight trait, 100 pepper seeds were weighted in analytical balance and 10 replicates were made per treatment to extrapolate to 1000 seeds weight (ISTA, 2020).

To determine hectoliter weight (HW, kg hL^{-1}) trait, seed was placed on a test tube, 100 mL capacity; seed was weighted on an analytical scale. Five replicates were performed and then units were converted according to the following formula (ISTA, 2020):

$$HW = \text{Sample weight (g)}/(\text{Base volume (mL)} \times 1000)$$

Seed length, seed width and seed thickness were measured with a vernier. Average (mm) of 25 replicates per treatment was registered (ISTA, 2020).

Physiological quality components

Standard germination. To determine germination, methodology of ISTA (2020) with some modifications was used. Twenty-five seeds were placed on sterile germination paper; another paper moistened with sterile distilled water was placed on top; a roll was made with the paper; three replicates were considered and placed in a germination chamber during an 8 to 15 d period at 25 °C in darkness. After 15 d the germination percentage was determined.

Accelerated aging. Distilled water (250 mL) was added to a beaker; a metal mesh was placed and seeds were placed on the mesh, 2 cm above water level. The beaker was foil covered sealed with tape and incubated at 42 °C during 96 h in darkness (ISTA, 2020). After that time standard germination test was performed with the previously described technique.

Normal and abnormal plants. Twenty-five seeds were germinated (three replicates) and placed in a germination chamber at 25 °C for 8 to 15 d in darkness. Data were taken after 15 d of germination: normal seedlings, abnormal seedlings and non-germinated seeds or dead seed. Normal seedlings were identified as those with epicotyl, hypocotyl, well-developed radicle and both cotyledons present; abnormal plants: no terminal bud, chlorotic or deformed cotyledons, absence of one or both cotyledons; dead seed: no germination presented (ISTA, 2020).

Radicle fresh and dry weight and aerial part of plantlets. The seed was set to germination during 15 d. Plantlet root and aerial part were sampled, and weighted with an analytical balance. Then they were dried out on a stove at 60 °C during 48 h. After, samples were weighed again. The root/aerial part proportion is the quotient between root dry weight and aerial part dry weight (Reyes-Pérez et al., 2013). Twenty-five plantlets at each replicate were weighed; three replicates were considered.

Protein extraction. The proteins extraction was performed using the methodology reported by Aguirre-Mancilla et al. (2020), with some modifications. Seed was ground into mortar to get a fine flour that was subsequently defatted by three washes (4:1, v/w) of a chloroform:methanol mix 2:1 (v/v); for 1 h in each wash; solvent was removed by centrifugation for 10 min at 11 500*g* and at 4 °C. After the third wash, the flour was allowed to dry at room temperature until the solvent was evaporated (about 12 h). To obtain albumin fraction 0.1 g flour from each sample was added to 1 mL distilled water, then stirred for 15 min and centrifuged for 10 min at 11 500*g* at 4 °C to obtain the supernatant. To obtain globulin fraction, precipitate from the previous extraction was added 1 mL 0.5 M NaCl in 50 mM Tris-HCl, pH 8, stirred for 15 min, then centrifuged for 10 min at 11 500*g*, 4 °C supernatant was recovered. To extract prolamin fraction, to the precipitate obtained from the previous fraction, 1 mL isopropyl alcohol 55% was added, sample was stirred and centrifuged as in previous fractions to get the supernatant. Finally, to get glutelin fraction 1 mL borate buffer (0.1 M sodium borate with 0.5% sodium dodecyl sulfate) pH 8 was added, mix was stirred and centrifuged under the above conditions. Protein fractions were quantified according to Bradford (1976) using calibration curve with bovine serum albumin (BSA) at concentrations of 0-500 μg mL^{-1}, six replicates were made for each protein fraction.

Electrophoretic protein profile. Electrophoretic pattern of seed storage proteins was determined by polyacrylamide gels (SDS-PAGE) (Schägger and von Jagow, 1987). Each protein fraction, 40 μg protein was diluted to a total volume of 20 μL with sample buffer (50 mM Tris, 4% SDS, 12% glycerol, 0.01% Coomassie blue, pH 6.8). The samples were loaded into the gel and ran for 2.5 h, then the gels were fixed (50:10:40 methanol:acetic acid:water) for 30 min and then stained (0.025% blue G and 10% acetic acid) for 1 h, then washed with bleaching solution (10% acetic acid) to remove dye excess. Gels image was taken in the photodocumenter GelDoc EZ with the program Image Lab software version 5.1 for proteins (Bio-Rad Laboratories, Hercules, California, USA).

Statistical analysis

Quantitative data were subjected to ANOVA using the SAS software Version 8 (SAS Institute, Cary, North Carolina, USA) and means comparison was realized by Tukey test ($p \leq 0.05$) when ANOVA showed significant differences among treatments.

RESULTS AND DISCUSSION

Physical quality components

Control seeds were superior to mutated seeds for 1000 seeds weight and hectoliter weight variables; seed weight was significantly different between T1P2 and T1P5; radiation affected negatively these variables (Table 1). Radiation treatments were significantly higher than the control for seed length and seed width; there were nonsignificant difference among the three treatments for seed thickness (Table 1). Although mutant seed treatments come from parents subjected to the same radiation dose (100 Gy) and kept under the same conditions, these results indicate that radiation had an effect on the seed physical characteristics and this effect was different for each type of mutant seed. The physical attributes possibly modified due to positive mutations were length and width of seed, they were significantly higher than control. Although the mutant size seeds were larger than the control, these characteristics were not reflected in greater weight, since the control was superior.

Manfreda and Acosta (2015) pointed out that seed length and seed width are inherited independent traits, so improving these traits in irradiated seeds can be searched for and selected in later generations. The phenotypic changes presented by mutant seeds are due to random genotypic alterations caused by radiation

Physiological quality components

The means comparison for germination percentage indicated that the control had a higher percentage of germination and germination with accelerated aging with respect to the mutant treatments (Table 2). The mutant seeds presented a lower germination percentage compared to the control; this coincided with Álvarez-Holguín et al. (2017) who report 65% germination of grass seeds subjected to a radiation dose of 100 Gy. Additionally, a greater deterioration in the quality of the seeds was found with the accelerated aging test. In the means comparison for the variable of normal seedlings, the control outperformed the mutants, which means that the abnormal seedlings were higher in the mutants (Table 2); the effects were not different between T1P2 and T1P5. Radiation had negative effects on germination process since the

Table 1. Means comparison by Tukey test of mutant seed and control for physical quality variables.

Treatments	1000 SW	HW	SL	SW	ST
	g	kg hL^{-1}	mm		
T1P2	5.26b	41.3b	4.3a	3.5a	0.852a
T1P5	3.38c	31.6c	4.2a	3.6a	1.3a
Control	6.34a	51.9a	4.1b	3.3b	1.1a

1000 SW: 1000 Seeds weight; HW: hectoliter weight; SL: seed length; SW: seed width; ST: seed thickness. T1P2 and T1P5: M1 seeds from different plants originating from seeds irradiated with 100 Gy; Control: seed from plant originating from non-irradiated seed.
Means with equal letters within each variable are not significantly different (Tukey, $p \leq 0.05$).

Table 2. Means comparison by Tukey test of physiological quality variables evaluated on mutant seeds as compared to control.

Treatments	G	GAA	NP	AP
	%			
T1P2	61.3b	22.67b	69.8b	30.4a
T1P5	56.0b	24.0b	76.2b	23.8a
Control	90.6a	57.3a	95.6a	4.4b

G: Germination; GAA: standard germination with accelerated aging; NP: normal seedlings; AP: abnormal seedlings; T1P2 and T1P5: M1 seeds from different plants originating from seeds irradiated with 100 Gy; Control: seed from plant originating from non-irradiated seed.
Means with equal letters within each variable are not significantly different (Tukey, $p \leq 0.05$).

number of normal seedlings was strongly reduced by its effect. The germination percentage was reduced 32.3% in T1P2 and 38.2% in T1P5 as compared to the control. Accelerated aging reduced germination percentage in the control by 42.7%; this test strongly affects germination in this crop. This test reduced germination percentage in T1P2 (60.4%) and in T1P5 (58.1%); a greater effect was observed in the germination percentage with accelerated aging, possibly due to a greater weakness of seed. Number of normal seedlings was reduced by 19.3% in T1P5 and by 27% in T1P2. Meitei et al. (2020) point out that the higher the doses of gamma radiation, the greater the number of chromosomal alterations with negative effects on the development and physiology of plants; however, mutations with desirable effects can be inherited over several generations; therefore, the evaluation of the morphological and physiological behavior in each generation is required to identify these changes.

Plantlet parts weight

In plantlet aerial part fresh weight and radicle fresh weight variables, control was superior to the mutants (Table 3). Aerial part dry weights and radicle dry weights variables, the control and the T1P2 mutant were significantly similar; both T1P2 and T1P5 mutants were significantly similar for these two variables. There were not differences for dry weight variables between control and one mutant treatment (T1P2). The radiation dose used caused a negative effect for the variables evaluated in only one of the mutants (T1P5) compared to the control, while the T1P2 mutant showed similar values to the control, this allows us to assume that radiation did not significantly affect the initial development of the mutant seedlings. Similarly, it was reported that common bean M1 plantlets, obtained with doses of 100 Gy, showed that root fresh weight and shoot fresh weight were equal to the control; however, root dry weight and shoot dry weight were significantly higher in the mutants than in the control (Ulukapi and Ozmen, 2018).

Reyes-Pérez et al. (2013) mentions that dry biomass is used as a measure for plant growth, because it reflects a balance between total photo assimilated production and respiration and in this sense, our results indicate that mutant seeds produced plantlets with less fresh and dry biomass as compared to the control. Jan et al. (2013), Ulukapi and Ozmen (2018) and Ulukapi (2021) report positive effect of low doses irradiation (100 Gy), in addition by the induction of genetic changes, also by inducing cytological, biochemical, physiological and morphogenetic changes in cells and tissues; these effects include the increase in chlorophyll, sugar and total C content. Variability in seed quality show that mutations occur because of radiation is individual and random events throughout the genome, where some characteristics are improved, while others are negatively affected Viana et al. (2019). But Aros et al. (2012) concluded that determining the optimal dose to induce positive mutations depends on the susceptibility of the plant and the tissue subjected to irradiation.

Storage protein content in seed

Total soluble protein content (mg g^{-1}) in pepper seed did not show significant differences between mutant and control seeds (Table 4). This shows that the total protein concentration was not affected by irradiation. Concentration of albumin, globulin, prolamin and glutelin protein fractions from seeds was also determined. Table 5 shows protein content values per fraction among genetic materials; alterations can be seen in protein fractions proportions of mutant treatments as related to control. For albumin, control was significantly superior to T1P2 and T1P5 mutants by 34% and 43%, respectively. For globulin, T1P2 treatment and control were significantly superior by 15% to T1P5. For prolamin, both mutant treatments (T1P2 and T1P5) were 60% and 44%, respectively, higher than control. For glutelin, mutant T1P5 had a protein content 73% higher than control and T1P2 treatment. Albumin content in control was higher than the irradiation treatments, but it

Table 3. Means comparison by Tukey test for plantlet parts weight evaluated on seeds mutant as compared to control.

Treatments	AFW	RFW	ADW	RDW	PDW/ADW ratio
	mg				
T1P2	600.00b	20.00b	53.33ab	13.33ab	0.2499
T1P5	506.67b	30.00b	36.66b	10.00b	0.2727
Control	816.67a	93.33a	63.33a	20.00a	0.3158

AFW: Aerial part fresh weight; RFW: radicle fresh weight; ADW: aerial part dry weight; RDW: radicle dry weight; T1P2 and T1P5: M1 seeds from different plants originating from seeds irradiated with 100 Gy; Control: seed from plant originating from non-irradiated seed.
Means with equal letters within each variable are not significantly different (Tukey, $p \leq 0.05$).

Table 4. Means comparison of total seed storage protein content by treatment.

Treatments	Total soluble protein content
	mg g^{-1} flour
T1P2	45.67a
T1P5	45.51a
Control	48.81a

T1P2 and T1P5: M1 seeds from different plants originating from seeds irradiated with 100 Gy; Control: seed from plant originating from non-irradiated seed.
Means with the same letters within each variable are not significantly different (Tukey, $p \leq 0.05$).

Table 5. Means comparison by Tukey test of protein content by fraction on mutant seeds compared to control.

Treatments	Albumin	Globulin	Prolamin	Glutelin
	mg g^{-1} flour			
T1P2	11.45b	21.66a	3.73a	8.81b
T1P5	9.92b	17.06b	3.35a	15.17a
Control	17.50a	20.20a	2.32b	8.77b

T1P2 and T1P5: M1 seeds from different plants originating from seeds irradiated with 100 Gy; Control: seed from plant originating from non-irradiated seed.
Means with the same letters are not significantly different (Tukey, $p \leq 0.05$).

was lower than these in the prolamin and glutelin content. Globulin content was significantly similar between the control and the T1P2 treatment. The protein fraction was differentially affected by radiation treatments. These results indicate that the genes that encode for the seed storage proteins of the albumin fraction, or the factors that participate in their expression, were altered by radiation; this affected the physiology of the seed, which showed a high percentage of abnormal seedlings and lower germination and vigor percentages compared to non-mutant seeds. Although seed storage proteins, generally do not carry out any enzymatic functions, enzymes with hydrolytic activity (proteases, amylases) are present in the seeds; therefore, another possibility is the mutation of the genes that encode for this type of proteins, which participate in the mobilization of nutrients towards the embryo in the process of germination and establishment of the seedling (Tan-Wilson and Wilson, 2012), which could also explain the low percentage of germination and vigor of the mutant seeds.

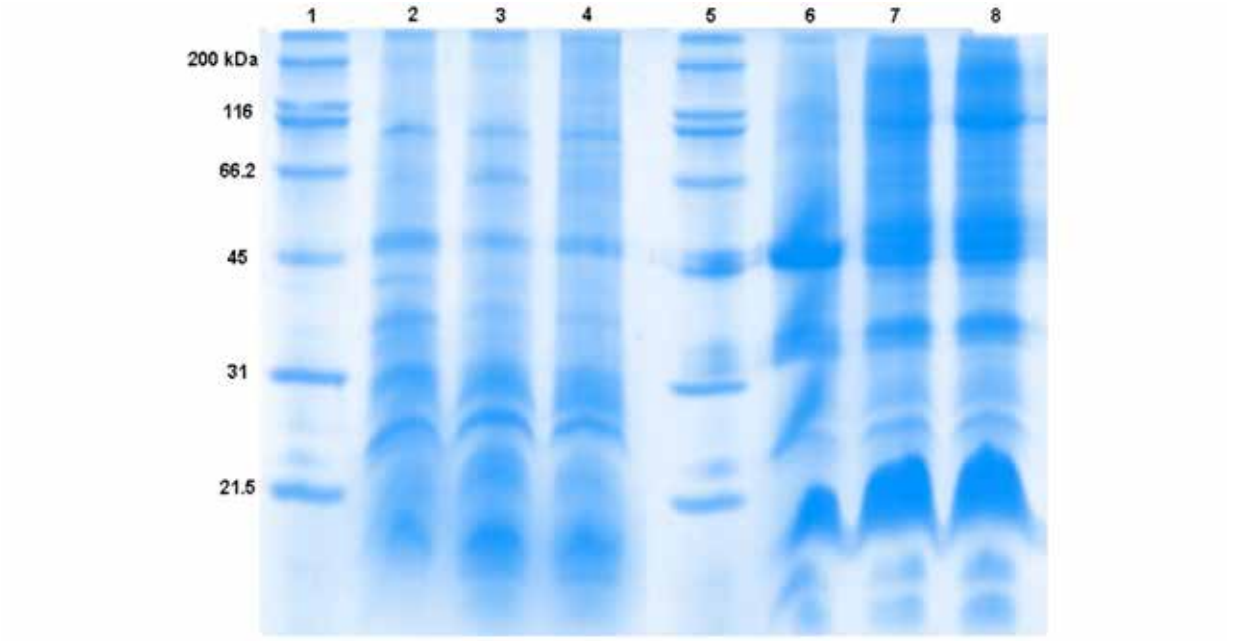
Electrophoretic pattern of protein fractions

The electrophoretic pattern from the different protein fractions showed differences between band patterns and their relative abundance between mutants and with the control. In albumin, T1P2 mutant showed a band at 66 kDa (Figure 1, lane 3), that control and T1P5 was absent (Figure 1, lane 1 and 4). In the control seeds, two bands were observed at 34 and 42 kDa (Figure 1, lane 2), which were absent in both mutants (Figure 1, lanes 3 and 4). And in the zone of low molecular weight (10 kDa) a band was generated in both mutant seeds (Figure 1, lanes 3 and 4), that the control seed was not observed (Figure 1, lane 2). In the case of the electrophoretic pattern of the globulin fraction, it was observed that both mutant seeds had more intense bands between 66 and 200 kDa than the control, a band at 49 kDa was also shown in mutant seeds (Figure 1, lanes 7 and 8) that was not observed in the control (Figure 1, lane 6). Lee et al. (2011) report that in mutant soybean seeds obtained by irradiation with 250 Gy, alterations and modifications were shown in glycinin proteins, it was also reported that some mutants increased protease inhibitors and in other mutants decreased this type of proteins. In these two protein fractions are present the proteins that participate in metabolic activation when the seed begins the germination process (Costa Nobre et al., 2016). These changes in the protein profile probably affected the components of the seed physiological quality of the mutated seeds (Table 2). Seed storage proteins are important substances that provide nutrients to the embryo so that germination can be triggered. If these proteins are poor in quantity or quality, the embryo may not germinate or the nascent seedling may have development problems (Fujiwara et al., 2002).

In the electrophoretic pattern of the prolamin fraction, the T1P2 mutant seed showed a band at 116 kDa (Figure 2, lane 3), the T1P5 seed was also observed with less intensity (Figure 2, lane 4), but it was not observed in the control seeds (Figure 2, lane 2). A change in the intensity of the 55 kDa band was also observed, in both mutant seeds, T1P2 and T1P5

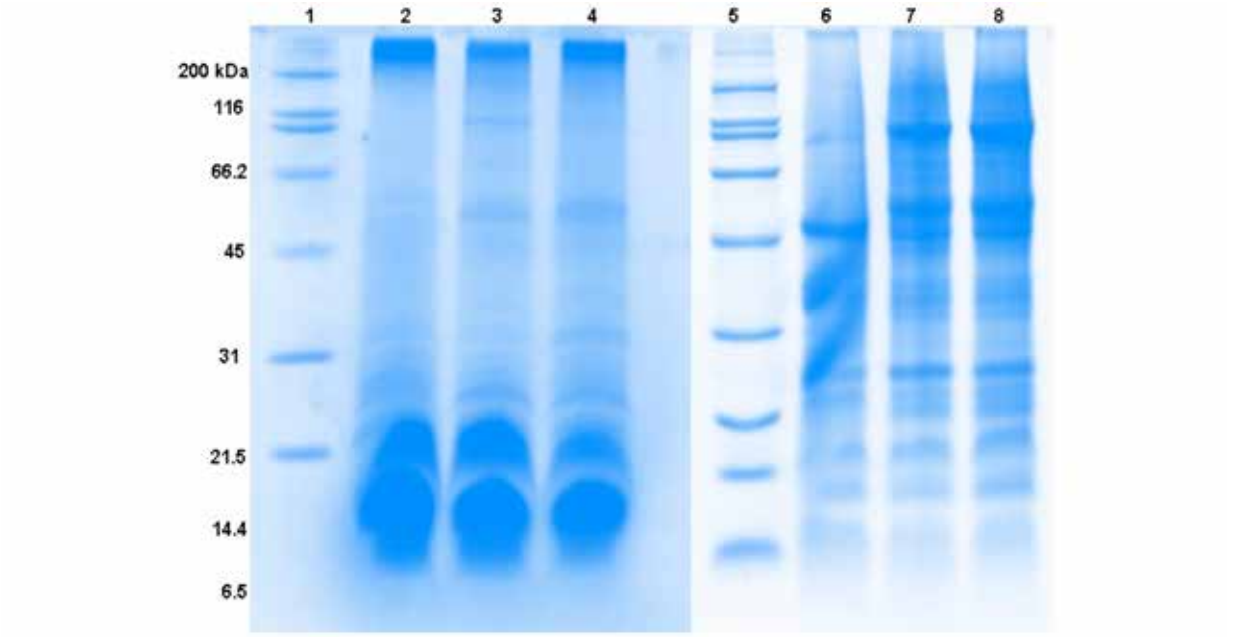
(Figure 2, lanes 3 and 4) was more intense than in the control seed (Figure 2, lane 2). And in glutelin fraction, both mutant seeds had an 80 kDa band that does not have the control (Figure 2, lanes 7 and 8); in addition, they had thicker bands of 110 kDa size than the control (Figure 2, lanes 7 and 8).

Figure 1. Electrophoretic profile of the albumin and globulin fractions (20 μg protein by lane).



1: Molecular weight marker in kDa; 2: control prolamin fraction; 3: T1P2 prolamin fraction; 4: T1P5 prolamin fraction; 5: molecular weight marker in kDa; 6: control glutelin fraction; 7: T1P2 glutelin fraction; 8: T1P5 glutelin fraction; T1P2 and T1P5: M1 seeds from different plants originating from seeds irradiated with 100 Gy.

Figure 2. Electrophoretic profile of prolamin and glutelin fractions (20 μg protein by lane).



1: Molecular weight marker in kDa; 2: control prolamin fraction; 3: T1P2 prolamin fraction; 4: T1P5 prolamin fraction; 5: molecular weight marker in kDa; 6: control glutelin fraction; 7: T1P2 glutelin fraction; 8: T1P5 glutelin fraction; T1P2 and T1P5: M1 seeds from different plants originating from seeds irradiated with 100 Gy.

In this work polymorphisms can be seen in the electrophoretic pattern of irradiated seeds reserve proteins with ^{60}Co, this can be reflected in differences presented by treatments in physical, physiological and yield quality components; Aguirre-Mancilla et al. (2020) points out that relative abundance of reserve proteins can alter seed properties such as vigor, this should be considered for plant breeding programs. The most abundant protein fractions were first globulins followed by albumins; this is consistent with Ramírez-Pimentel et al. (2016), who sustains that dicotyledons present albumins and globulins as main reserve proteins. Mouzo et al. (2018) points out that seeds reserve proteins may be used as a study system of genetic diversity and hybridization of these species, being glutelin the most suitable fraction for this, which may lead to further researchers since in an indirect way we can assume that if seed reserve protein content changed, then it means there was a change in irradiated pepper seeds genome.

Meitei et al. (2020) mention that gamma ray mutagenesis is a feasible tool in plant breeding, because it can induce favorable changes in the plant genome, but it is important to evaluate the effects of radiation doses, since high doses can produce adverse results in the phenotype, in addition to the fact that each plant species responds differently to the effects of radiation.

CONCLUSIONS

The mutant seeds obtained from plants selected for their ability to survive water stress showed different physical characteristics among themselves and to a greater degree compared to the control; indicating that, although the parents (M0 seeds) received the same radiation dose, the mutations caused were random among individual seeds. The different seed quality tests to which the mutant seeds were subjected turned out to be lower than the control. Although the total soluble protein content of the mutant and control seeds was not affected, the proportions of the seed storage proteins of the mutant seeds were affected, and this partly explains why germination and vigor were notably affected.

It is necessary to continue with the process of evaluating the following generations of mutants, in order to continue selecting and tracking characters that help enhance the adaptability of the crop under adverse conditions, as well as increase the physiological quality of the seeds that allows a better crop establishment.

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