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Response of melon accessions to doses of Co^{60} gamma rays and their effects on the morphology of the M1 generation[1]

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ABSTRACT - The aim of this study was to verify the response of the melon accessions MR1 and C14 to gamma radiation in terms of germination and morphology.The melon accessions came from the germplasm collection of the Federal Rural University of the Semi-arid Region, and were submitted to five doses of gamma radiation (0, 100, 200, 300, 400 Gy) in four replications, each comprising 25 seeds, at the Center for Nuclear Energy in Agriculture of the University of São Paulo. The seeds were grown in a greenhouse at UFERSA employing a completely randomised design. The germination potential, rate and index were evaluated during the first stage of the trial. During the second stage, the germinated plants were transplanted into eight-litre buckets containing substrate and sand, in four replications of 15 plants each. The inflorescence, leaf blade and fruit were evaluated. The IMAGE J image analysis software was used to obtain the leaf and fruit data. The germination potential varied for the gamma-ray doses being tested, while the germination rate and index were not affected; phenotypic variations were seen in the leaves. The gamma-ray doses had no effect on the germination process.

Key words: Irradiation. Genetic variability. Mutation. Phenotypes.

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INTRODUCTION

The melon (*Cucumis melo* L.) belongs to family Cucurbitaceae. The vegetable is cultivated in various regions around the world, due to its wide adaptability (Kesh; Kaushik, 2021). Melon cultivation is socioeconomically most important in the northeast of Brazil, where especially in the semi-arid region, the melon accounts for 90% of domestic production (Silva *et al.*, 2024). The states of Rio Grande do Norte and Ceará stand out in terms of exports, accounting for up to 80% of the total for the country (Hortifrut Brasil, 2021).

In order to meet the demands of the agricultural market, genetic improvement has proved to be an important alternative in obtaining new genotypes resistant to biotic and abiotic stress.

Paul Ernest *et al.* (2020) and Tulmann Neto *et al.* (1995) suggest induced mutations as a promising alternative in breeding. This has significantly contributed to the creation and incorporation of new genes of agronomic interest.

Gamma rays are electromagnetic waves that are considered to be one of the main mutagenic agents, causing changes at various levels in plants, and stimulating biological responses by interacting with the atoms or molecules of the cells, affecting everything from DNA to cytoplasmic organelles, and modifying the phenotype of the plants (Kovács; Keresztes, 2002). The free radicals produced by this interaction have been reported to a have different effects on plant morphology, anatomy, biochemistry and physiology depending on the dose of radiation (Ashraf *et al*., 2004).

The effect of gamma radiation on agricultural production has been reported by researchers around the world. In general, the sensitivity of seeds to the effects of mutagens on germination, plant development and survival is assessed first (Hazra *et al*., 2021; Paul Ernest *et al*., 2020; Velkov; Tomlekova; Sarsu, 2016). The fertility of the M1 generation should be taken into account when testing mutation frequency in response to the dose of radiation, since in studies of mutation creation, M2 progeny should be generated by reproduction (Jo; Kim, 2019).

Borzouei *et al* (2010) studied the effects of gamma radiation on germination and the physiological characteristics of wheat seedlings using doses of 100-400 Gy. They found that different doses of gamma radiation have different effects on the biochemical characteristics of the plants, such as increasing the level of proline and chlorophyll, and stimulating germination and growth in the seedlings.

Beyaz (2020), evaluating the effect of pre-treatment with gamma radiation on growth in the common vetch, reported positive results for germination and seedling development. Paul Ernest *et al.* (2020) evaluated the sensitivity of two watermelon cultivars (Kaolack and Crimson) to different doses of gamma radiation (100-600 Gy) and saw a reduction in values for the parameters under evaluation, i.e. germination rate, survival rate and shoot length, for increases in the irradiated dose.

The expressed response depends on the dose of radiation, which can condition the gain or loss of desirable characteristics. Although the generation of new varieties by gamma radiation is random, these can be quickly identified and selected, accelerating the process of establishment and release (Riviello-Flores *et al*., 2022).

Considering the effects of gamma radiation on plants, the aim of the present study was to evaluate the germination response of seeds of two melon accessions, MR1 and C14, irradiated with different doses of gamma radiation, and their effect on some of the main characteristics in the morphology of the plants.

MATERIALS AND METHODS

Location and experimental design

The accessions were irradiated at the Center for Nuclear Energy in Agriculture of the University of São Paulo (CENA/USP) and the studies were conducted in a greenhouse of the Department of Agronomic and Forestry Sciences (DCAF), at the Federal Rural University of the Semi-arid Region (UFERSA), in Mossoró, Rio Grande do Norte.

The district of Mossoró is located at 5º12’48’’ S, 37º18’44’’ W at an altitude of 37 m above sea level. According to the Köppen classification, the climate in the area is of type BSwh’, hot and dry with a rainy season in the summer that lasts until the autumn (Carmo Filho; Oliveira, 1995). The average temperature at the site was between 31 ºC and 33 ºC, with a relative humidity between 52.6% and 53.9%.

A completely randomised design (CRD) was adopted in a factorial scheme. The first factor consisted of five doses of gamma radiation (0, 100, 200, 300 and 400 Gy), and the second factor comprised two melon accessions (C14 and MR1), with four replications, which gave rise to the plants of the M1 cycle.

M0 seeds from two melon accessions were irradiated at five different doses of gamma radiation, with four replications. To assess the germination response, each replication included 25 seeds, giving a total of 100 seeds per accession irradiation treatment. To assess the morphology of the M1 cycle plants, each replication consisted of 15 plants, giving a total of 60 plants for each accession irradiation treatment.

Plant material

Seeds from two melon accessions from the Active Germplasm Collection of UFERSA, accession MR-1 (subspecies *momordica*) and accession C-14 (subspecies *agrestis*) (Figure 1), were used in the study. The MR-1 strain is of the *momordic* type, its fruit is oblong or flattened with a yellow-coloured skin with dark green veins and a creamy pulp (Figure 1 A) (Albuquerque, 2014). Ag-15591Ghana, known as C14, is a wild melon native to Ghana, and belongs to the *agrestis* group. The fruit is small and oval-shaped, with dark green stripes and a low soluble-solids content (< 5 ºBrix) (Figure 1 B).

The C14 and MR-1 accessions have no commercial value for the fruit crop in the northeast, they are, however, sources of resistance to *Macrophomina phaseolina* and *Pseudoperonospora cubensis*, respectively.

Gamma irradiation

Seeds from the melon accessions were sent to CENA/USP in Piracicaba, São Paulo, where, based on the available literature, they were irradiated at five different levels (0, 100, 200, 300 and 400 Gy) of Cobalt-60 (Co^{60}) radiation using a Gammacell-220 irradiator.

Conducting the experiment

Seed germination

Following irradiation the seeds were sown in trays of 50 cells filled with commercial substrate and washed sand in a 1:1 ratio (one part soil to one part substrate), sterilised in an autoclave (2 hours at 120 ºC for two consecutive days, with an interval of 24 hours between each sterilisation) and moistened with water at 60% of the holding capacity (Brasil, 2009). The trays were placed in a greenhouse and irrigated daily to maintain the appropriate conditions for germination. The number of emerged normal seedlings (cotyledons forming a 90° angle) was counted daily until the 15 th day after sowing.

The number of germinated seeds was recorded over 15 days, when the following were measured: germination potential, rate of twinning and germination index, where:

Germination potential (%) = (Number of germinated seeds on the 5th day / total number of seeds) x 100%

Germination Rate (%) = (Number of germinated seeds on the 15th day / total number of seeds) x 100%

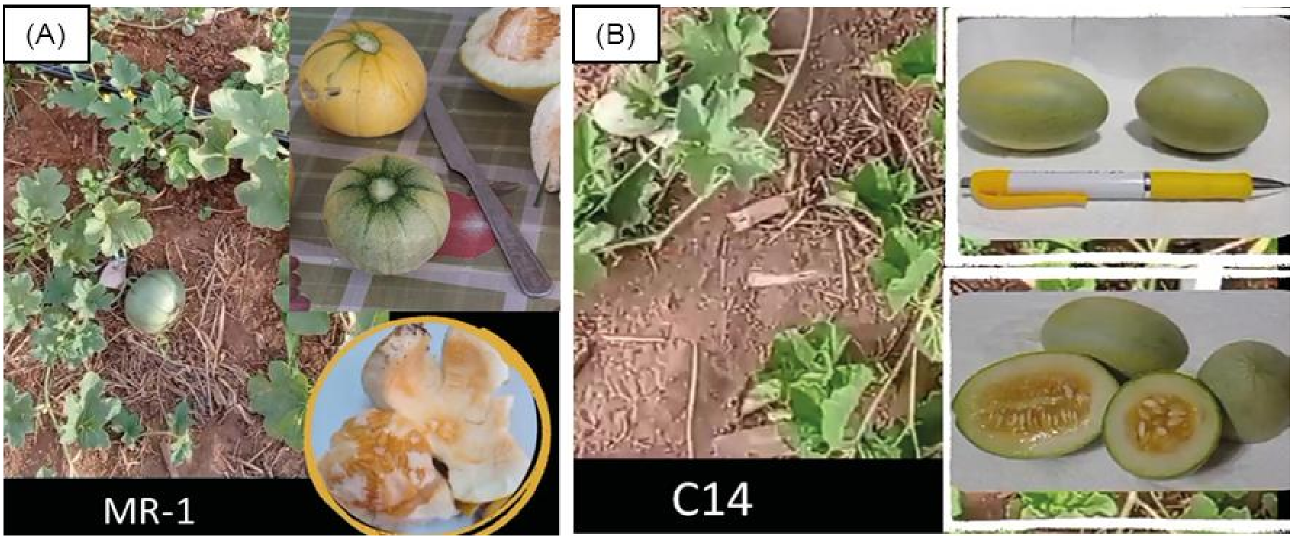
Germination index = ∑ (Gt/Dt), where Gt represents the number of germinated seeds on day *t* and Dt represents the number of days of germination.

Morphological characteristics

From the germination trial, 15 M1 plants were selected per treatment/replication and transplanted into 8-litre buckets containing substrate and sand in a 1:1 ratio. The plants were grown in a greenhouse until fruit was obtained, resulting in a total of 600 plants.

Two flowers were collected during full flowering at an interval of 25 days to obtain the inflorescence data. For data on the leaf blade, three leaves located in the basal, median and apical regions of the stem of each plant were highlighted, and the samples placed on a table and photographed. To analyse the fruit data, fruit samples (originating from manual pollination) were collected and photographed. The images were analysed using the IMAGEJ software to obtain the data. The seeds were counted manually.

Figura 1 - Acessions from the germplasm collection of UFERSA. (A) MR-1 (subspecies *momordica*); (B) C-14 (subespecies *agrestis*)



The variables under evaluation related to the inflorescence were the number of female flowers, number of male flowers, number of bisexual flowers, number of flowers with anomalies, number of flowers with up to four petals, number of flowers with more than five petals, and number of anthers. The leaf-blade variables were length (cm) and width (cm). The variables associated with the fruit included weight (g), width (cm), length (cm), pulp thickness (cm), number of seeds and number of fruit.

Statistical analysis

The data were analysed using the Snedecor F-test with the aid of the R software, and the mean values were compared using the Scott-Knott test at 5% probability ($\alpha = 0.05$), a multivariate analysis of principal components.

RESULTS AND DISCUSSION

The accession had an effect on all the characteristics related to germination (Table 1), while the dose differed for germination potential only. The interaction between the two factors under study was non-significant, indicating a lack of distinct genotype behavior on germination at the applied doses of gamma radiation. Because of this, comparisons should only be made at the level of the principal effects.

Accession C-14 presented a higher mean value for germination percentage, germination rate and germination index compared to the estimate for accession MR-1 (Table 1). For germination percentage, the doses were divided into two groups: the first group, with highest mean values, consisted of doses 0, 100 and 200, while the second group comprised doses 300 and 400. For the other characteristics, the doses were grouped together.

This study showed the low sensitivity of melon accessions to the doses of gamma radiation under test, with a difference for germination potential only. Doses 100 and 200 did not differ from the control, unlike doses 300 and 400, which generated lower mean values, showing a slightly harmful effect which delays germination in accessions C14 and MR1. The low sensitivity of cucurbits has been previously reported, with gamma rays having the ability to improve germination parameters at low doses. Jaipo *et al.* (2019) evaluated the effects of radiation on germination parameters in the cucumber and okra, where the best values for germination index and germination rate were seen in plants that received a dose of 50 Gy. In this study, dose 100 resulted in a 10% increase in germination potential. Considering that the sensitivity response is different between species, cucurbits show low sensitivity, with no harmful effects up to a dose of 200 Gy in the case of the accessions under evaluation (Table 1).

The accessions showed variations between each other for germination potential, germination rate and germination index. Similar behavior was described by Dutta *et al.* (2021), who evaluated the use of gamma radiation in breeding bitter gourds (*Momordica charantia* L.), a cucurbit of commercial

Table 1 - Mean values of three characteristics related to seed germination from two melon accessions submitted to four doses of gamma radiation

Factor	Mean Value (Characteristics)		
	GP	GR	GI
Accession			
MR-1	61.00 b	96.00 b	4.57 b
C-14	95.20 a	99.50 a	10.91 a
Dose			
0	78.50 a	97.00 a	6.45 a
100	87.00 a	98.00 a	8.34 a
200	84.50 a	98.50 a	7.68 a
300	68.00 b	99.00 a	8.50 a
400	72.50 b	97.50 a	7.75 a
F (Accession)	81.75**	15.38**	83.84**
F (Dose)	3.55*	0.57^{ns}	1.08^{ns}
F (H x D)	1.73^{ns}	0.48^{ns}	1.06^{ns}

**, *: Significant by the Snedecor F-test at ($p < 0.01$) and ($p < 0.05$), respectively. ^{ns}: Not significant. Mean values followed by the same letter do not differ by Scott-Knott test ($p > 0.05$). GP: Germination Percentage (%); GR: Germination rate; GI: Germination index importance in India, and found that germination percentage in the M1 generation varied for the four genotypes under study. The Special Boulder genotype was the most tolerant to the mutagenic, while the Meghna-2 genotype was the most susceptible to radiation.

Studies comparing the responses of two varieties of watermelon to five doses of gamma radiation (100, 200, 300, 400 and 600 Gy) showed significant behaviour for the Kaolack variety at a dose of 600 Gy, this variety obtained the lowest survival rate; while for the Crimson variety the lowest rate was seen at a dose of 300 Gy (Paul Ernest *et al*., 2020).

A significant effect was seen on the characteristics related to the leaf for the principal effects of the factors Accession and Dose (Table 2). There was, however, no significant effect for the interaction between the above factors.

The mean values for length and width of the leaf blade were higher in the C-14 accession than in the MR-1 accession. For both variables, two groups of mean values were formed relative to the doses. Doses 0 and 400 showed higher mean values, and doses 100, 200 and 300 were characterised by small leaves. Chen *et al.* (2018) found morphological changes in the plant architecture, leaves, floral organs and fruit; however, the leaves had the highest proportion of mutations, with 33.6%. Studying the variability induced by gamma radiation in the eggplant (*Solanum melongena* L.), found significant differences between treatments for the characteristics under analysis, among them the length and width of the leaves. He also points to these characteristics as an indicator of the genetic variation that exists in the total variation presented by the materials. In this study, changes in lobe morphology were seen in both accessions (Figure 2).

There was a significant accession effect on the variables related to flowering, but only for the number of female flowers and the number of male flowers (Table 3), with the MR-1 accession having highest mean values for the numbers of female and male flowers. In this study, there were no significant variations for the number of flowers in terms of sex expression or changes in floral morphology intensified by the use of gamma radiation. Corroborating these data, Velkov, Tomlekova and Sarsu (2016) evaluated the sensitivity of the Bojura variety of watermelon to the mutagenic agents Co^{60} and ethyl methanesulfonate (EMS) and reported no changes in the structure of the flowers.

However, alterations in floral morphology using mutagens have been found in some studies with cucurbits. Chen *et al*. (2018) conducted research using the EMS mutagen in the cucumber to obtain a mutant library, and selected the mutant lines from morphological changes in the plant architecture, leaves, floral organs, fruit and other characteristics in the M1, M2, and M3 generations. The mutations in the floral organs included both the shape of the flower and sex expression.

According to Zhang *et al*. (2012) and Reddy and Anadurai (1992), sterile plants or the changes in flower structure in mutant offspring is one of the markers associated with determining the efficacy of the dosage.

Figure 2 - Effects of gama rays on the leaf blade of the MR1 (A) and C14 (B) accessions

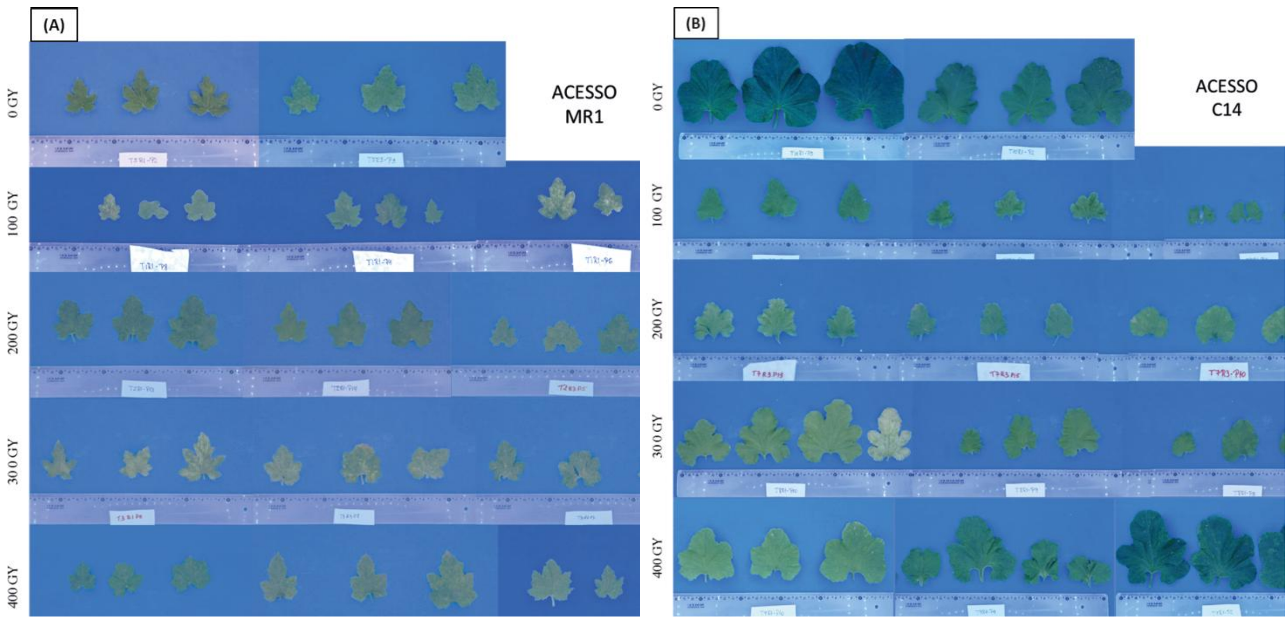


Table 2 - Mean values of two characteristics related to the leaf blade in two melon accessions submitted to four doses of gamma radiation

	Mean Values (Characteristics)	
	Leaf blade length (cm)	Leaf blade width (cm)
Accession		
MR-1	3.82 b	3.97 b
C-14	5.53 a	5.31 a
Dose		
0	5.90 a	5.62 a
100	3.82 b	3.98 b
200	4.48 b	4.53 b
300	4.55 b	4.29 b
400	6.08 a	5.89 a
F (Accession)	21.12**	18.47**
F (Dose)	3.55*	3.64*
F (H x D)	0.61^{ns}	1.25^{ns}

**, *: Significant by the Snedecor F-test at ($p < 0.01$) and ($p < 0.05$), respectively. ns: Not significant. Mean values followed by the same letter do not differ by Scott-Knott test ($p > 0.05$)

Table 3 - Mean values of six characteristics related to flowering in two melon accessions submitted to four doses of gamma radiation

	Mean Values (Characteristics)					
	NFF	NMF	NFA	NF4	NF5	NAT
Accession						
MR-1	2.60 a	38.65 a	2.75 a	1.40 a	0.30 a	3.00 a
C-14	0.50 b	24.60 b	2.95 a	1.20 a	0.31 a	3.20 a
Dose						
0	2.25 a	26.38 a	1.50 a	1.13 a	0.50 a	3.00 a
100	1.38 a	27.38 a	2.88 a	1.38 a	0.38 a	3.13 a
200	1.50 a	37.38 a	2.63 a	1.88 a	0.23 a	3.00 a
300	1.75 a	39.25 a	4.38 a	1.13 a	0.21 a	3.25 a
400	0.88 a	27.75 a	2.88 a	1.00 a	0.21 a	3.13 a
F (Accession)	14.57**	11.57**	0.98^{ns}	0.77^{ns}	0.17^{ns}	1.58^{ns}
F (Dose)	0.46^{ns}	2.06^{ns}	2.25ns	2.56^{ns}	2.34^{ns}	1.49^{ns}
F (H x D)	1.51^{ns}	1.46^{ns}	2.47^{ns}	1.25^{ns}	0.37^{ns}	2.11^{ns}

**, *: Significant by F-test ($p < 0.01$) and ($p < 0.05$), respectively (Wobbrock *et al.*, 2011). ns: Not significant. Mean values followed by the same letter do not differ by Scott-Knott test ($p > 0.05$). NFF: Number of female flowers; NMF: Number of male flowers; NFA: Number of flowers with anomalies; NF4: Number of flowers with up to four petals; NF5: Number of flowers with more than five petals; NAT: Number of anthers

The only significant effect on the fruit characteristics evaluated in the present study was from the accession (Table 4). The C-14 accession had larger, wider fruit, with thicker pulp, but a smaller number of seeds per fruit. Dutta *et al.* (2021) worked with cucurbits and obtained phenotypic variations of the fruit; while Velkov, Tomlekova and Sarsu (2016) reported no variations for the fruit characteristics of the treated plants.

The first principal component (PC-1) explained 54.10% of the total variation, while the second component accounted for 21.50%, giving a total of 75.60% (Figures 2). The variables that most contributed to CP-1 were mean fruit weight (MFW), mean fruit length (MFL), pulp thickness (PTH), germination index (GI), number of female flowers (NFF), number of seeds per fruit (NSF) and width of the leaf blade (LBW). The variables that most contributed to CP-2 were number of flowers with anomalies (NFA) and number of flowers with more than five petals (NF5).

Table 4 - Mean values of five characteristics related to the fruit in two melon accessions subjected to four doses of gamma radiation

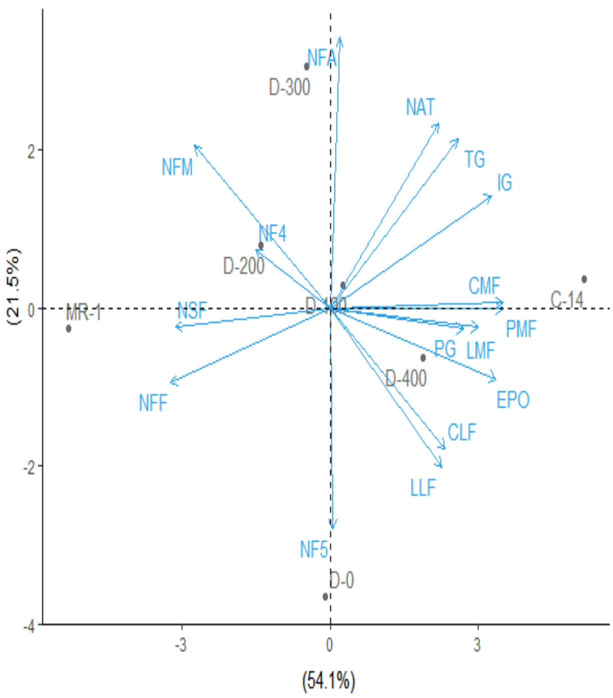
Factor	Mean Values (Characteristics)				
	MFW	MFL	MFW	PTH	NSF
Accession					
MR-1	10.25 b	2.41 b	2.81 b	0.35 b	65.06 a
C-14	18.92 a	3.63 a	3.29 a	0.53 a	17.76 b
Dose					
0	13.34 a	2.99 a	2.92 a	0.47 a	32.73 a
100	14.37 a	3.11 a	3.07 a	0.46 a	46.64 a
200	12.81 a	2.75 a	2.98 a	0.40 a	40.82 a
300	13.29 a	2.94 a	2.87 a	0.41 a	32.02 a
400	17.52 a	2.98 a	3.36 a	0.44 a	41.40 a
F (Accession)	14.30**	35.60**	6.21**	29.21**	25.22**
F (Dose)	0.59^{ns}	0.45^{ns}	0.75^{ns}	0.67^{ns}	0.24^{ns}
F (H x D)	1.27ns	0.43^{ns}	1.42^{ns}	0.56^{ns}	0.41^{ns}

**, *: Significant by the Snedecor F-test at ($p < 0.01$) and ($p < 0.05$), respectively. ^{ns}: Not significant. Mean values followed by the same letter do not differ by Scott-Knott test ($p > 0.05$). MFW: Mean fruit weight (g); MFL: Mean fruit length (cm); MFW: Mean fruit width (cm), PTH: Pulp thickness (cm); NSF: Number of seeds per fruit

The CP-1 variables are associated with the characteristics of fruit, germination and flowering, which mainly correspond to the differences between the accessions and the leaf blade variables associated with the doses of gamma radiation and the accession. The morphological diversity of the melon has been widely studied and represents great diversity, especially at the level of the fruit. The shape and size of the fruit, skin colour, pulp colour, the presence of a gelatinous sheath around the seeds, and seed size, can be used to define horticultural groups and subgroups (Pitrat, 2016), and was useful in this study to discriminate phenotypic diversity in MR1 and C14 (Figure 3) at the doses of 400 and 200 Gy (Figure 4).

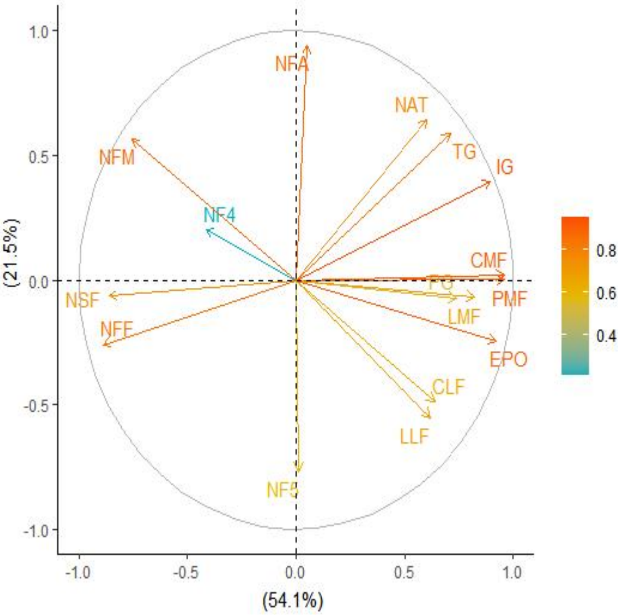
No variations in leaf morphology have been reported in cantaloupe treated with gamma rays. In cucurbits, the changes that occur following gamma radiation treatments are associated with chlorotic abnormalities in the upper leaves during the advanced stages of development (Velkov; Tomlekova; Sarsu, 2016). The diversity in characteristics associated with flowering in the melon can help to group melon types (Pandey *et al.*, 2020), and in this study made it possible to discriminate between accessions in the M1 generation, especially for the variable, number of flowers, with CP-2 anomalies at a dose of 300 Gy (Figure 4), and the number of female and male flowers that made it possible to discriminate between accessions. The variables related to germination, such as germination rate and germination index, made it possible to discriminate between the germination rates, especially between C14 and MR1, although the doses were not clearly indicated by these variables, unlike the study by Jaipo *et al*. (2019), where changes were seen from 50 Gy onwards.

Figure 3 - Principal components involving 16 variables evaluated in two melon accessions subjected to four doses of gamma radiation



GP: Germination Percentage (%); GR Germination rate; GI: Germination index; MFW: Mean fruit weight (g); MFL: Mean fruit length (cm); MFW: Mean fruit width (cm), PTH: Pulp thickness (cm); NSF: Number of seeds per fruit; NFF: Number of female flowers; NMF: Number of male flowers; NFA: Number of flowers with anomalies; NF4: Number of flowers with up to four petals; NF5: Number of flowers with more than five petals; NAT: Number of anthers; LBL: Leaf blade length (cm) and LBW: Leaf blade width

Figure 4 - Dispersion of melon accessions and doses of gamma radiation and their relationship to the variables



GP: Germination Percentage (%); GR: Germination rate; GI: Germination index; MFW: Mean fruit weight (g); MFL: Mean fruit length (cm); MWF: Mean fruit width (cm), PTH: Pulp thickness (cm); NSF: Number of seeds per fruit; NFF: Number of female flowers; NMF: Number of male flowers; NFA: Number of flowers with anomalies; NF4: Number of flowers with up to four petals; NF5: Number of flowers with more than five petals; NAT: Number of anthers; LBL: Leaf blade length (cm) and LBW: Leaf blade width

The MR-1 accession stood out with a larger number of female and male flowers, as well as a larger number of seeds per fruit (Figure 3). The C-14 accession stood out for the characteristics related to germination (GP, GR and GI), mean fruit weight (MFW), fruit length and width (MFL and MFW), pulp thickness (PTH), and width and length of the leaf blade (LBW and LBL) (Figure 3).

CONCLUSION

The doses of gamma radiation under test did not generate any physiological damage that had a negative effect on the processes of germination. The accessesions under evaluation showed no radiosensitivity up to a dose of 400 Gy. Germination potential was slightly affected at doses of 300 and 400 Gy. The leaf blade was also affected at doses of 100, 200 and 300 Gy, resulting in a reduction in the length and width of the leaves. The variables under evaluation made it possible to discriminate between the accessions but only some of the effects caused by the gamma rays.

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