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Article

Gamma radiation in roselle seeds to induce morphological variation and selection of mutants

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Abstract

The objective was to determine the LD_{50} and RC_{50} and induce morphological variation in the roselle variety UAN-8 by gamma rays to select mutant plants of agronomic interest in the M_2 generation. The radiation doses used were: 0, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1 000 Gy. The experiments were conducted under greenhouse and field conditions in Río Grande, Villa de Tututepec, Oaxaca, in 2018. The experimental design used was randomized blocks with four repetitions. In M_1, seedling emergence, survival, height and percentage of plants were evaluated. In M_2, morphological variation was recorded, and mutant plants were selected. Plant survival and height data were analyzed using nonlinear regression to determine the median lethal and reductive doses (LD_{50} and RC_{50}). The LD_{50} and RC_{50} were found at 395.48 and 453.2 Gy, respectively. The M_2 seeds of this variety produced plants with morphological variability in doses from 100 to 300 Gy. From these plants, it was possible to identify six promising mutant genotypes. The genotype identified as S7 L13 presented desirable morphological characteristics such as a higher number of red calyces per plant and absent or very weak pubescence, compared to the plants of the parental genotype.

Keywords:

gamma radiation, mutagenesis, radiosensitivity.

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Introduction

Roselle (*Hibiscus sabdariffa* L.) is an annual plant of the Malvaceae family, grown mainly for its calyces. The consumption of roselle calyces has increased due to its antioxidant (Frank *et al*., 2012), hypotensive activity (Herrera *et al*., 2004) and treatment of the lipid profile (Gurrola-Díaz *et al*., 2010), as well as preventive treatment for cancer (Muhammad and Shakib, 1995; Pacheco-Oviedo *et al*., 2019). In Mexico, the state of Oaxaca is the third producer of roselle by sowing area with 1 457 ha, with an average yield of 350 kg ha^{-1} (SIAP, 2019). The sowing of roselle
is carried out under rainfed conditions in 20 municipalities, mainly on the cost of Oaxaca. The traditional varieties for sowing are Criolla, Sudan, Tempranilla and Yersey acriollada.

The genotype UAN-8 was selected within 60 genotypes that were evaluated in the coast of Oaxaca in different locations, for its yield greater than 600 kg ha^{-1} of dry calyces, resistance to stem rot caused by *Phythopthora parasitica* Dastur and for its high content of bioactive compounds. This variety also presents excellent quality of dry calyces and because these are three times larger than the landrace variety, the harvest per day is higher (Ovando *et al*., 2018). Nevertheless, this genotype presents fruits and calyces with a medium degree of pubescence, which makes harvesting difficult, since this is done manually, and it causes discomfort after a while of handling the calyces.

The genotype UAN-8 was used to generate a material of little to no pubescence through crosses or mutagenesis induced with gamma radiation. This last method has the advantage of generating genetic variability and thus being able to select materials with good yield and quality characteristics, in less time in the process by using a single parent and generating variations in a few traits, compared to conventional methods of genetic improvement (Oladosu *et al*., 2016). In this regard, Harding and Mohamad (2009) report for roselle an RC_{50} of 754 Gy for the Terengganu variety and 773.8 Gy for Arab. In another roselle variety, Hanafiah *et al*. (2017) determined that the LD_{50} for seed germination was 477.8 Gy. Also, Díaz-López *et al*. (2016) determined that doses of 50 Gy affected 28% the germination of a roselle collection from the coast of Oaxaca.

To begin a program of genetic improvement by mutagenesis induced with gamma radiation, it is necessary to determine the optimal dose that generates genetic variation with the highest probability of success, associated with the median lethal and reductive doses (LD_{50} and RC_{50}). This optimal dose is different even between varieties of the same species (Olasupo *et al*., 2016), which is why the study of radiosensitivity for the genotype of interest is important. Due to the above, the objective was to determine the LD_{50} and RC_{50}, and induce morphological variation in the roselle variety UAN-8 by gamma rays to select mutant plants of agronomic interest in the M_2 generation.

Materials and methods

Irradiation of plant material

The irradiation of roselle seeds of the UAN-8 variety was carried out at the Moscafrut irradiation plant of SENASICA-SADER in Metapa de Domínguez, Chiapas, Mexico, with the Gamma Beam 127 MDS Nordion equipment with storage source of 50 g of ^{60}Co dry, with a dose ratio of 0.029 Gy s^{-1}. Seeds of roselle of the UAN-8 variety, with 11.2% moisture on average, were exposed to 10 doses of gamma radiation: 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1 000 Gy, in addition, non-irradiated seeds as a control treatment. Seventy-five seeds per dose were used. The doses used were in accordance with what was reported by Harding and Mohamad (2009).

Radiosensitivity of roselle to gamma rays of Cobalt 60

The evaluation of the sensitivity of UAN-8 roselle seeds to gamma radiation was carried out at the Costa Oaxaqueña Experimental Site of the Valles Centrales de Oaxaca Experimental Field-

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INIFAP, located in Río Grande, Villa de Tututepec, Oaxaca, whose geographical coordinates are 97° 25'19.37" west latitude, 19° 59' 38.1" north latitude and an altitude of 7 m. The germination of the irradiated seeds was carried out in unicel (expanded polystyrene) trays of 200 cavities with dimensions of 2.5 wide and long and 6 cm deep, with peat moss® substrate. At 15 days after sowing in trays, the emergence of seeds was evaluated (the seedling whose cotyledons were above the surface of the substrate was considered as emerged).

In addition, 20 roselle seedlings per treatment were randomly selected, which were transplanted in the field under the real frame sowing system with a distance of 1 m between plants and furrows. The experimental plot was 220 m^2, using a completely randomized block experimental design with four repetitions. Five plants distributed in a 5 m long furrow were taken as repetition, of which three plants were the experimental unit.

Radiation doses were considered as treatment. The mineral fertilization to the soil was divided into two parts, the first application was made at 30 days after sowing (das), using 10 g $plant^{-1}$ of the formula N17-P17-K17, while the second application was made at 60 das, 10 g $plant^{-1}$ of the formula N46-P00-K00. Irrigation was carried out by drip. The management of pests such as the ant (*Atta* spp.) was performed with Imidacloprid®. The control of weeds was carried out manually.

At the time of harvesting the roselle of the control treatment, plant survival, height and percentage of plants with seed were evaluated. Plant survival was calculated using the formula PS= (number of living plants/five plants at the beginning of the study)*100. Plant height was measured from the base of the stem to the terminal apex of the plant, three plants per repetition were taken from the center of the furrow. The percentage of plants with seeds was calculated with the formula PPS= (plants with at least one seed with normal morphology/number of plants of the treatment)*100. That seed that was not empty, that was well-formed and brown in color was considered a normal seed. Finally, the lowest and highest number of seeds per plant were recorded.

Morphological variation and selection of mutants in the M_2 generation

Twenty seeds of roselle were randomly selected from each M_1 plant that produced seed, from the treatments of 100 (20 plants), 200 (19 plants) and 300 Gy (6 plants), to generate the M_2 population where the parental variety was included as a control (10 plants). Only these doses were considered because from 400 Gy, the seeds obtained were empty and undeveloped. The sowing was carried out in the field at the Costa Oaxaqueña Experimental Site, on August 12, 2018. One seed per sowing point was deposited at 2 cm depth, with the real frame sowing arrangement with 1 m distance between plants and furrows. The agronomic management was similar to that used in the M_1 generation.

The experimental design used was completely randomized blocks with four repetitions (five plants per repetition). Based on visual observations in the field, morphological characteristics of stem, flower and calyx that were different from the parental were recorded. While at harvest, plants with a greater number of calyces (under full competition) and little pubescence in calyces, with respect to the control, were selected. The selected plants were characterized based on 11 morphological descriptors of plant, flower and calyx (SAGARPA-SNICS, 2014). The colors were recorded based on the Munsell plant tissue color book (Munsell Color, 2012).

Data analysis

The data on survival and height of plant of roselle variety UAN-8 were analyzed by Anova and comparison of means with the Dunnett test, 0.05. The LD_{50} and RC_{50} were determined using the parameters of the Logistic Power model.

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Results and discussion

Radiosensitivity of roselle seeds to gamma rays

The emergence of roselle seedlings of the UAN-8 genotype was not affected by gamma radiation ($p > 0.05$), which was found between 78 and 88%. While the survival of roselle plants was affected by radiation ($p < 0.01$), from doses of 400 to 700 Gy, it was less than 50% and from 800 Gy there was no survival (Table 1). The plants that did not survive only had the cotyledonary leaves. The reduced plant survival can be attributed mainly to DNA malformations, produced by gamma radiation (Raut *et al*., 2021). In this regard, in mitotic cells of *Catharanthus roseus* L., a greater number of aberrations are reported with increasing gamma radiation dose (Murugan *et al*., 2015).

Table 1. Emergence, survival and height of plant of roselle variety UAN-8 as a function of the dose of ^{60}Co gamma radiation.

Radiation dose (Gy)	Emergence (%)	Plant survival (%)	Plant height (cm)
0	82	100	111.7
100	88	100	108.3
200	82	95	103.7
300	79	80	82.1
400	87	35**	67.6
500	83	45**	36*
600	82	10**	46*
700	82	15**	31.1*
800	79	0**	-
900	78	0**	-
1 000	79	0**	-

* = statistically significant difference with respect to the control, according to Dunnett, $p < 0.05$; ** = $p < 0.01$.

The radiation produced a negative effect on the height of the UAN-8 roselle plants ($p < 0.05$) obtained with seeds with the dose of 500 Gy, these had lower height (<50%) compared to the control (Figure 1). In contrast, Hanafiah *et al*. (2017) did not obtain significant negative effects on the plant height of roselle variety Roselindo 2, in doses of 100 to 600 Gy. Lagoda (2012) indicates that radiation destroys many enzymes, which produces low cell division and plant growth. For their part, Momiyama *et al*. (1999) reported that the negative effect of radiation on corn plant height can be attributed to auxin destruction.

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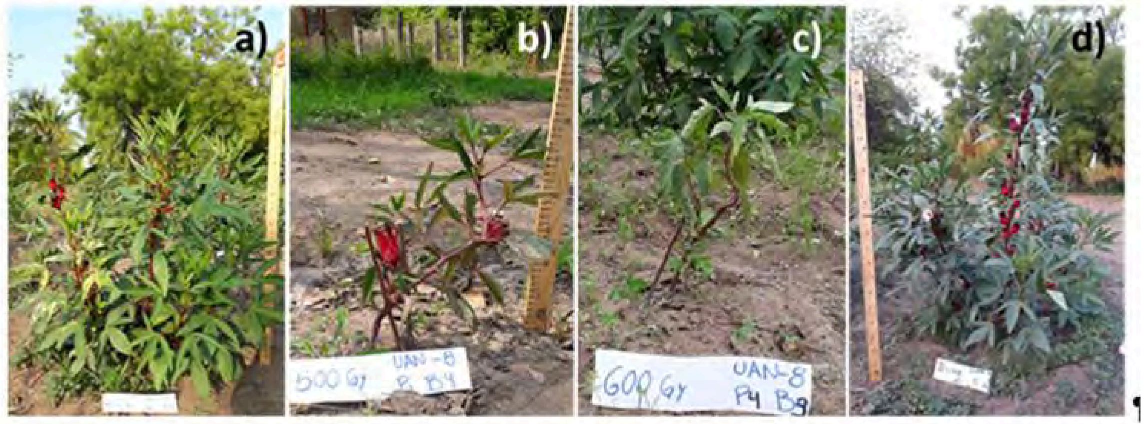
Figure 1. Appearance of UAN-8 roselle plants in the M_1 generation with doses of 200 (a), 500 (b) and 600 Gy (c) and control (d).



Gamma radiation also influenced the percentage of roselle plants that produced seeds. The roselle plants of the treatments 0, 100 and 200 Gy showed seeds with normal morphology (it was well formed, brown and not empty), observing a stimulation in the production of seeds per plant, as happened with the dose of 100 Gy, while in higher doses, more than 60% of the surviving plants produced abnormal seeds.

At doses of 600 and 700 Gy, the effect was more severe, only 10% in the surviving plants produced seeds with normal morphology (Table 2 and Figure 2). The decrease or null production of seeds due to the effect of gamma radiation has been attributed to the increase of sterile pollen (meiotic abnormalities such as inversions and translocations), lack of reproductive structures in the flower and abortion of the embryo before maturity (Kodym *et al*., 2012).

Table 2. Percentage of M_1 plants of UAN-8 roselle with seed, and minimum and maximum of seeds per plant as a function of the dose of ^{60}Co gamma radiation.

Dose (Gy)	No. of plants evaluated	Plants with seed (%)	No. of seeds per plant	
			Minimum	Maximum
0	20	100	140	750
100	20	100	50	1139
200	19	100	43	221
300	16	37.5	29	151
400	10	10	4	-
500	9	11.1	3	-
600	2	0	-	-
700	3	0	-	-

Total number of plants of four blocks.

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Figure 2. Appearance of capsules and seeds of UAN-8 roselle in the M_1 generation with doses of 500 Gy (a and b) and control (c).

Studies of radiosensitivity of the roselle varieties Terengganu and Arab showed the median lethal dose for plant height at 754 and 773.8Gy two weeks after sowing the seeds (Harding and Mohamad, 2009). Hanafiah *et al*. (2017) determined the LD_{50} for germination of seeds of the roselle variety Roselindo 2 at 477.8 Gy of ^{60}Co gamma radiation. The LD_{50} and RC_{50} for UAN-8 was found at 396.48 and 453.2 Gy, respectively.

The results obtained with this variety suggest that with lower doses, morphological variants of agro-industrial interest can be obtained, and that during the selection process the time may be shorter. However, in the M_1 generation of the treatments of 400 and 500 Gy, they produced 10% of roselle plants with few seeds and considering the type of reproduction of this species (sexual), doses close to 300 Gy are suggested to generate genetic variability with a greater probability of success (Table 3).

Table 3. LD_{50} and RC_{50} with ^{60}Co gamma radiation for survival and height of plant of roselle variety UAN-8, estimated by the Logistic Power model.

Variable	R^2	Equation	LD_{50}	RC_{50}
Survival	0.96	$Y= 100.84/(1+(x/393.81)^{3.93})$	395.48	-
Plant height	0.96	$Y= 112.51/(1+(x/450.42)^{2.51})$	-	453.2

Morphological variation and selection of mutant plants in the M_2 generation

In a genetic improvement program assisted by induced mutagenesis, the selection of potential mutant plants begins in the M_2 generation and continues in subsequent generations (Oladosu *et al*., 2016). In the M_2 population of the roselle genotype UAN-8 at 100, 200 and 300 Gy, morphological changes were observed in stem, flower and calyx (Figure 3). Five roselle plants with bright pink flowers (5RP 5/10) were observed (three plants in 100 Gy and two plants in 200 Gy) (Figure 3c and 3d), which contrast with the light pink color of the parental (2.5R 8/4) (Figure 3a).

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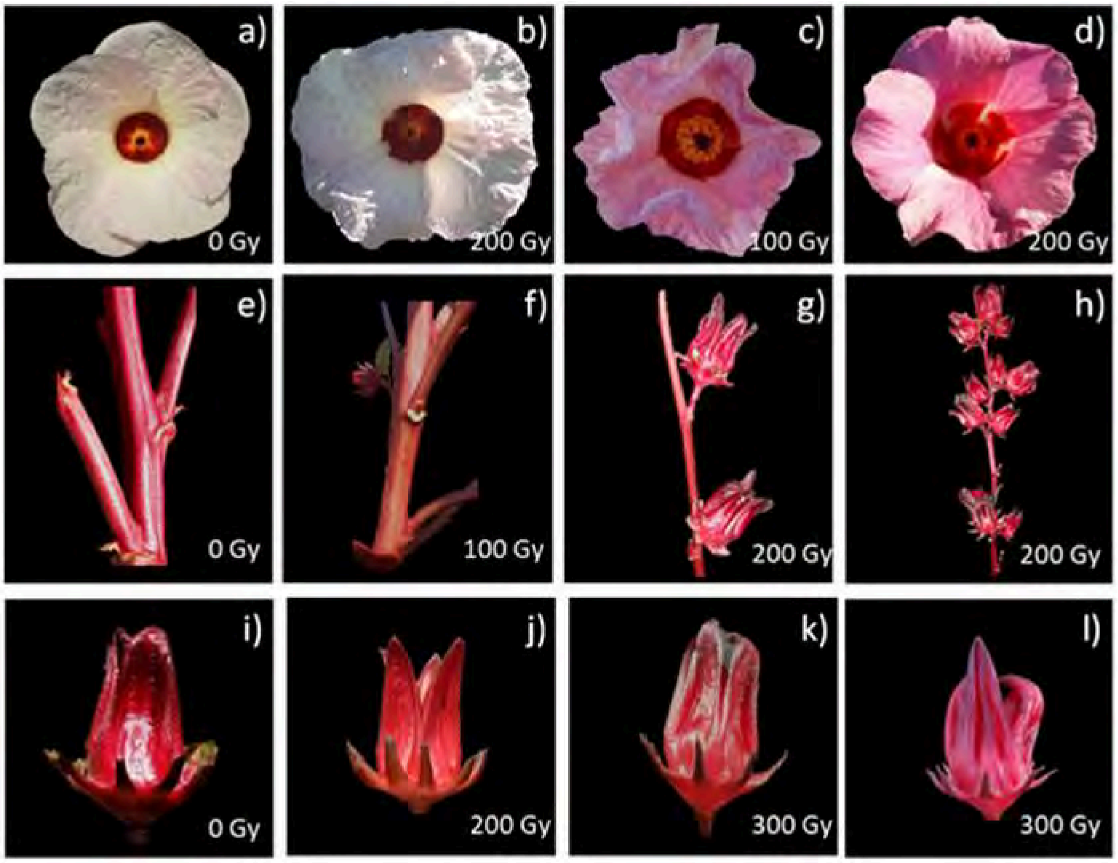
Figure 3. Appearance of the flower, stem and calyx of the roselle genotype UAN-8 (a, e, g, i) and the M_2 generation (b, c, d, f, h, j, k and l).



In addition, at 200 Gy, a plant with a six-petaled flower (3b) was observed, which differs from the parental with four petals (Figure 3a). Four plants with paler red stems were identified, three plants in 100 Gy and two plants in 200 Gy; (Figure 3f), while the parental with red color (Figure 3e). At 200 Gy, a plant with a different branch was identified, which had two to three calyces per production point (Figure 3h), while the parental only had one calyx per axil (Figure 3 g).

Regarding the calyces, four plants that presented pink calyces (Figure 3l) and three with salmon-colored calyces with small capsules and abnormal seeds were identified, both in 100, 200 and 300 Gy (3j), the parental presented red calyces (Figure 3i). Likewise, two plants with calyces with little or very weak pubescence were identified in 200 and 300 Gy (Figure 3k).

Based on the selection criterion of a greater number of calyces per plant compared to the control (under full competition), five individuals were identified in the treatments of 100, 200 and 300 Gy, whose morphological characteristics of plant and calyx are shown in Table 4. The plants identified as S7 L13 and S8 L14 of doses of 100 Gy were the ones that presented the highest number of calyces compared to the control (76.19 and 58.20%), red and pubescence similar to the parental. In the treatment of 200 Gy, plants S1 L4 and S2 L8 had 48.14 and 40.21% more calyces based on the control, red and medium and absent or weak pubescence.

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Table 4. Morphological traits of mutant roselle plants selected in the M_2 generation of the UAN-8 variety.

Trait	Control	100 Gy		200 Gy		300 Gy	
		S7 L13	S8 L14	S1 L4	S2 L8	S1 L2	S3 L1
GH	Erect to extended	Erect to extended	Erect to extended	Erect to extended	Erect to extended	Erect to extended	Erect to extended
SC	Red	Red	Red	Red	Red	Pink	Red
PH	177.2	189.5	172.8	200.1	143.9	186.7	131.5
HBFF	10.6	5.5	4.1	3.2	5	19.15	3.9
DF	90	90	91	92	91	87	98
BPP	34	57	55	55	45	41	14
CL	5.78	5.41	5.93	5.16	5.73	5.48	5.1
CD	3.27	3.11	3.17	3.13	3.18	2.99	3.17
CC	Red	Red	Red	Red	Red	Red	Pink
CPP	2	333	299	280	265	268	91
CP	Medium	Medium	Medium	Medium	Absent or very weak	Medium	Absent or very weak

GH= growth habit; SC= stem color; PH= plant height (cm); HBFF= height from branch to first fruit (cm); DF= days to flowering; BPP= branches per plant; CL= calyx length (cm); CD= calyx diameter (cm); CC= calyx color; CPP= calyces per plant; CP= calyx pubescence.

At 300 Gy, the plant S1 L2 was identified with 40% more calyces than the control, that were red and with pubescence similar to the parental. While in the plant labeled as S3 L1, fewer calyces per plant with respect to the control were observed, it was selected for presenting absent or weak pubescence. The characteristic of little pubescence has also been obtained due to the effect of gamma radiation in the M_1 generation of sesame variety Escoba at 300 Gy (Mussi *et al*., 2016).

Conclusions

In the M_1 generation, the LD_{50} and RC_{50} with gamma rays of Cobalto 60 for seeds of the roselle variety UAN-8 was found at 396.48 and 453.2 Gy, respectively. The M_2 seeds of this variety produced plants with morphological variability in doses from 100 to 300 Gy. From these plants, it was possible to identify six promising mutant genotypes. The genotype identified as S7 L13 showed desirable morphological characteristics such as a higher number of red calyces per plant and absent or very weak pubescence, compared to the plants of the parental genotype.

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