

Genetic improvement of Chickpea cultivars (*Cicer arietinum* L.) by gamma irradiation

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ABSTRACT

The study was aimed to determine the radiosensitivity of three Chickpea varieties (DN-12, DI-18 and DI-21). Ten different radiation doses were tested on seeds: 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 Gy, in a source of ^{60}Co gamma rays. Once irradiated, 100 seeds per dose and a non-irradiated control were pre-germinated to evaluate the effect seven days post-germination. Stem height and root length of seedlings after 15 days and their survival were assessed after 30 days. Germination percentage and mean lethal dose (LD_{50}) were determined for each cultivar, and medium growth (GR_{50}) and dose-effect curves established for the evaluated characters and cultivars. Optimal irradiation doses for the breeding process were chosen. Afterwards, 1000 irradiated seeds per treatment were selected and donor cultivars were seeded in the field and harvested without making selection and planted again. The respective healthy plants were evaluated for the total number of branches, pods and grains, number of pods with two grains and total mass of grains. The irradiation dosage tested had no effect on germination, while seedling and roots height significantly decreased with dose increase, and a drastic reduction was seen in survival for cultivars DI-18 and DI-21 with 400 Gy and for DN-12 with 500 Gy. Then, 200, 300 and 400 Gy doses were selected to irradiate seeds from the three cultivars and they were evaluated in field planting experiments. Field evaluation provided variability, and six lines were selected due to superior performance and the lack of pest damage.

Keywords: Chickpea, radiosensitivity, gamma radiations, grains, seed

RESEARCH

RESUMEN

Mejoramiento genético de cultivares de garbanzo (*Cicer arietinum* L.) mediante el tratamiento con radiación gamma. Se determinó la radiosensibilidad de tres cultivares de garbanzo (DN-12, DI-18 y DI-21), a radiación gamma aplicada a semillas con una fuente de ^{60}Co en diez dosis de 100 a 1000 Gy con intervalos de 100 Gy. Se pre-germinaron 100 semillas por dosis y un control no irradiado para evaluar la germinación por siete días. A los 15 días se evaluó la altura del tallo y la longitud de la raíz, y a los 30 días la supervivencia. Se determinaron el porcentaje de germinación, las curvas dosis-efecto para los caracteres y cultivares evaluados, la dosis letal media (LD_{50}) para cada cultivar y el crecimiento medio (GR_{50}) para cada carácter y cultivar, y se seleccionó las dosis de radiación para el proceso de reproducción. Después irradiar 1000 semillas por tratamiento, los cultivares donantes se sembraron en campo y se cosecharon sin realizar la selección, se plantaron nuevamente y se seleccionaron plantas sanas, en las que se evaluó varios caracteres agronómicos. La radiación no tuvo efecto en la germinación, y la altura de las plántulas y las raíces disminuyó significativamente con el aumento de la dosis, con reducción drástica en la supervivencia de los cultivares DI-18 y DI-21 irradiados con 400 Gy y DN-12 con 500 Gy. La selección y siembra en campo de las semillas de los tres cultivares irradiados con dosis de 200, 300 y 400 Gy aportó variabilidad, y se seleccionaron seis líneas superiores a los cultivares parentales y que no presentaron daños de plagas.

Palabras clave: radiosensibilidad, radiaciones gamma, garbanzo, granos, semilla

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Introduction

Legumes are a primary source of food and, when combined with cereals, provide a nutritionally balanced amino acid composition ideal for humans. Chickpea (*Cicer arietinum* L.) is one of the most important legumes for food in the world. It constitutes a relevant source of energy, proteins, minerals, vitamins, fiber, and it also contains phytochemicals potentially beneficial for health [1].

Chickpea is grown in 40 countries, reaching nearly 11 million hectares, with a production exceeding 9 million tons yearly, for an average yield of 0.8 ton/ha. Contradictorily, available cultivars are susceptible to pests and their productivity has decreased in recent years, probably due to the narrow genetic base of crops [2-5].

In order to palliate such limitations, hybridizations have been widely used worldwide for genetic improvement of Chickpea, with some success. An alternative method of proven efficacy comprises the induction of mutations by irradiation, called mutation breeding, for the introduction of genetic variability in economically relevant crops [6, 7], shortening the time required to obtain new cultivars in comparison to traditional methods.

Mutation breeding has been used in recent years as a valuable supplement to traditional plant breeding methods for crop improvement, particularly by gamma irradiation. Over the 70 past years, more than 2252 mutant varieties including cereals, oil seeds, pulse

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vegetables, fiber fruits and ornamentals have been officially released in more than 50 countries [8,9].

Taking advantage of these precedents, mutation breeding by gamma irradiation was used to introduce genetic variability in Chickpea, aimed to improve yields and other main cultivar morphologic characters. Hence, this work was aimed to determine the radio sensitivity of three cultivars' seed of Chickpea against gamma radiations with ^{60}Co , in order to select potentially superior genotypes in donor plants.

Materials and methods

Seeds from three Chickpea cultivars were assayed: DN-12 obtained at the Institute of Fundamental Research on Tropical Agriculture (INIFAT), and DI-18 and DI-29 as obtained from the International Institute for Research in Dry Areas (ICARDA) in the Syrian Arab Republic.

Mature seeds of three cultivars of Chickpea (DN-12, DI-18 and DI-21), were harvested in the field, at the Base Scientific Technological Unit (UCTB) located in Los Palacios, belonged to National Agricultural Sciences Institute (INCA), in April 2016. They were irradiated with a ^{60}Co MPX Gamma 25-M radiation source, with a dose power of 0.28 Gy/s and doses of 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 Gy.

Once irradiated, 100 seeds per dose and a non-irradiated control were pre-germinated in Petri dishes at 30 °C through a completely randomized design. After seven days, the number of germinated seeds was determined. Seedlings were further inspected on day 15 post-germination and stem height and root length measured (cm) by using a graduated ruler. Survival was assessed on day 30. Dose-effect curves for the evaluated characters and cultivars were determined with data obtained and germination percentage values.

Gamma irradiation mean lethal doses (LD_{50}) were determined for each cultivar, and mean growth rate (GR_{50}) for each cultivar and character. Regression curves were obtained from survival data, length of roots and stems with the irradiation dose, with the aid of the statistical package SPSS version 11.0 on Windows (Microsoft Corporation). Regression curves were calculated using the general formula ($y = a - bx$), where y was replaced by half the value of the control to obtain the dose (x). Then, these values were used to select irradiation doses able to introduce genetic variability.

Subsequently, 1000 irradiated seeds per treatment or donor cultivars were seeded in the fields located at areas of the UCTB Los Palacios, on Hydromorph Gley Nodular Petroferric soil [10]. Seeds were planted on November 2016, in plots of 5.6 m² (2.8 × 2.0) following a random blocks design, where 10 seeds were planted per linear meter with a planting distance of 0.70 m between rows and 0.10 m between plants.

The cultural attentions were made according to the Technical Instructions for the Cultivation of the Chickpea in Cuba [11]. All the material (M1) was harvested, without making selection and field planted once again on November 2017, following the same procedure as described above, but healthy plants were selected (pest-free). The total number of branches, pods

and grains, number of pods with two grains and the total mass of grains (g) were evaluated.

Data obtained were processed through a Principal Components and Clusters Analysis [12]. The statistical package SPSS version 11.0 on Windows was used and simultaneous selection was made on all the characters evaluated, with independent criteria.

Results and discussion

In the first four days evaluated, the germination process started on day 1 and showed no differences between cultivars for the non-irradiated seeds, for doses from 600 to 1000 Gy (Figure 1). For doses from 100 to 500 Gy, germination began on day 2 with more than 70 % of the seeds germinated.

After seven days, there were similar levels of germination among the irradiation doses tested for all the cultivars (Figure 2), with values higher than 75 %. Several authors have suggested that germination of seeds both *in vivo* [13] and *in vitro* [14] of most species is not altered by irradiation. However, a reduction in the percentage of germination with increasing gamma doses was previously reported in peas [15]. Gamma radiation increases enzymatic activation, and thereby the cell division rate and can stimulate the germination process [14].

On the other hand, the growth of stems and roots (Figure 3) were gradually affected with the increase in irradiation doses in the three evaluated Chickpea cultivars. Likewise, there was a significant difference between cultivars, since DN-12 showed the highest values for both characters and remarkable for the length of the roots.

No gradual reduction in response to the increase irradiation dose was found in survival of the three

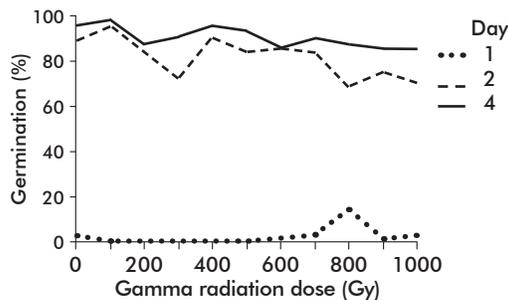


Figure 1. Percentage of germination of total seeds from three Chickpea cultivars irradiated with ^{60}Co and the non-irradiated control.

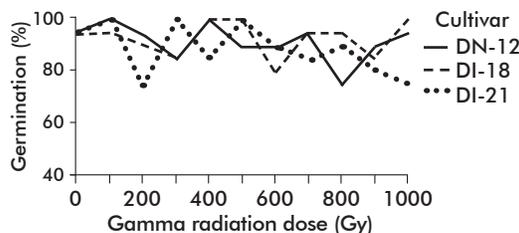


Figure 2. Seed germination percentage of three Chickpea cultivars (DN-12, DI-18 and DI-21) irradiated with ^{60}Co and the non-irradiated control (0 Gy) after seven days.

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Chickpea cultivars irradiated with ⁶⁰Co, 30 days after germination (Figure 4). Only few differences were detected for 100 to 300 Gy.

At the 400 Gy dose, there was a drastic reduction in survival for cultivars DI-18 and DI-21, this effect only seen in cultivar DN-12 at a 500 Gy dose. This corroborates the differential effect that irradiation levels have among different cultivars of the same species. Hence, this remarks the relevance of determining the suitable radiation dose for a particular cultivar on mutation breeding process.

Other authors found that survival in Chickpea plants decreased significantly from treatment with doses higher than 100 Gy, with plant mortality reaching almost 90 % at 650 Gy doses, and LD₅₀ set to 150 Gy. Moreover, they observed modifications of the leaves between 250 and 700 Gy [8].

Gamma radiation can cause biochemical and physiological changes, including the inhibition of growth and germination [5]. They have been also stated that stem height and root length are important characters for the determination of radiosensitivity curves [13].

The regression equations of the dose-effect curves, for the characters and cultivars evaluated (Table 1), contributed different values for the LD₅₀ and GR₅₀ among cultivars and for characters evaluated for the same cultivar. Apparently, DI-21 is the cultivar most sensitive to irradiation, with an LD₅₀ of 392 Gy and GR₅₀ of 292 Gy for stem height and 309 Gy for root length.

These results demonstrate the importance of determining the radiosensitivity of each cultivar that is going to be used in the improvement by mutations, every cultivar responds differently to the mutagenic agents. It is also evidenced that seed germination is not a suitable indicator of this sensitivity, as previously reported with other crops like wheat [9]. Considering the abovementioned results, the doses of 200, 300 and 400 Gy were selected to irradiate seeds of the three cultivars without generating deleterious effects.

The dose of the mutagen should be high enough as to increase the probability of inducing a mutation, but below the level harmful to cells and tissues as to cause lethality. Hence, biological populations are normally irradiated at levels below the LD₅₀ value. Since mutation induction is a random event and recovery from it is dependent upon survival of the plant, this strategy increases the probability to obtain a viable mutant. The radiosensitivity varies with the species, culture procedures, the physiologic conditions of the plant and its organs, the manipulation of the irradiated material before and after the mutagenic treatment, and should be determined through experimentation [5].

The field evaluation of the M2 generation provided variability enough as making selection possible. When analyzing the values and Eigen vectors, resulting from the Principal Components Analysis (Table 2), it was observed that the first two components extracted 79.4 % of the variability. In the first component, the most important variables were the total number of pods and grains, as well as the total mass of grains, all positive, while in the second component the total number of branches (positive) and the number of pods with two grains (negative) provided the greatest contribution.

Distribution of the individuals according to the Principal Components Analysis and the contribution

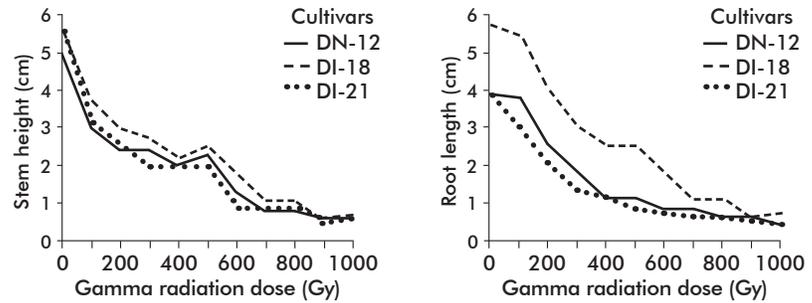


Figure 3. Effect of gamma irradiation on agronomic characters of Chickpea. A) Stem height (cm). B) Root length (cm) of three chickpea cultivars (DN-12, DI-18 and DI-21) irradiated with ⁶⁰Co and the not irradiated control (0 Gy) after 15 days post-germination.

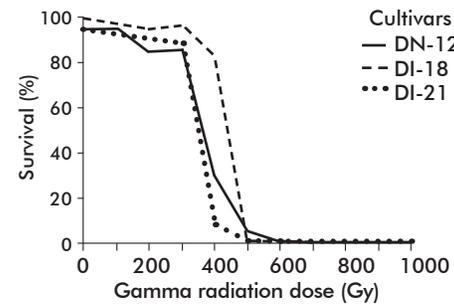


Figure 4. Percentage of survival of three cultivars of Chickpea (DN-12, DI-18 and DI-21) irradiated with ⁶⁰Co and the control not irradiated (0 Gy) at 30 days.

Table 1. Regression equations of the dose-effect curves, mean lethal dose (LD₅₀) and mean stem and root growth rate (GR₅₀) of three cultivars of Chickpea (DN-12, DI-18 and DI-21) irradiated with ⁶⁰Co

Cultivars	Regression equation	LD ₅₀ (Gy)	GR ₅₀		
			Stem (Gy)	Regression equation	Root (Gy)
DN-12	y = 109.2 - 0.132x	449	374	y = 5.218 - 0.005x	474
DI-18	y = 95.68 - 0.119x	405	344	y = 3.322 - 0.003x	457
DI-21	y = 93.22 - 0.118x	392	292	y = 2.877 - 0.003x	309

Table 2. Eigen values and percentage of contribution and accumulated of components 1 and 2 and their correlations with the original components

Parameter	Principal components	
	C1	C2
Eigen value	3.03	0.94
Total contribution (%)	60.57	18.86
Cumulative (%)	60.57	79.43
Total branches	0.40	0.76
Total pods	0.91	0.06
Pods with two grains	0.46	-0.60
Total grains	0.96	-0.06
Total mass of grains	0.95	-0.03

of the first and second components, in which the characters evaluated had a strong contribution, are shown in figure 5.

Individual dispersion was evidenced, taking into account these characters, corroborating the variability generated in the vegetal material by irradiation. Moreover, in the upper right quadrant, distant from the donor cultivars, are located the best genotypes with the highest values for total number of branches,

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Pods and grains, as well as the total mass of grains. In the lower right quadrant are those genotypes with the highest number of pods with two grains.

On the basis of the automatic classification (conglomerates), the lines were grouped into 10 classes (Table 3) and class four was highlighted with the three best lines possessing the highest values of the total branches, pods and grains and the mass total of the grains, which exceeded the average of the experiment and parental cultivars, those that were located in classes two (DI-21), five (DI-18) and eight (DN-12).

Second, class three is presented, with individuals had high values for these characters and also the highest number of pods with two grains.

The gamma radiation can produce mutations that are able to create new and improved variants as compared to their parental donor cultivars [8]. Successful results with this breeding technique are known in wheat, with M3 lines selected for its precocity, smaller height, favorable to avoid lodging and superior grain yield, the authors concluding that irradiations caused variability enough for choosing superior variants for crops breeding [9].

In Chickpea, nine selected lines exhibited the highest mean values for total number of pods and seeds per plant as well as yield per plant [1]. Moreover, the maximal variability was observed for the yield component (pods per plant, number of seeds per plant and yields) with a very high coefficient of variation [1]. By performing a path coefficient with the data obtained, there was found that the total number of seeds per plant had the highest positive direct effect, followed by 100 seed weight, characters that function as performance indicators of yield at early breeding stages.

Conclusions

In summary, six lines were selected that outperform the cultivars that originated them and did not present pest damage. This facilitates the subsequent evaluation and selection process in the following generations. Moreover, irradiation gamma had no effect on germination but seedlings and root height diminished significantly with dose increase and there was a drastic reduction in survival with 400 and 500 Gy doses.

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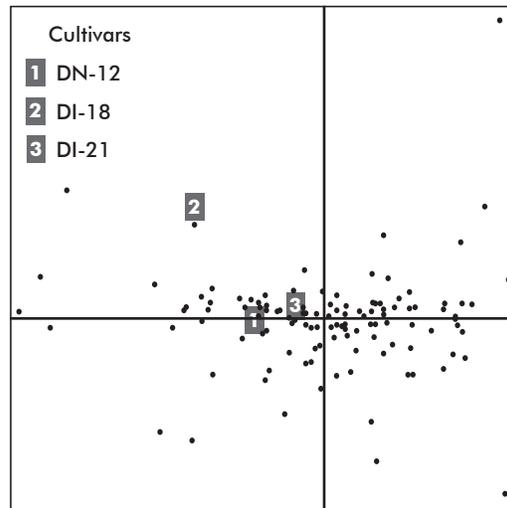


Figure 5. Graphical representation of the performance of three chickpea cultivars irradiated with ^{60}Co and the non-irradiated control, in the M2 generation, according to the components considered.

Table 3. Average values of the characters evaluated in each class established on the basis of existing diversity through Principal Component Analysis

Evaluated characters	Classes									
	1	2	3	4	5	6	7	8	9	10
Total branches	4	3	3	5	3	4	4	3	4	3
Total pods	83	65	101	159	48	67	102	27	131	8
Pods with two grains	3	3	3	4	2	1	3	1	0	0
Total grains	35	37	35	74	24	19	48	12	46	3
Total mass of grains	11.8	12.9	11.8	23.0	7.8	6.5	16.0	4.1	15.4	1.6
Number of lines	15	24	15	3	31	10	10	18	1	3
Location of tester	-	DI-21	-	-	DI-18	-	-	DN-12	-	-

Differences were found between cultivars, indicating that radiosensitivity varies and seedling height, roots height and survival were important characters to determine radiosensitivity curves in Chickpea.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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