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Original Article

Radiosensitivity of rice (*Oryza sativa* L. var CR5272) to gamma irradiation in Costa Rica

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ABSTRACT

Induced mutations have been used for the genetic plant breeding. A previous step to this is the material radiosensitivity study to be worked on, since it allows observing the effect on the plant material and defining the adequate dose. The aim of this work was to determine the radiosensitivity of rice seeds (*Oryza sativa* L. var CR5272), subjected to gamma irradiation with ^{60}Co. Seven doses were evaluated: from 100 to 700 Grays (Gy) with increments of 100 Gy, compared with a control without irradiation (T0). The variables were evaluated: at seven days, germination percentage and at 21 days, stem and root length. Variable values decreased as the irradiation dose increased. The highest germination and stem length occurred at 100 and 200 Gy; however, neither had a statistical difference with respect to the control; root length increased by 12.74 %, with 100 Gy irradiation, in relation to the non-irradiated treatment. The median lethal dose (LD_{50}) was established at 674 Gy for germination percentage, 380 Gy for stem length and 274 for root length. The correlation between radiation levels and stem and root length (R=0.92 and R=0.85) was significant, but not for germination (R=0.54). Radiosensitivity was achieved for rice seeds (*Oryza sativa* L. var CR5272) and a range of 300-400 Gy of irradiation was established as an effective dose, adequate to induce favorable mutations in this variety.

Key words: cobalt, LD, plant breeding, mutation

INTRODUCTION

Rice (*Oryza sativa*) is considered one of the most important food crops in the world, as it is the staple food for more than half of the world's population. The accelerated population growth, the reduced amount of available resources and land, as well as the climatic uncertainty that causes new breeds of diseases and pests, are a threat to food security; therefore, it is urgent to increase crop diversity with improvements in efficiency, to face future challenges, for which tools have been sought for the modification and genetic breeding of rice, among them the radiation application [1,2].

Ionizing radiation use is a technique that generates alterations in plant material, which allows inducing genetic variability, which is very efficient in obtaining mutants that allow the breeding of crops in specific traits with desired characteristics of agronomic importance [3,4]. Gamma rays are a physical mutagenic agent that have a low wavelength and high penetration power, causing genetic mutations in living organisms, which are randomly distributed in the genome [3].

Gamma radiation application is used to extend the genetic variability and create new varieties with superior genotypes in a short time, so that its application is efficient, it must be carried out tests that allow determining the optimal irradiation dose, which will depend on the physical characteristics of the vegetal material, when evaluating the radiosensitivity of tissues to different intensity, what is sought is to become familiar with radiation effects on it, as well as to evaluate the survival percentage of materials to doses [5].

There is a greater probability of producing effective mutations for plant breeding with doses where 50 % of the irradiated individuals die, this is known as mean lethal dose [6]. On the other hand, the genome suffers multiple impacts with high doses that commonly produce aberrations or negative changes [7].

The effectiveness in the production of mutations decreases as the dose of gamma rays increases [8], since these can cause alteration of physiological characters. The biological effect of gamma rays is due to the interaction of atoms or molecules in the cell, particularly in water, to produce free radicals [9].

These radicals can damage or change important components of plant cells and have been reported to differentially affect plant morphology, anatomy, biochemistry, and physiology, depending on the radiation dose [10].

More than 2200 crop varieties were released at the end of the last century using irradiation mutagenesis; 434 of them are rice varieties [11]. In Costa Rica, in the 1990s, this technique was used to obtain the CAMAGO-8 mutant, which was selected for its tolerance to *Pyricularia* and for its high yield; this variety was planted for several years on growers' farms in the Guanacaste region, until tolerance to the fungus was not maintained [12].

In Costa Rica for the period 2017/2018 the national consumption of milled rice was 239 707 t [13] with a per capita consumption of around 50 kg, it is estimated that world rice consumption by 2020 will be763 million tons, with an increase of 852 million tons by 2035 [14,15], These values reveal the importance of this cereal in the global diet and that of Costa Ricans, making it a fundamental element of food security, which is why it is important to have genetic improvement programs that help to optimize the rice cultivation quality and thus supply future demand. For this reason, the objective of this study was to determine the radiosensitivity of rice seeds of var. CR5272 and the LD_{50} for this cultivar.

MATERIALS AND METHODS

This research was carried out at the facilities of Agricultural Sciences School of the National University (UNA). Seeds of cultivar CR5272 (*Oryza sativa* cv CR5272) with 92 % germination and moisture between 11-13 % were irradiated with seven doses of gamma rays (100, 200, 300, 400, 500, 600 and 700 Gy) and a control without irradiation.

Seeds were irradiated using a cobalt source (^{60}Co) with an irradiation rate of 60 Gy/minute in a Gammacell of the Gamma Irradiation Laboratory of the Technological Institute of Costa Rica (ITCR according its acronyms in Spanish), Cartago, Costa Rica. A total of 160 seeds were used for each treatment.

After irradiation, seeds were sown using the wrist method, which consisted of placing the seeds on sheets of newspaper 36.5 cm long x 26 cm wide, with the longest part of the paper 1 cm from the edge and with a spacing of 0.9 cm per seed. Once seeds were placed, the paper was moistened with distilled water and the paper was rolled into a cylinder shape and placed in a 3 liter beaker with water to three quarters of the paper sheet, these were placed in a germination chamber for seven days, the chamber was maintained at 85 % humidity and 30 °C. After germination, all seeds were placed in the greenhouse until 21 days were completed.

A completely randomized experimental design (CRD) was implemented with four replicates per treatment, each replicate consisting of 40 seeds. Each experimental unit consisted of one plant. Seed radiosensitivity was evaluated by means of germination percentage, stem length (from the base to the apex of the longest leaf) and root length (from the base to the most distal part of the longest root) of seedlings. The evaluation was carried out at seven days for germination and at 21 days for stem and root length.

To determine treatment effect on the variables evaluated, an analysis of variance of mean comparison was performed for stem, root and germination length, by means of the LSD Fisher test ($p<0.05$), using the INFOStat statistical package. The variables were evaluated using a DMR in R to determine the LD_{50} with a non-linear three-parameter model.

RESULTS AND DISCUSSION

The analysis of variance indicated that irradiation between 100 Gy and 500 Gy did not show significant effects on rice plant germination. At that radiation range, germination was similar to the unirradiated control, with no significant difference ($p>0.05$) (Figure 1a). In a trial with 13 irradiated rice cultivars, they found that increasing the dose of gamma radiation had no significant effect on seed germination at any dose [16]. Radiation increases plant sensitivity to gamma rays, the stimulatory effect of gamma rays on germination may be attributed to RNA activation or protein synthesis and occur during the initial stage of germination after seed irradiation, increasing radiation dose may be responsible for lower germination failure; however, certain genotypes possess different sensitivity to gamma irradiation [16,17].

The 600 Gy and 700 Gy treatments did show significant differences ($p>0.05$) with respect to the control (radio inhibitory effect) (Figure 1a), at 700 Gy ($p<0.0001$) the germination percentage decreased up to 66 % (Figure 1d). This agrees with the results obtained when irradiating two rice varieties, the highest dose (500 Gy) caused the greatest inhibition in germination with 53.75 and 51.54 %, respectively, for rice varieties ADT-37 and ADT (R)-45 [18]. In another class of grasses, such as weeping grass, germination decreased from 98 to 36 % when increasing the irradiation dose from 300 to 400 Gy, and in African grass it went from 98 % germination in the control without irradiation to values of 1 % when using 900 Gy [19-21]. High doses of irradiation can cause lesions at the genic and chromosomal level that are lethal for the cells in division, causing physiological damages that inhibit the vital functions of cells, such as damage to the embryonic tissues, abnormal cell division and mutation, this can cause the death of the embryo in the seed, which causes a decrease in germination [22,23].

The dose at which germination had the closest value to LD_{50} was found at 674 Gy according to statistical analysis, in another study for rice variety IBD1 the LD_{50} was found at dose 564.5 Gy [1], while in a trial with two rice varieties (MRQ74 and MR269) LD_{50} was found between 351 and 365 Gy, LD_{50} was at 300.3 Gy and 300 Gy for rice cultivars ADT-37 and ADT (R)-45, respectively; while LD_{50} for the cultivar Anna (R)4 in India was 376.5 Gy [18,24,25].

Regarding stem height, the data show that the dose increment of 0-200 Gy in radiation, did not present significant differences with respect to the control ($p>0.05$), but as the irradiation doses increased, the height of the plants decreased; from 300 Gy the decrease in plant height was 28 %, while at a dose of 700 Gy the decrease in height was 87 %. Other results showed that two rice lines (G10 and G16), as well as the cultivar Baas Selem, decreased plant height as radiation was increased from 200 Gy to 500 Gy [2], while in a trial with the varieties ADT-37 and ADT (R)-45 the decrease in height corresponded to 63.15 and 65.37 %, respectively, at the dose of 500 Gy [18]. This indicates that high doses of gamma radiation in seeds have a negative effect on plant growth, this may be due to the fact that gamma radiation causes destruction of important compounds for plant development, such as enzymes, auxins and ascorbic acid, which causes damage in the process of cell division and elongation [20,22,26]. High doses of radiation can also affect the structure of DNA, causing anomalies in the nucleotide sequence which, in turn, induces defective transcriptions, inactivates protein products, resulting in the vegetative growth inhibition [22,23].

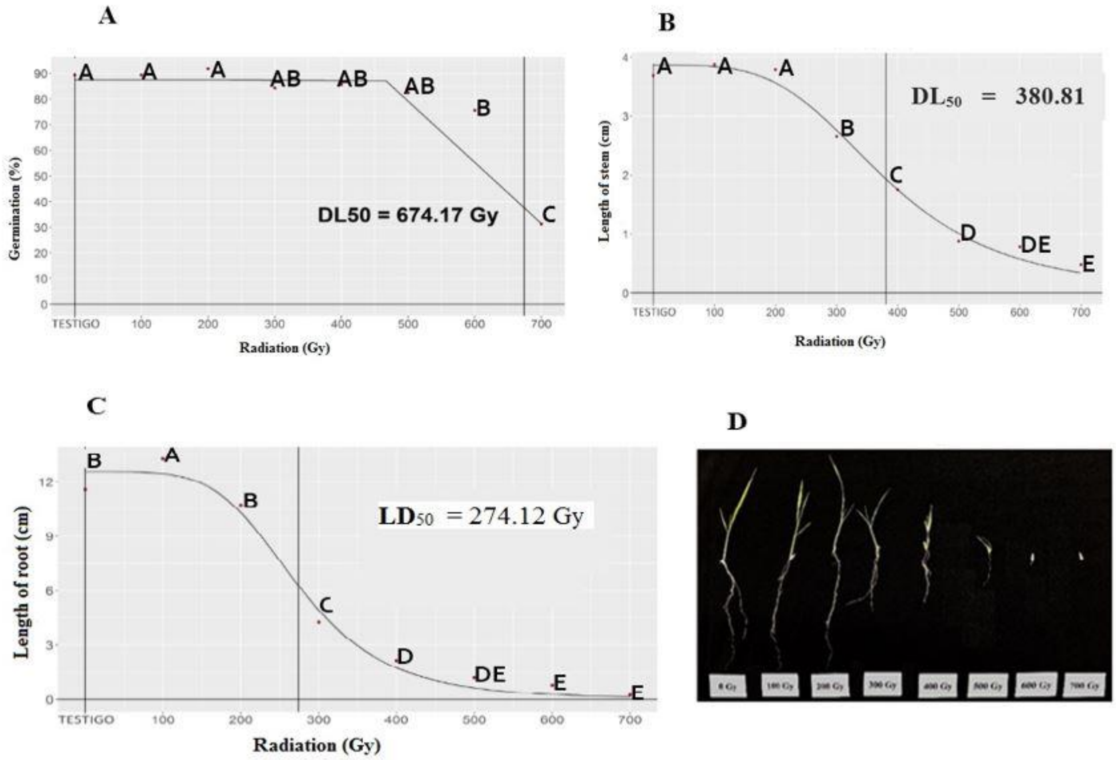
The median lethal dose (LD_{50}) for plant length evaluated by statistical analysis was found to be 380 Gy (Figure 1b). In contrast, for the rice variety MR284 LD_{50} was 400 Gy [27].

According to the analysis of variance, significant differences were found between the control and the 100 Gy treatment ($p<0.0001$), since the seeds irradiated with this dose presented the greatest root length

(Figure 1c). This same situation occurred in other crops such as tomato, where a positive response to irradiation was observed in root length of 37 %, in relation to the control at low doses, while in a study with sunflower plants for oil production, 200 Gy dose was the one that induced the greatest stimulation in the root length indicator; likewise, in forest trees, a significant improvement in root length was observed at low radiation doses [(28-30)].

It has been demonstrated that radiation at low doses is elemental in plants, since it can increase their stimulation and development, by using with greater efficiency the biochemical-metabolic pathways by means of free radicals, ions and excited molecules [(31)], this causes radioresistance in plants, which is the repair capacity of their system to overcome the harmful effect of stressors and their successful establishment in adverse conditions [(29)].

As with the previous two variables, in the present study with rice, a reduction in root length was observed as the gamma dose was increased from 300 Gy. The smallest root length (0.25 cm) occurred at 700 Gy and was 97.8 % smaller than the control size ($p<0.0001$). In another rice study with the Anna (R) variety, root length was reduced by 73.32 % with the 400 Gy dose, in the rice varieties ADT-37 and ADT (R)-45 root length also decreased, as radiation doses increased, the greatest root reduction was observed at the highest dose of 500 Gy, with 66.81 and 64.73 % reduction, respectively [(18,25)]. On the other hand, exposure to high radiation can cause chromosomal damage, which results in cells that cannot develop well and therefore do not favor plant development and growth, high radiation doses also reduce mitotic activity in meristematic tissues and reduction of moisture content in seeds, leading to a reduction in root length [(25,32)]. The mean lethal dose for root length was 274 Gy (Figure 1c), according to statistical analysis. In contrast, for two pumpkin varieties, the LD50 was found to be at a dose of 161-177 Gy, based on root length [(33)]. However, for other crops higher doses are required, as in millet the LD_{50} was given at 500 Gy [(6)].



Different capital letters mean statistical differences. LSD Fisher ($p<0.05$)

Figure 1. ^{60}Co irradiation of rice (*Oryza sativa* L.var CR5272) seeds and its effect on: germination percentage (a); stem length (b); root length (c) and sensitivity of CR5272 from 0 to 700 Gy (d)

The results of the present study indicate that, of the three parameters studied, stem length and root length can be used with the same reliability to estimate the appropriate doses of gamma irradiation for the treatment of rice seeds in a breeding program. The germination indicator is excluded, because its LD_{50} is at the 674 Gy dose which was one of the highest and where germination was decreased by 66 %, apart from the fact that this radiation dose significantly affected the other two parameters (Table 1). Therefore, it was decided to establish a range, comprising the weighted average of the parameters, stem length and root length, and it was determined that the LD_{50} for the cultivar CR5272 (*Oryza sativa* L. spp. Indica cv CR5272) is in the range of 300 to 400 Gy, which is very similar to that established in other rice varieties, found between 280 Gy and 350 Gy [34]. With the range established for cultivar CR5272, it will be possible to obtain the greatest amount of useful mutants, with minimal damage to plant survival.

Table 1. Summary of LD_{50}s obtained from dose-response analysis by nonlinear model for each parameter

	Parameter	LD_{50} (Gy)	S.E	T-Value	P-Value
4-	Germination	674,17	8,5136	79,1879	2,2e-16 ***
5-	Stem	380,81	7,157919	53,201	2,2e-16 ***
6-	Root	274,12	6,45163	42,489	2,2e-16 ***

Significant codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

CONCLUSIONS

This study made it possible to determine the radiosensitivity of rice seeds of var. CR5272, and the LD_{50} was established in the range of 300 to 400 Gy, as a dose that could generate changes in the genetic traits allowing the selection of mutants in initial phases.

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