



## **Effects of Gamma Rays on Cocona (*Solanum sessiliflorum* Dunal)**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author CATB designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors ASA and MRAB carried out the field experiment and assessed morphoagronomic characteristics. Author DFSF contributed in the discussion of findings. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Harvesting hairy fruits of current cocona (*Solanum sessiliflorum* Dunal) is a painful task because the hair is itching. Therefore, growers would be interested in hairless fruit type planting materials. Breeding for this character depends on the amount of genetic variety present within the species. In the case of limited genetic variability occurring naturally, one can be created using mutagenic agents. Gamma rays were used in the course of the present study on cocona seeds of genotype CUB-08 at 100, 150, 200, 300 and 400 Gy. Irradiated seeds were sown in styrofoam seedling trays, then transplanted in the open field, three months later, following a randomized block design with four replications and seven plants per plot, in the Agricultural experimental station of the National Institute for Amazonian Research (Instituto Nacional de Pesquisas da Amazônia).

The 100 and 150 Gy radiations led to germinating vigor and fruit pilosity variability whereas 200 Gy decreased the germination rate, and 300 and 400 Gy were totally deleterious. Therefore, gamma rays 100-150 Gy could be used to enhance genetic diversity for fruit pilosity and for fruit number as well.

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## 1. INTRODUCTION

Cocona (*Solanum sessiliflorum* Dunal) is a traditional Amazonian fruit. Its flavor is like an unknown citrus mixture. This fruit displays pilosity, which is a typical characteristic of this species, making it harder to be harvested on account of the itching it inflicts onto the pickers' exposed skin parts. This phenotype would have to keep on exhibiting its natural genetic variability so as to overcome this drawback, which will enable it to be improved through manual crossings or backcrossings. However, cocona's fruit pilosity variability must be induced since it is limited naturally. The use of gamma radiation, to come up with desirable mutants, has shown to be the successful way of increasing any crop's genetic diversity [1,2].

Radiation dose and number of genetic mutations have shown to be directly related, yet higher doses induce chromosomal mutations [3], preventing the seeds from germinating and, thus leading to the embryo death. Conversely, lower doses have shown to be unable to induce significant mutations [1]. Therefore, it is essential to be able to determine the accurate dose to be used. In the case of cocona, there seems to be no published information concerning the optimal dose to be used, therefore, one must seek for it on kin species.

In eggplant, a species phylogenetically related to cocona, 300 Gy of gamma radiation applied to its seeds increased the fruit size and mass [4]. In another similar work in eggplant, 160 Gy displayed to be appropriate dose for induce favorable mutations [1].

Gamma radiation used in plants, is generally produced by radioactive elements such as cobalt-60 and cesium-137. It produces ions inside the cells causing irreparable damage to cellular nucleus or genome mutations to gene mutations, which are the main inductors of new varieties. The gene mutations for plant breeding are more useful because they don't damage most of the genetic material.

Plant breeding programs have been used whole plants or different plant tissues for irradiation, such as, seeds, tubers, stems, buds, bulbs, pollen, *in vitro* plants, embryos, microspores and callus [5]. Yet, seeds have been preferred for their easy to transport, handle and store, i.e. can be irradiated, stored and germinated at any time.

The present study has irradiated cocona seeds with different doses ranging from 100 to 400 Gy for determining the optimal dose that creates variability.

## 2. MATERIALS AND METHODS

The experiment was carry out at National Institute for Amazonian Research (Instituto Nacional de Pesquisas da Amazônia) (Manaus, AM) in the Agronomical Experimental Station (Manaus, AM), which is located at Km 14 AM-10 roadway (2.9964 S and 60.0236 W).

Cocona seeds of genotype "CUB-08" were gamma-irradiated with different doses i.e. 100, 150, 200, 250, 300 and 400 Gy from a 5010 Gy/h cobalt-60 source at Center for Nuclear Energy in Agriculture (Centro de Energia Nuclear Aplicada à Agricultura, CENA/USP, Piracicaba, SP). The control was not irradiated.

The seeds were then sent to Manaus-AM and sown in 128-cell styrofoam trays filled with Plantmax® mineral-organic substrate. After three weeks, the seedlings were transplanted into plastic cups filled with the same Plantmax® substrate. Then, three months later; they were transplanted to the open field in a randomized complete block design, with four replications and seven plants per plot. Plants were grown for five months and their fruits harvested every week for two months.

The assessed fruit characteristics were yield, pilosity (1 = No pilosity and 3 = Excessive pilosity), weight, diameter, number per plant, and ripeness after seven days (1 = firm; 3 = rotten). Furthermore, stem pilosity was assessed, as well.

Data were submitted to analyses of variance (ANOVA) and means were compared by the Duncan Test ( $P < 0.05$ ). These analyses were performed using SAS 9.1.3, procedure PROC GLM (SAS Institute, Cary NC). Moreover, a biplot graphic was done based on correlations and principal components analysis using JMP 10 software (SAS institute, Cary, NC).

## 3. RESULTS AND DISCUSSION

Mutagenic agents which are grouped into chemical, physical and biological ones may be recommended for inducing genetic variability in

plants [5]. Physical radiations that include gamma radiations have been a very popular, plant breeding mutagenic agent, and gamma radiation has shown to be the most utilized. However, there are no former studies addressing radiation dosages in cocona. On account of both cocona and eggplant being phylogenetically related [6], this research was inspired by a previous work on eggplant [4], which demonstrated that 300 Gy of gamma radiation would induce non deleterious variability, thus the tested dosages for cocona were determined from 100 to 400 Gy.

The experiment was initially planned to be conducted with 20 plants per plot, yet only seven plants per plot were used due to their seeds' general germinating vigor loss, which was evident in the control treatment (0 Gy). This indicated the seeds have likely been submitted to temperature stress during their transport (Manaus-São Paulo-Manaus). In fact, studies have shown that cocona seeds are thermosensible [7], where variations of temperature from 20 to 30°C increase the germination percentage. This reduced plot size is close to the 10 plants per plot used for *Capsicum annum* [8]. Therefore, the 7 plants per plot used in this study was acceptable.

The ANOVA revealed radiation effect on the fruit pilosity and on the number of fruits per plant. It did not detect any radiation effect of the rest of the characters such as, stem, fruit's pilosity, number, yield, mass, length, diameter, L/D ratio and ripening time. Highlighting the high coefficient of variation for fruit yield, which indicates that variations in plot size affect the experimental precision.

The 150 Gy radiation significantly decreased fruit pilosity (Table 1). Furthermore, it induced no effect on the other traits, such as the number of plants per plot, the stem's pilosity, fruit yield, fruit mass, fruit length (L), fruit diameter (D), fruit length to diameter ratio (L/D) and fruit ripeness. This result is also shown on biplot graphic (Fig. 1), which explained 89% of total variation. The 150 Gy was found in the fruit pilosity vector's opposite direction, i.e. this dose decreased the character expression. Moreover, fruit yield and number vectors orthogonal projections point toward 150 Gy, suggesting this dose tended to increase these characteristics. Therefore, this dose shows to be more adequate for inducing the fruit pilosity's genetic variability and increasing fruit yield.

The 150 Gy radiation was very close to the 160 Gy that created genetic diversity for plant dry and fresh weights in a eggplant genotype [1]. But, researchers observed low effect of the same radiation dose in another eggplant genotype, displaying the presence of genotype x radiation interaction. Thus, our results would be valid mainly for CUB-08 cocona genotype. Other genotypes would need different doses.

Our findings have shown 100 and 150 Gy contributed to significantly increase the germinating vigor and decrease fruit pilosity as compared with 0 Gy (Table 1). This positive effect can be explained by gamma rays ability to modify both cell physiology and morphology. For instance, irradiation can increase polygalacturonase and pectin-methyl-esterase activity resulting in pectin degradation [9]. Another study on *in vitro* culantro (*Eryngium foetidum* L.) showed that 40 Gy-gamma rays increased flavonoids, flavonone, anthocyanin, vitamin C and folic acid content, and decrease flavonols and pyridoxine [10]. These facts suggest that combination of some metabolites may break down seed dormancy and consequently increase the germinating vigor. On the other hand, pilosity decrease can be explained by genetic factor like that in nectarine/peach case [11]; however, the present work suggested that cocona pilosity was not controlled by a single gene.

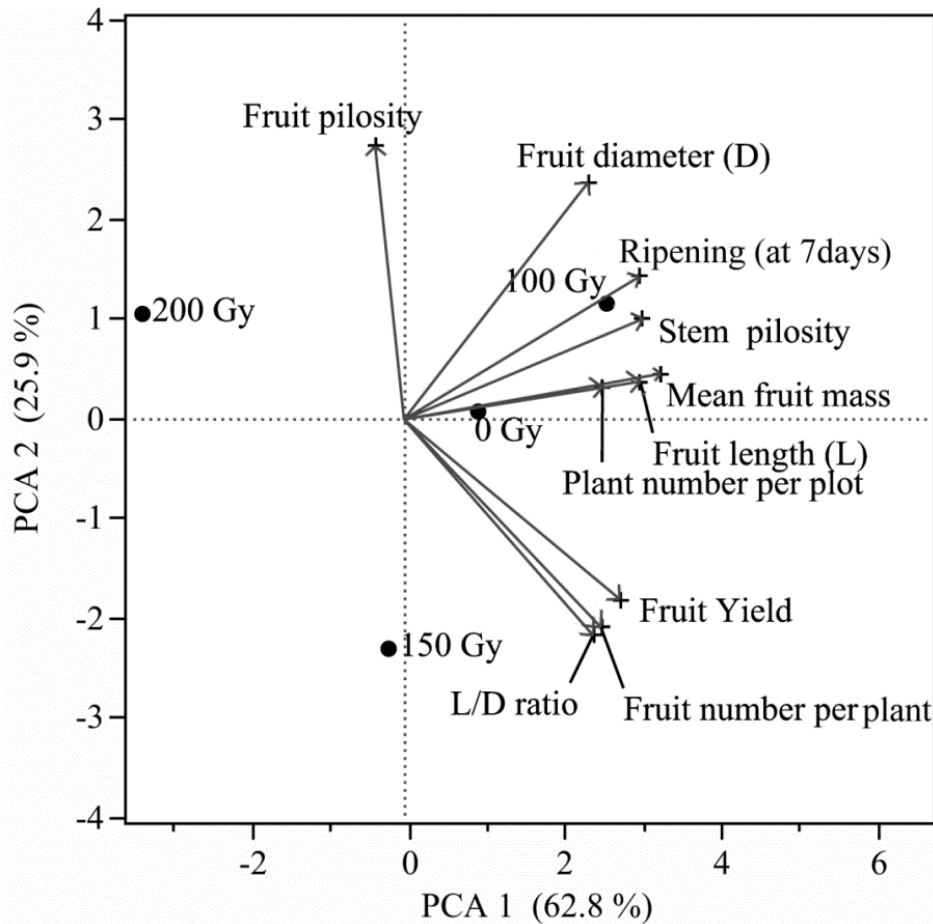
In contrast, 200 Gy decreased germination rate. They suggest 100 to 200 Gy doses not to be deleterious for these characters. However, 200 Gy tends to reduce fruits number per plant and yield (Table 1).

Biplot graphic (Fig. 1) explained about 89% of total variation. In consequence, this graphic represents most part of data and their interactions. It showed three equidistant radiation doses (100, 150 and 200 Gy), suggesting their different and contrasting effects. Yet, non-irradiation (0 Gy) is near the vector origin, indicating natural environment's lower effect on creating variability of morphoagronomic characteristics. Hence, gamma rays have shown to be able to inducing agronomical characters' variation in cocona. On the other hand and in absolute terms, 150 Gy was related with high fruit yield and fruit number per plant. Whereas 100 Gy was related with high values of fruit mass and diameter in absolute terms also. These findings indicate 100 to 150 Gy could be the optimal doses to create variability for fruit yield and its components.

**Table 1. Effect of different gamma radiations on the morphoagronomical traits of cocona (*Solanum sessiliflorum* Dunal)**

<b>Radiation dosages</b>	<b>Plant number per plot</b>	<b>Stem pilosity</b>	<b>Fruit pilosity (cm)</b>	<b>Fruit Yield (t ha<sup>-1</sup>)</b>	<b>Mean fruit mass (g)</b>	<b>Fruit length [L] (cm)</b>	<b>Fruit diameter [D] (cm)</b>	<b>L/D ratio</b>	<b>Ripeness (at 7 days)</b>
0 Gy	3.00 b	2.08	2.28 a	7.19	152.66	8.60	6.40	1.34	1.46
100 Gy	5.75 a	2.20	2.18 ab	6.65	167.44	8.48	6.62	1.31	1.62
150 Gy	4.00 ab	2.05	2.00 b	7.42	146.90	7.98	6.08	1.35	1.18
200 Gy	2.75 b	2.00	2.25 a	2.07	132.85	7.62	6.22	1.22	1.13
Mean	3.87	2.08	2.16	5.83	149.96	8.17	6.33	1.29	1.34
F treatments)	5.45	0.84	4.07	2.32	1.88	1.48	1.86	1.41	1.93
P-value (F test)	0.02	0.51	0.05	0.14	0.20	0.28	0.21	0.30	0.20
CV (%)	30.10	9.31	5.80	59.95	13.93	9.19	5.40	6.93	25.01

CV: Coefficient of variation. Mean values followed by the same letter were not significantly different



**Fig. 1. Biplot graphic of gamma radiation doses over cocona (*Solanum sessiliflorum* Dunal) seeds and some morphoagronomic characters vectors**

This biplot has also shown 200 Gy being in opposite direction of the characteristics' vectors indicating this radiation dose effect was deleterious for cocona for both plant development and fruit yield.

Dose recommendation of gamma ray to induce non-deleterious mutations depends on species and vegetative material. For example, in tomato seeds it was 50 to 150 Gy [12] and in potato meristems 2.5 Gy [13]. Therefore, each species and material needs a specific recommendation.

Doses from 250 to 400 Gy were highly deleterious leading to non-germination of their irradiated seeds.

In conclusion, the optimal dosages for inducing mutations in cocona have shown to range from 100 to 150 Gy, although 150 Gy has shown to be more effective, in addition to not modifying any of

the fruit's agronomical traits such as, yield, mass, length and diameter. Gamma radiation doses above 200 Gy showed to be highly deleterious on account of hampering the seed germination.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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