

Short communication

Effects of gamma rays on cocona (*Solanum sessiliflorum* Dunal)

ABSTRACT

Harvesting hairy fruits of current cocona (*Solanum sessiliflorum*) 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. **Aims:** The present work aims to assess the gamma rays effects on cocona (*Solanum sessiliflorum*).

Study design: Cocona seeds of genotype CUB-08 genotype were exposed to gamma rays (at 0, 100, 150, 200, 300 and 400 Gy. Irradiated seeds were sown in styrofoam seedling trays, then seedlings were transplanted in the open field, three months later, following a randomized block design with four blocks and seven plants per plot.

Place and Duration of Study: The present study was developed in the Agricultural experimental station of the Instituto Nacional de Pesquisas da Amazônia at the Agricultural experimental station, which is located on Km 14 AM-10 roadway, from April 2016 to December 2016.

Methodology: Fruits were harvested from November to December 2016. Fruit characteristics such as yield, pilosity (1=No pilosity and 3=Excessive pilosity), weight, diameter, number per plant, and ripening after seven days (1=firm; 3=rotten) were assessed. Furthermore, stem pilosity was assessed, as well.

Results: Results showed 200 Gy dose to decrease the germinating vigor, whereas 300 and 400 Gy were highly deleterious and prevented seeds from germinating. In contrast, The 100 and 150 Gy radiations doses did not affect germinating vigor, but just created led to germinating vigor and fruit pilosity variability whereas 200 Gy decreased the germination rate, and 300 and 400 Gy were totally deleterious. Therefore,

Conclusion: The gamma ray 100-150 could dose may be used to enhance this species' genetic diversity for fruit pilosity and for fruit number as well.

Keywords: CUBiu; peach tomato; irradiation; mutation; plant breeding

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

Formatted: Font: Not Bold

Formatted: Body, Left, Space After: 0 pt, Don't keep with next

Comment [O1]: To write the full name in English and the original name in parentheses

23 variability so as to overcome this drawback, which will enable it to be improved through
24 manual crossings or backcrossings. However, cocona's fruit pilosity variability must be
25 induced since it is ~~hard to be found~~ limited naturally. The use of gamma radiation, to come up
26 with desirable mutants, has shown to be the successful way of increasing any crop's genetic
27 diversity [1,2,3,4] [1, 2].

29 Radiation dose and number of genetic mutations have shown to be directly related, yet
30 higher doses induce ~~chromosomal~~ mutations [5] [3], preventing the seeds from germinating
31 and, thus leading to the embryo to die ~~death~~. Conversely, lower doses have shown to be
32 unable to induce significant mutations [2] [1]. Therefore, it is essential to be able to ~~calculate~~
33 determine the accurate dose to be used. In the case of cocona, there seems to be no
34 published information concerning the optimal dose to be used, therefore, one must seek for
35 it on kin species.

37 Eggplant, a species phylogenetically related to cocona, ~~has enabled to determine that 300~~
38 Gy of gamma radiation applied to its seeds ~~increases~~ increased the fruit size and mass, ~~when~~
39 ~~it is applied to its seeds~~ [6] [4]. In another similar work in eggplant, 160 Gy displayed to be
40 appropriate dose for induce favorable mutations [2] [1].

42 Gamma radiation ~~that is used in plants~~, is generally produced by radioactive elements such
43 as cobalt-60 and cesium-137 [7]. ~~Its short waves, at picometer level, have high penetration~~
44 ~~power, which produces~~ ions inside the cells causing irreparable damage to cellular nucleus
45 ~~or~~ It is considered the genome mutations to gene mutations ~~mutations~~ main inductors ~~for~~
46 ~~creating of new varieties, from 1971 to 2008~~ [7], ~~its genetic effects on cellular nucleus go from~~
47 ~~genome mutations to gene mutations~~ [8]. The gene mutations for plant breeding are more
48 useful because they don't damage most of the genetic material.

49 ~~In~~ Plant breeding programs have been used ~~whole plants or~~ different plant tissues for
50 irradiation, such as ~~whole plants~~, seeds, tubers, stems, buds, bulbs, pollen, in vitro plants,
51 embryos, microspores and callus [8] [5]. Yet, seeds have been preferred for their easy to
52 transport, handle and store, i.e. can be irradiated, stored and germinated at any time.

55 The present study has irradiated cocona seeds with different doses ranging from ~~0-100~~
56 400 Gy for determining the optimal dose ~~to bring about their~~ that creates variability.

58 2. MATERIAL AND METHODS

60 The experiment was carry out at Instituto Nacional de Pesquisas da Amazônia (Manaus, AM)
61 in the Agronomical Experimental Station (Manaus, AM), which is located ~~on~~ at Km 14AM-10
62 roadway (2.9964S and 60.0236 W).

63 Cocona seeds of genotype "CUB-08" ~~genotype~~ were gamma-irradiated with different doses
64 i.e. 100, 150, 200, 250, 300 and 400 Gy ~~from a 5010 Gy/h cobalt-60 source~~ at the Centro de
65 Energia Nuclear Aplicada à Agricultura (CENA/USP, Piracicaba, SP). The control was not
66 irradiated ~~with different doses i.e. 0, 100, 150, 200, 250, 300 and 400 Gy with a cobalt-60~~
67 ~~source, which itself emitted 5010 Gy/h.~~

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

Comment [O2]: To write the full name in English and the original name in parentheses

Comment [O3]: To write the full name in English and the original name in parentheses

74 The assessed fruit characteristics were yield, pilosity (1=No pilosity and 3=Excessive
 75 pilosity), weight, diameter, number per plant, and ripeness after seven days (1=firm;
 76 3=rotten). Furthermore, stem pilosity was assessed, as well.

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

Comment [04]: To indicate the name of the developer and the city of development of the JMP 10 software

84 3. RESULTS AND DISCUSSION

85
 86 Mutagenic agents which are grouped into chemical, physical and biological ones may be
 87 recommended for inducing genetic variability in plants (Bado et al. 2015)[5]. Physical
 88 radiations that include gamma radiations have been a very popular, plant breeding
 89 mutagenic agent, and gamma radiation has shown to be the most utilized one [7]. However,
 90 there are no former studies addressing radiation dosages in cocona. On account of both
 91 cocona and eggplant being phylogenetically related [9], this research was oriented
 92 through inspired by a previous work on eggplant [6], which demonstrated that 300 Gy of
 93 gamma radiation would induce non deleterious variability, thus the tested dosages for
 94 cocona were determined from 100 to 400 Gy.

95 The experiment was initially planned to be conducted with 20 plants per plot, yet only seven
 96 plants per plot were used due to their seeds' general germinating vigor loss, which was
 97 evident in the control treatment (0 Gy). This indicates the seeds to have likely been
 98 submitted to temperature stress during their transport (Manaus-São Paulo-Manaus). In fact,
 99 studies have shown that cocona seeds are thermosensible [40, 447], where variations of
 100 temperature from 20 to 30 °C increase the germination percentage. This reduced plot size is
 101 close to that the 10 plants per plot being used for other Solanaceae family species:
 102 *Capsicum annuum* (40 plants) [42, 138]. Therefore, the 7 plants per plot plant number used in
 103 this study is was acceptable.

104 Dose of The ANOVA reveal radiation effect on the fruit pilosity and on the number of fruits
 105 per plant. It did not detect any radiation effect of the rest of the characters such as, stem,
 106 fruit's pilosity number, yield, mass, length, diameter, C/D ratio and ripening time.

Comment [05]: It is difficult to believe that there was no radiation effect on fruit that varied from 2.07 t.ha⁻¹ at 200 Gy to 7.42t.ha⁻¹ at 150 Gy

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

Comment [06]: Do you mean L/D ratio?

116 **Table 1. Effect of different gamma radiations on the morphoagronomical traits means of cocona**
 117 **(*Solanum sessiliflorum* Dunal) - Manaus 2016.**

Radiation dosages	Plant number per plot	Stem pilosity	Fruit pilosity ^a (cm ²)	Fruit Yield (t ha ⁻¹)	Mean fruit mass (g or kg ²)	Fruit length (L)(cm ²)	Fruit diameter (D)(cm)	L/D ratio	Ripeness ^b (at 7 days)
0 Gy	3.00 b ^a	2.08 a	2.28 a	7.19 a	152.66 a	8.60 a	6.40 a	1.34 a	1.46 a
100 Gy	5.75 a	2.20 a	2.18 ab	6.65 a	167.44 a	8.48 a	6.62 a	1.31 a	1.62 a

Formatted: Not Superscript/ Subscript

	150 Gy	4.00 ab	2.05 a	2.00 b	7.42 a	146.90 a	7.98 a	6.08 a	1.35 a	1.18 a
118 ¹ =No pilosity-e	200 Gy	2.75 b	2.00 a	2.25 a	2.07 a	132.85 a	7.62 a	6.22 a	1.22 a	1.13 a
119 ³ =Excessive	Mean	3.87	2.08	2.16	5.83	149.96	8.17	6.33	1.29	1.34
120 pilosity- ² 1=firm;	CV ³ (%)	30.10	9.31	5.80	59.95	13.93	9.19	5.40	6.93	25.01

121 ³=rotten;³

122 Coefficient of variation

123 ⁴Equal letters indicate there not to have been significant radiation differences, by the Duncan test (P=0.05) C.V.

124 Coefficient of variation, mean values followed by the same letter were not significantly different

125

126

127 The 150 Gy radiation was very close to the 160 Gy that created genetic diversity se findings
 128 shown to be similar to found for plant dry and fresh weights in a eggplant genotype (160 Gy)
 129 [21]. But these researchers observed low effect of the same radiation dose in another
 130 eggplant genotype, displaying the presence of genotype x radiation interaction. Thus, our
 131 results would be valid mainly for CUB-08 cocona genotype. Other genotypes can would need
 132 different doses.

133 Given these four treatments, Our findings have shown 100 and 150 Gy to contributed
 134 significantly increase the germinating vigor and decrease fruit pilosity as compared with 0 Gy
 135 (Table 1). This positive effect can be explained by gamma rays ability to modify both cell
 136 physiology and morphology. For instance, irradiation can increase polygalacturonase and
 137 pectin-methyl-esterase activity resulting in pectin degradation [149]. Another study on *in*
 138 *vitro* culantro (*Eryngium foetidum* L.) showed *in vitro* culantro (*Eryngium foetidum* L.) that
 139 40 Gy-gamma rays increased flavonoids, flavonone, anthocyanin, vitamin C and folic acid
 140 content, and decrease flavonols and pyridoxine [1510]. These facts suggest that combination
 141 of some metabolites may break down seed dormancy and consequently increase the
 142 germinating vigor. On the other hand, pilosity decreasing decrease can be explained by
 143 genetic factor such as in nectarine/peach case [1611]; however, the present work suggests
 144 suggested that cocona pilosity to be was not controlled by a single gene.

145 In contrast, 200 Gy decreased germination rate and vigor. The rest of characters such as, stem,
 146 fruit's pilosity, number, yield, mass, length, diameter, C/D ratio and ripening time have shown
 147 no significant radiation dose induced differences. They suggest 100 to 200 Gy doses not to
 148 be deleterious for these characters. However, 200 Gy tends to reduce fruits number per
 149 plant and yield (Table 1).

150 Biplot graphic (Figure 1) explained about 89% of total variation. In consequence, this graphic
 151 represents most part of data and their interactions. It showed three equidistant radiation
 152 doses (100, 150 and 200 Gy), suggesting their different and contrasting effects. Yet, non-
 153 irradiation (0 Gy) is near the vector origin, indicating natural environment's lower effect for
 154 on creating variability of modifying the morphoagronomic characteristics. Hence, gamma rays
 155 have shown to be able to inducing modify agronomical characters' variation in cocona. On
 156 the other hand and in absolute terms, 150 Gy was related with high fruit yield and fruit
 157 number per plant. Whereas 100 Gy was related with high values of fruit mass and diameter
 158 in absolute terms also. These findings indicate 100 to 150 Gy being could be the optimal
 159 range of doses that to creates variability for fruit yield and its components in cocona
 160 genotype CUB-08. The graphic showed

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

164 Dose recommendation of gamma ray to induce non-deleterious mutations depends on
 165 species and vegetative material. For example, in tomato seeds it was recommended 50 to

Formatted: Font: Not Italic, Not Superscript/ Subscript

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Normal, Justified

Comment [07]:

Comment [08]: Complete the staement "...such as ... in nectarine/paech case". Such as what?

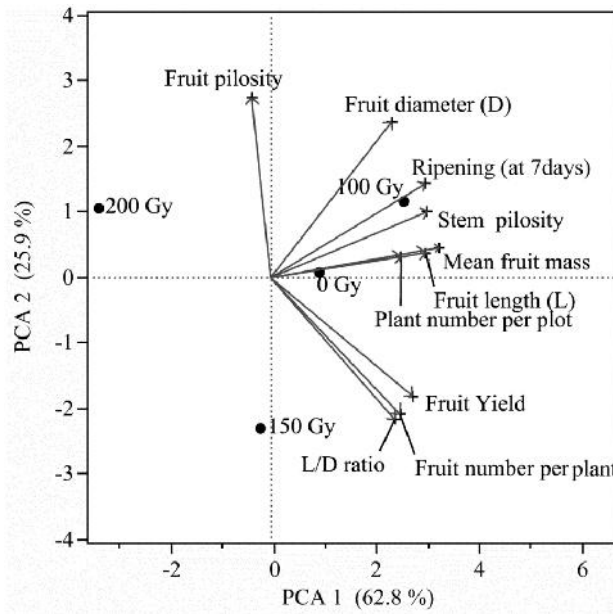
Comment [09]: You don't estimate germination vigor. You are just guessing. What you have estimated was germination rate

Comment [010]: Do you mean L/D ratio?

Comment [011]: 100 Gy radiation and 150 Gy radiation are not statistically different.

166 | 150 Gy [17-12], in grasspea seeds 100 Gy [18]. Yet, in banana plants and shoots was
 167 | recommended 20 to 30 kGy [19], in fig plantlets 30 kGy [20] and in potato meristems 2.5 Gy
 168 | [24-13]. Therefore, each species and material needs a specific recommendation.

169



170

171 | **Fig. 1. Biplot graphic of gamma radiation doses over cocona (*Solanum sessiliflorum*)**
 172 | **seeds and some morphoagronomic characters (vectors)**
 173

174 | Doses from 250 to 400 Gy were highly deleterious leading to as shown by their
 175 | seeds' germinating vigor loss non-germination of their irradiated seeds and, experimental
 176 | treatments number reduction to four (0, 100, 150 and 200 Gy). These findings suggest
 177 | genetic variability in cocona to be able to be induced by using doses of less than 250 Gy.

178

179 | **4. CONCLUSION**

180

181 | In conclusion, Gamma radiation doses applied on cocona seeds above 200 Gy showed to be
 182 | highly deleterious on account of hampering the seed germination. The optimal dosages for
 183 | inducing mutations on in cocona have shown to range from 100 to 150 Gy, yet, if the
 184 | objective is to induce high variability, although 150 Gy has shown to be more effective, in
 185 | addition to not modifying any of the fruit's agronomical traits such as, yield, mass, length and
 186 | diameter. Gamma radiation doses above 200 Gy showed to be highly deleterious on account
 187 | of hampering the seed germination.

188

189

190

191

192 **COMPETING INTERESTS**

193

194 Authors have declared there not to be any competing interests.

195

196

197

197 **REFERENCES**

198

199

200 1. Taheri S, Abdullah TL, Ahmad Z, Sahebi M, Azizi P. Phenotypic and molecular effects of
201 chronic gamma irradiation on *Curcuma alismatifolia*. European Journal of Horticultural
202 Sciences. 2016; 81(3): 137-47.

Available: <https://doi.org/10.17660/eJHS.2016/81.3.1>

Field Code Changed

203

204

205

2-1 Ulukapi K, Ozdemir B, Onus AN. Determination of proper gamma radiation dose in
mutation breeding in eggplant (*Solanum melongena*). Advances in Environmental and
Agricultural Science. 2015;1(1): 149-53.

206

207

208

3. Dhillon RS, Saharan RP, Jattan M, Rani T, von Wuehlisch G. Molecular characterization
of gamma-rays induced mutants in *Jatropha curcas* L. Indian Journal of Biotechnology.
2014;13(1): 67-74.

209

210

211

4-2 Shin JM, Kim BK, Seo SG, Jeon SB, Kim JS, Jun BK, et al. Mutation breeding of sweet
potato by gamma-ray radiation. African Journal of Agricultural Research.
2011;6(6):1447-54.

Formatted: Numbered + Level: 1 +
Numbering Style: 1, 2, 3, ... + Start at: 1 +
Alignment: Left + Aligned at: 0" + Indent at:
0.25"

212

213

214

215

216

5-3 Zhang J, Jiang Y, Guo YL, Li GR, Yang ZJ, Xu DL, Xuan P. Identification of Novel
Chromosomal Aberrations Induced by Co-60-gamma-Irradiation in Wheat
Dasyphyrum villosum Lines. International Journal of Molecular Sciences. 2015;16(12):
29787-96.

Available: <https://doi.org/10.3390/ijms161226134>

217

218

4. Ramaswamy N, Sayed S. Studies on the effect of gamma rays on eggplant. Progressive
Horticulture. 1977;9:77-79.

Formatted: Indent: Hanging: 0.25"

219

220

5. Mba C, Shub QY. Gamma Irradiation. In: Shu QY, Forster BP, Nakagawa H, editors.
Plant mutation breeding and Biotechnology. Rome: FAO; 2011.

Formatted: Indent: Left: 0.2", No bullets or
numbering

221

222

223

8-5 Bado S, Foster BP, Nielen S, Ali AM, Lagoda PJL, Till BJ, Laimer M. Plant mutation
breeding: current progress and future assessment. p. In: Janick J, editor. Plant
breeding reviews, Volume 39. Hoboken: John Wiley & Sons; 2015.

Formatted: Numbered + Level: 1 +
Numbering Style: 1, 2, 3, ... + Start at: 5 +
Alignment: Left + Aligned at: 0.25" + Indent
at: 0.5"

224

225

226

227

9-6 Bohs L. A chloroplast DNA phylogeny of *Solanum* section *Lasiocarpa*. Systematic
Botany. 2004;29(1):177-87.

Available: <https://doi.org/10.1600/036364404772974310>

Formatted: Indent: Left: 0", Hanging: 0.2",
Numbered + Level: 1 + Numbering Style: 1, 2,
3, ... + Start at: 5 + Alignment: Left + Aligned
at: 0.25" + Indent at: 0.5"

228

229

230

231

10. Lopes JC, Pereira MD. Germinação de sementes de cubiu em diferentes substratos e
temperaturas. Revista Brasileira de Sementes. 2005. 27(2):146-50. Portuguese.

Available: <http://dx.doi.org/10.1590/S0101-31222005000200021>

Formatted: Indent: Left: 0", Hanging: 0.2",
Numbered + Level: 1 + Numbering Style: 1, 2,
3, ... + Start at: 5 + Alignment: Left + Aligned
at: 0.25" + Indent at: 0.5"

232

233

234

11-7 Santos LA, Bueno CR, Clement CR. Influencia da temperatura na germinação de
sementes de cubiu (*Solanum sessiliflorum* Dunal) no escuro. 2000; 30(4):671-75.
Portuguese.

Field Code Changed

Formatted: Indent: Left: 0", Hanging: 0.2",
Numbered + Level: 1 + Numbering Style: 1, 2,
3, ... + Start at: 5 + Alignment: Left + Aligned
at: 0.25" + Indent at: 0.5"

- 235 | 12. Lorentz LH, Lúcio AD. Tamanho e forma de parcela para pimentão em estufa plástica.
 236 | Ciência Rural. 2009; 39(8): 2380-87. Portuguese.
 237 | Available: <http://dx.doi.org/10.1590/S0103-84782009005000202>
- 238 | 13.8. Padrón RAR, Lopes SJ, Renedo VSG. Estimation of the optimal plot size and
 239 | number of replications in a field pepper crop experiment with varying irrigation depths and
 240 | application. Scientia Horticulturae. 2018; 237:96-104.
 241 | Available: <https://doi.org/10.1016/j.scienta.2018.03.052>
- 242 | 14.9. Kovács E, Keresztes A. Effect of gamma and UV-B/C radiation on plant cells.
 243 | Micron. 2002; 33(2):199-210.
 244 | Available: [https://doi.org/10.1016/S0968-4328\(01\)00012-9](https://doi.org/10.1016/S0968-4328(01)00012-9)
- 245 | 15.10. Aly AAE. Biosynthesis of phenolic compounds and water soluble vitamins in culantro
 246 | (*Eryngium foetidum* L.) plantlets as affected by low doses of gamma irradiation.
 247 | Analele Universitatii din Oradea, Fascicula Biologie. 2010; 17(2):356-61.
- 248 | 16.11. Vendramin E; Pea, G; Dondini, L; Pacheco, I; Dettori, MT; Gazza, et al. 2014. A
 249 | unique mutation in a MYB gene cosegregates with the nectarine phenotype in peach.
 250 | PloS one. 2014;9: e90574-e90574.
 251 | Available: <https://doi.org/10.1371/journal.pone.0090574>
- 252 |
 253 | 17.12. Sikder S, Biswas P, Hazra P, Akhtar S, Chattopadhyay A, Badigannavar A M,
 254 | D'souza SF. Induction of mutation in tomato (*Solanum lycopersicum* L.) by gamma
 255 | irradiation and EMS. Indian Journal of Genetics and Plant Breeding. 2013; 73(4): 392-99.
 256 | Available: <https://doi.org/10.5958/j.0975-6906.73.4.059>
- 257 | 18. Ramezani P, Siavoshi, M, More Ad, Ebrahimi M, Dastan S. Gamma rays and EMS
 258 | induced flower color mutation in grasspea (*Lathyrus sativus* Linn.). Journal of Agricultural
 259 | Sciences. 2017; 23(4): 423-27.
- 260 | 19. Pestana RKN; Amorim EP, De Oliveira e Silva S, Neto AT. Gamma radiation for in vitro
 261 | mutagenesis in banana, cultivar Terra Maranhão. Pesquisa Agropecuária Brasileira.
 262 | 2010; 45(11): 1328-30.
 263 | Available: <http://dx.doi.org/10.1590/S0100-204X2010001100015>
- 264 | 20. Ferreira EA, Pasquali M, Tulmann Neto, A. 2009. In vitro sensivity of fig plantlets to
 265 | gamma rays. Scientia Agricola. 2009; 66(4): 540-42.
 266 | Available: <http://dx.doi.org/10.1590/S0103-90162009000400017>
- 267 | 21.13. Issa, F. H.; Najjalhasnawi, A.; Shehab Sabah, S. Influence of gamma radiation on
 268 | in vitro growth microtubersation and hormonal content of some potato (*Solanum tuberosum*
 269 | L.) cultivars. Plant Archives. 2018; 18(2): 2317-23.
 270 | Available: <http://dx.doi.org/10.13140/RG.2.2.23925.01761>

Field Code Changed

Formatted: Indent: Left: 0", Hanging: 0.2",
 Numbered + Level: 1 + Numbering Style: 1, 2,
 3, ... + Start at: 5 + Alignment: Left + Aligned
 at: 0.25" + Indent at: 0.5"

Field Code Changed

Formatted: Indent: Left: 0", Hanging: 0.2",
 Numbered + Level: 1 + Numbering Style: 1, 2,
 3, ... + Start at: 5 + Alignment: Left + Aligned
 at: 0.25" + Indent at: 0.5"

Field Code Changed

Formatted: Indent: Left: 0", Hanging: 0.2",
 Numbered + Level: 1 + Numbering Style: 1, 2,
 3, ... + Start at: 5 + Alignment: Left + Aligned
 at: 0.25" + Indent at: 0.5"

Formatted: Indent: Left: 0", Hanging: 0.2",
 Numbered + Level: 1 + Numbering Style: 1, 2,
 3, ... + Start at: 5 + Alignment: Left + Aligned
 at: 0.25" + Indent at: 0.5"

Field Code Changed

Formatted: Indent: Left: 0", Hanging: 0.2",
 Numbered + Level: 1 + Numbering Style: 1, 2,
 3, ... + Start at: 5 + Alignment: Left + Aligned
 at: 0.25" + Indent at: 0.5"

Formatted: Indent: Left: 0", Hanging: 0.2",
 Numbered + Level: 1 + Numbering Style: 1, 2,
 3, ... + Start at: 5 + Alignment: Left + Aligned
 at: 0.25" + Indent at: 0.5"

Formatted: Indent: Left: 0", Hanging: 0.2",
 Numbered + Level: 1 + Numbering Style: 1, 2,
 3, ... + Start at: 5 + Alignment: Left + Aligned
 at: 0.25" + Indent at: 0.5"