Mutation induction as a strategy to overcome the restricted genetic base in Physalis

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Abstract – The restricted genetic base in physalis in Southern Brazil is a challenge that requires genetic breeding for the achievement of fruits with superior agronomic quality. Therefore, genetic changes were induced in physalis for the selection of populations with superior fruit quality. To that end, seven populations of physalis were submitted to gamma irradiation (0, 100 and 200 Grays – Gy), which provided 21 populations. Significant population difference was observed, which indicates the existence of variability between at least two populations of physalis. The contrasts showed difference for Colômbia01, Colômbia02, Caçador and CAV. The mutation induction was effective at causing genetic variations in these populations. For Colombia01 (100 Gy), it was observed reduction of 3.97 mm and 2.56 mm (200 Gy) in the transverse fruit diameter (DTF). In the Colombia02 population (200 Gy), there was an increase of 2.99 mm in the longitudinal fruit diameter (DLT) and 4.90 mm in the DTF. For CAV (200 Gy), it was found the increase of 1.81 ºBrix. Mutation induction was beneficial in these cases, but fruit quality is still below the potential of the crop, when compared to fruits from Andean countries. It is possible to suggest that quantitative traits, such as fruit mass (MF) and total soluble solids (SST), and the degree of ploidy in physalis (2n = 4x = 48) reduce the mutagenic agent ability to cause variations.

Index terms: small fruit, gamma radiation, Cobalt⁶⁰, selection.

Indução de mutação como estratégia para a superação da restrita base genética em Physalis

Resumo – A restrita base genética em fisális é um desafio a ser superado pelo melhoramento da cultura, na região Sul do Brasil, de modo a obter frutos com qualidade agronômica superior. Diante disso, objetivou-se induzir alterações genéticas em fisális para a seleção de populações com qualidade de fruto superior. Para tanto, sete populações de fisális foram submetidas à irradiação gama (0; 100 e 200 Grays – Gy), resultando em 21 populações. Houve diferença significativa para população, revelando a existência de variabilidade entre, pelo menos, duas populações. Por meio dos contrastes detectou-se diferença para: Colômbia01, Colômbia02, Caçador e CAV. A indução mutação foi eficiente em causar variações genéticas nestas populações. Para Colômbia01 (100 Gy), observou-se redução de 3,97 mm e 2,56 mm (200 Gy) no diâmetro polar do fruto (DPF). Já na população Colômbia02 (200 Gy), acréscimo de 2,99 mm no diâmetro equatorial do fruto (DEF) e de 4,90 mm no DPF. Para CAV (200 Gy), o acréscimo foi de 1,81 ºBrix. A indução de mutação foi benéfica nestes casos, porém a qualidade de frutos ainda é aquém do potencial da cultura, quando comparada a frutos oriundos de importação de países Andinos. Pode-se considerar que caracteres quantitativos, dentre os quais massa do fruto (MF) e sólidos solúveis totais (SST), e o grau de ploidia em fisális (2n = 4x = 48) reduzem a eficiência do agente mutagênico em causar variações.

Termos para indexação: pequeno fruto, radiação gama, Cobalto⁶⁰, seleção.
**Introduction**

Physalis (*Physalis peruviana* L., Solanaceae) is a species marketed in the group of small fruits that has been cultivated in Brazilian regions in recent years. The country is not self-sufficient in the production of such fruits. The development of productive genotypes and fruits of superior quality are challenges to be solved by genetic breeding programs of the culture, in order to help in the expansion and the self-sufficiency of the production. Studies developed in Southern Brazil demonstrate restricted variation between and within populations in traits related to the phenological cycle and fruit size (TREVISANI et al., 2016). The restricted genetic variability is worrisome both for the evolution process of the species and for the work of breeders, especially considering that the cultivation of the species is increasing. This is a great obstacle to the processes of selection of superior genetic constitutions, including the obtaining of tastier and larger fruits.

Variations between individuals in the same species have genetic and environmental causes (ALLARD, 1971; BRIGGS; KNOWLES, 1967). Since they are inheritable, genetic variations arise the interest of breeders and can be created by means of mutations or amplified by means of hybridization (recombination of traits). Mutation has the power to create new alleles that do not exist in the gene pool of a species. It can be induced artificially with the purpose of accelerating the process for obtaining variants with desirable traits, since natural mutations are rare, casual and difficult to identify (ALLARD, 1971; BADO et al., 2015). It is considered a strategy relatively fast and inexpensive, compared to artificial hybridization, due to the difficulty of the recombination process and the lack of desirable traits in the parents.

The increasing number of registered varieties derived from mutation induction demonstrates that this strategy is very promising for the breeding of several species. By the year 2014, there were about 2800 varieties of more than 200 species of plants, including around 60 fruitful species (MALUSZYNSKI; 2001, IAEA, 2014). Successful plant breeding with the use of induced mutation (chemical or physical) can be verified in a wide range of species, including banana and apple, in which the variations obtained are essentially related to resistance to pathogens and fruit skin color (BISHOP, 1959; MALNOY et al., 2008).

In addition to the search for traits governed by few (qualitative) genes, in species marketed in the small fruit group, economically important traits are related to fruit size and flavor. Variation in fruit mass, diameters and sweet taste is important in the selection of genotypes for the fresh fruit market (FERREIRA et al., 2010). Varieties were created to increase fruit size by using gamma radiation, which is the case of the apple cultivar Golden Haidegg (in 1986), medlar cultivar Shiro-mogi (in 1981), peach cultivar Magnif 135 (in 1968) and the sweet cherry cultivars Lapins and Suburst (PREDIERI, 2001). Sweet cherry mutant plants are under analysis for higher sugar concentration and longer production cycle (SALVI et al., 2015).

Therefore, based on the hypothesis that gamma radiation is effective at causing variations in traits governed by a high number of genes, such as fruit mass, and providing the selection of populations with agronomically superior traits, this work aimed to induce genetic changes in physalis by using the mutagen agent Cobalt60 in order to select populations with superior fruit quality.

**Material and Methods**

- **Location of the experiment and genetic constitutions assessed**

The experiment was carried out in the experimental area of the Instituto de Melhoramento e Genética Molecular (Institute of Breeding and Molecular Genetics) (IMEGEM) of UDESC, in the municipality of Lages-SC, under greenhouse conditions. The experimental climatic conditions were maintained constant, with mean temperature of 23ºC and relative humidity of 75%, measured with the use of a portable digital thermo hygrometer.

Among the set of physalis populations that constitute germplasm of the IMEGEM, seven populations were selected to represent the genetic variability of physalis currently cultivated, originating from different locations (Colombia01, Colombia02, Caçador, Lages, Fraiburgo, CAV and Peru). The selection of the populations from different geographic regions, under specific edaphoclimatic conditions, is associated to a likely genetic divergence between them, caused by natural and artificial selection. To obtain the mutant populations, seven populations of physalis were subjected to gamma irradiation (physical method) with the mutagenic agent Cobalt60 at the doses of 0, 100 and 200 Grays (Gy), which resulted in 21 populations or treatments. The determination of the irradiation doses for the culture is based on the work carried out by Caro-Melgarejo et al. (2012).

The gamma irradiation gave rise to the populations in the M1 generation. Then, it was used to advance the generation and obtain the populations in M2 generation, in which the agronomic traits were assessed. It is important to point out the predominance of the physiological effects found in the M1 generation caused by the mutagenic agent on the seed. The genetic effects are verified from the M2 generation.
- Experimental design and statistical analysis

The experiment was conducted in a greenhouse in a completely randomized design, consisting of five replicates (vessel with one plant) per treatment (population of physalis), which totaled 105 observations. The following traits were measured: fruit mass (MF) in grams, capsule mass (MC) in grams, seed mass (MS) in grams, total soluble solids (SST) in °Brix, transverse fruit diameter (DTF) in millimeters and longitudinal fruit diameter (DLF) in millimeters. The evaluations were carried out during the productive cycle of the crop, in January and February. The average of three harvests was used for data analysis.

The mathematical model considered only the population factor as the cause of controlled variation, so that: \[ Y_{ik} = \mu + \text{pop}_i + e_{ik}, \] where \( Y_{ik} \) refers to the vectors of the averages of the evaluated traits; \( \mu \) is the expected overall mean effect; \( \text{pop}_i \) is the effect of the i-th population factor; and \( e_{ik} \) is the effect of the experimental error.

The graphical dispersion of the 21 physalis populations was performed based on the canonical scores of the first two canonical discriminant functions (FDC1 and FDC2) using the CANDISC procedure of the SAS, based on the set of traits evaluated. The hypotheses between treatments were tested using multivariate analysis of variance (MANOVA).

The MANOVA result reveals only the effect of global variation between the treatments, but there is a structure for the comparison between treatments that should be investigated. For such, non-orthogonal multivariate contrasts were performed, and each population was compared at dose zero versus the respective population at doses 100 Gy and 200 Gy, according to the following scheme: C1: Colômbia01_0 vs. Colômbia01_100; C2: Colômbia01_0 vs. Colômbia01_200; C3: Colômbia02_0 vs. Colômbia02_100; C4: Colômbia02_0 vs. Colômbia02_200; C5: Caçador_0 vs. Caçador_100; C6: Caçador_0 vs. Caçador_200; C7: Lages_0 vs. Lages_100; C8: Lages_0 vs. Lages_200; C9: Fraiburgo_0 vs. Fraiburgo_100; C10: Fraiburgo_0 vs. Fraiburgo_200; C11: CAV_0 vs. CAV_100; C12: CAV_0 vs. CAV_200; C13: Peru_0 vs. Peru_100; C14: Peru_0 vs. Peru_200.

The standardized canonical coefficients (CCP) were analyzed for each contrast, in order to identify which traits present greater canonical weight for population differentiation. The CCPs are interpreted as follows: i) positive values indicate the effect of the separation between the treatments. Traits with higher CCP values show greater weight in the differentiation and, ii) negative values can be interpreted similarly, but in the opposite direction of the effect, whereas negative values reduce the effect of the trait under study (HAIR et al., 2007). Given the occurrence of positive values for the CCPs, the differences between the averages of interest were estimated to give support to the interpretation of the results.

Results and discussion

The multivariate analysis of variance showed significant difference for the population factor (Table 1). It demonstrates that there is some difference between at least two populations, among the 21 populations of physalis analyzed, considering the set of traits simultaneously. Genetic variability is determinant for the success of the selection of promising genetic constitutions (ALLARD, 1971; CECARELLI, 2009). It provides breeders the conditions to select plants better adapted to the conditions of cultivation, either considering tolerance to biotic and abiotic stresses (JAIN, 2010) or the search for traits related to quality. In the case of small fruits, quality is essentially related to the appearance and taste of the fruit (DE LIMA et al., 2014; HURTADO-SALAZAR et al., 2015), and breeders are interested in obtaining genotypes with larger fruits with an adequate ratio between sugars/acidity and color intensity.

Selection via natural or man-made processes and the low frequency of spontaneous mutations may restrict the genetic basis for many traits of economic relevance (FURROW; FELDMAN, 2013). In physalis populations cultivated in the Southern Brazil, restricted genetic variability was observed for flower and fruit emergence and fruit size (TREVISANI et al., 2016). This is a major hindrance for the likely gains obtained from selection because it prevents the adoption of efficient breeding methods, due to the lack of variation, and may jeopardize the species under cultivation conditions by exposing it to adverse factors.

In the present case, the multivariate dispersion of the 21 populations of physalis (original and mutant populations), represented by the scores of the first and second canonical discriminant linear functions (FDC1 and FDC2), revealed the occurrence of genetic variability among the populations. Together, the canonical functions captured 85% of the total variance (Figure 1). According to Cruz et al. (2012), satisfactory interpretations of the variability manifested by the treatments can be reached with 80% of the cumulative variance.

Figure 1 shows the divergence among the 21 populations for the set of traits assessed. The visual analysis of the graph indicates the existence of variability between mutant populations and non-irradiated populations. For such, the graphical analysis was extremely important to clarify whether the hypothesis on the effectiveness of the mutagenic agent at causing genetic changes in physalis was corroborated or not. Therefore, the analysis of the coordinates of the graph does not show marked divergence when populations with and without dose are compared. For example, the Fraiburgo population (5.0) is close to its respective mutant population at doses 100 Gy (5.1) and 200 Gy (5.2), which is also true for the Caçador population (doses 0, 100 and 200 Gy).

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Table 1 - Summary of the multivariate analysis of variance, by means of four statistical tests, for the population factor, considering the traits total soluble solids (SST), seed mass (MS), capsule mass (MC), fruit mass (MF), transverse fruit diameter (DTF) and longitudinal fruit diameter (DLF). UDESC-IMEGEM, Lages SC, year 2014/2015.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Statistical Test</th>
<th>Test value</th>
<th>Value F</th>
<th>NGL(1)</th>
<th>DGL(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilks’ Lambda</td>
<td>0.01*</td>
<td>4.28</td>
<td>120</td>
<td>417.8</td>
<td></td>
</tr>
<tr>
<td>Pillai’s Trace</td>
<td>2.64*</td>
<td>2.99</td>
<td>120</td>
<td>456</td>
<td></td>
</tr>
<tr>
<td>Population</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hotteling-Lawley</td>
<td>11.43*</td>
<td>6.62</td>
<td>120</td>
<td>292.25</td>
<td></td>
</tr>
<tr>
<td>Roy’s Maximum Root</td>
<td>7.57*</td>
<td>28.78</td>
<td>20</td>
<td>76</td>
<td></td>
</tr>
</tbody>
</table>

H<sub>0</sub>: μ<sub>1</sub> = μ<sub>2</sub> = ... = μ<sub>k</sub>, H<sub>A</sub>: at least two μ’s are different.

(1) Numerator Degrees of Freedom. (2) Denominator Degrees of Freedom

* Ho rejected at 5% error probability by the F test.

Figure 1 - Multivariate dispersion of the 21 physalis populations coordinated by the first and second canonical discriminant linear function, capturing 85% of the accumulated variance in the eigenvalues. Canonic scores obtained for total soluble solids (SST), seed mass (DM), capsule mass (MC), fruit mass (MF), fruit transverse diameter (DTF) and fruit longitudinal diameter (DLF). UDESC-IMEGEM, Lages SC, year 2014/2015.

*Physalis populations: (1.0) Colômbia01 dose 0; (1.1) Colômbia01 dose 100; (1.2) Colômbia01 dose 200; (2.0) Colômbia02 dose 0; (2.1) Colômbia02 dose 100; (2.2) Colômbia02 dose 200; (3.0) Caçador dose 0; (3.1) Caçador dose 100; (3.2) Caçador dose 200; (4.0) Lages dose 0; (4.1) Lages dose 100; (4.2) Lages dose 200; (5.0) Fraiburgo dose 0; (5.1) Fraiburgo dose 100; (5.2) Fraiburgo dose 200; (6.0) CA V dose 0; (6.1) CA V dose 100; (6.2) CA V dose 200; (7.0) Peru dose 0; (7.1) Peru dose 100; (7.2) Peru dose 200.
The multivariate contrasts were performed in order to statistically test the differences between the irradiation levels (Table 2). The contrast analysis showed that, out of the 14 physalis mutant populations, six populations (43%) presented variations when compared to the original population free from mutation, namely, Colômbia01 (doses 100 and 200 Gy), Colômbia02 (doses 100 and 200 Gy), Caçador (dose 200 Gy) and CAV (dose 200 Gy). In the other populations, the mutagenic agent and the doses used were not effective at causing phenotypic changes in the traits of the physalis fruit.

Radiosensitivity varies according to several factors, including species and/or cultivar (genetic constitution), genome repair mechanisms, physiological condition of the plant and the organ before and after treatment with the mutagenic agent, water content in the material, storage conditions after irradiation, radiation dosage, degree of ploidy of the individual and DNA content of the genome (PREDIERI, 2001; SHU; LAGODA, 2007). However, the mechanisms by which plants recognize and respond to the irradiation doses to which they are exposed have not been clarified (PRISTOV et al., 2013).

Table 2 – Multivariate contrasts and standardized canonical coefficients (CCP) for total soluble solids (SST), seed mass (MS), capsule mass (MC), fruit mass (MF), transverse fruit diameter (DTF) and longitudinal fruit diameter (DLT). Wilk’s Statistics (U). UDESC-IMEGEM, Lages SC, year 2014/2015.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>U</th>
<th>SST</th>
<th>MS</th>
<th>MC</th>
<th>MF</th>
<th>DTF</th>
<th>DLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.75*</td>
<td>-0.35</td>
<td>0.26</td>
<td>0.63</td>
<td>-0.87</td>
<td>0.95</td>
<td>-0.26</td>
</tr>
<tr>
<td>C&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.76*</td>
<td>0.11</td>
<td>0.27</td>
<td>0.51</td>
<td>-0.79</td>
<td>1.12</td>
<td>-0.55</td>
</tr>
<tr>
<td>C&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.84*</td>
<td>0.47</td>
<td>0.21</td>
<td>0.01</td>
<td>-0.84</td>
<td>-0.14</td>
<td>1.09</td>
</tr>
<tr>
<td>C&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.86*</td>
<td>-0.02</td>
<td>-0.08</td>
<td>0.03</td>
<td>-1.01</td>
<td>0.38</td>
<td>0.83</td>
</tr>
<tr>
<td>C&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.85&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>-0.46</td>
<td>0.11</td>
<td>-0.05</td>
<td>0.27</td>
<td>1.07</td>
<td>-0.04</td>
</tr>
<tr>
<td>C&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0.78*</td>
<td>-0.30</td>
<td>0.17</td>
<td>0.05</td>
<td>0.56</td>
<td>0.91</td>
<td>0.03</td>
</tr>
<tr>
<td>C&lt;sup&gt;7&lt;/sup&gt;</td>
<td>0.93&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.78</td>
<td>-0.19</td>
<td>0.37</td>
<td>-0.53</td>
<td>-0.07</td>
<td>-0.26</td>
</tr>
<tr>
<td>C&lt;sup&gt;8&lt;/sup&gt;</td>
<td>0.95&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.59</td>
<td>-0.05</td>
<td>0.46</td>
<td>0.98</td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>C&lt;sup&gt;9&lt;/sup&gt;</td>
<td>0.98&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>-0.13</td>
<td>-0.26</td>
<td>1.27</td>
<td>0.34</td>
<td>0.24</td>
<td>-0.16</td>
</tr>
<tr>
<td>C&lt;sup&gt;10&lt;/sup&gt;</td>
<td>0.95&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.92</td>
<td>-0.75</td>
<td>1.14</td>
<td>0.71</td>
<td>0.10</td>
<td>-0.26</td>
</tr>
<tr>
<td>C&lt;sup&gt;11&lt;/sup&gt;</td>
<td>0.88&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>-0.74</td>
<td>0.54</td>
<td>-0.73</td>
<td>-0.11</td>
<td>0.80</td>
<td>-0.11</td>
</tr>
<tr>
<td>C&lt;sup&gt;12&lt;/sup&gt;</td>
<td>0.81*</td>
<td>1.56</td>
<td>-0.55</td>
<td>0.18</td>
<td>1.99</td>
<td>-0.28</td>
<td>0.02</td>
</tr>
<tr>
<td>C&lt;sup&gt;13&lt;/sup&gt;</td>
<td>0.90&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.89</td>
<td>-0.43</td>
<td>0.52</td>
<td>0.79</td>
<td>0.01</td>
<td>-0.33</td>
</tr>
<tr>
<td>C&lt;sup&gt;14&lt;/sup&gt;</td>
<td>0.85&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.84</td>
<td>-0.09</td>
<td>0.07</td>
<td>0.48</td>
<td>-0.19</td>
<td>-0.44</td>
</tr>
</tbody>
</table>

<sup>1</sup>C<sub>1</sub>: Colômbia01_0 vs. Colômbia01_100; C<sub>2</sub>: Colômbia01_0 vs. Colômbia01_200; C<sub>3</sub>: Colômbia02_0 vs. Colômbia02_100; C<sub>4</sub>: Colômbia02_0 vs. Colômbia02_200; C<sub>5</sub>: Caçador_0 vs. Caçador_100; C<sub>6</sub>: Caçador_0 vs. Caçador_200; C<sub>7</sub>: Lages_0 vs. Lages_100; C<sub>8</sub>: Lages_0 vs. Lages_200; C<sub>9</sub>: Fraiburgo_0 vs. Fraiburgo_100; C<sub>10</sub>: Fraiburgo_0 vs. Fraiburgo_200; C<sub>11</sub>: CAV_0 vs. CAV_100; C<sub>12</sub>: CAV_0 vs. CAV_200; C<sub>13</sub>: Peru_0 vs. Peru_100; C<sub>14</sub>: Peru_0 vs. Peru_200.

* Ho rejected at 5% error probability by the Wilks’ Lambda test.
Zaka et al. (2002) claim that it is very difficult to establish the extreme limits of ionizing irradiation by gamma rays (low and high dose). There are no studies in physalis indicating the efficient doses of irradiation for causing changes or the lethal doses of the mutagenic agent applied to the seed. Caro-Melgarejo et al. (2012) investigated the effect of gamma irradiation on vegetative buds of *P. peruviana*, under *in vitro* conditions, and found that the dose of 300 Gy inhibited the development of buds and root primordia. However, tissues grown under *in vitro* conditions are more sensitive to treatments with irradiation when compared to seeds (Lu et al., 2007).

The results of the present study revealed that the dose of 200 Gy was not lethal in any of the populations of physalis. It was evident that the populations Colômbia, Caçador and CAV presented greater sensitivity to the mutation doses applied and the differences detected were due to changes in the phenotypic means of one or more measured traits. It is noteworthy that the results obtained cannot affirm that doses higher than those used are ineffective at causing greater variations in the plant genome, when the effects observed in the phenotype of the individual are either positive or negative.

In the contrasts C₁ and C₃, the standardized canonical coefficients (CCP) showed that the traits MS, MC and DTF performed the differentiation of the population Colômbia01 when submitted to the doses of 100 and 200 Gy (Table 2). It is necessary to investigate whether the change was beneficial (positive) and then verify if the magnitude of the variation is satisfactory for the selection of larger fruits, for example. Therefore, differences between the averages for each trait demonstrate a reduction of 0.25 (C₃) and 0.36 (C₃) grams in the capsule mass and reduction of 3.97 (C₅) and 2.57 (C₅) mm in the transverse diameter, significant at the 5% level of error probability by the t-test.

From the perspective of plant breeding, the selection of fruits with lower capsule mass to the detriment of a larger fruit is a promising alternative for selection. Therefore, it is also verified that the fruit mass was not changed by mutation induction, as it brings populations close (Table 2). However, a reduced fruit transverse diameter is observed, which is a non-promising fact. Therefore, the selection of fruits with smaller transverse diameter does not prove to be a favorable strategy for the breeding of physalis, and the reduction of approximately 4.00 mm is considered significant for a fruit with an average of 20.00 mm in diameter.

The analysis of the contrasts C₄ and C₁₂ reveal that the traits SST and DLT differentiated the populations. These traits are very important in the breeding of physalis and should be considered at the time of selection, together with the trait MF revealed by the contrasts C₆ and C₁₂ (Table 2). According to the result, the doses of the mutagenic Cobalt⁴⁰ were effective at causing variations in the populations Colômbia02, Caçador and CAV in fruit traits of commercial value, which provided the selection and direct use as new cultivars and even the indication in hybridization processes for the development of segregating populations. This fact is proven by the differences between the means, since the contrasts C₄ and C₁₂ were increased in 4.16 mm in the DLT for C₄ and 1.81º brix for the contrast C₁₂. Such variations are extremely important in the selection of fruits with greater diameter and higher sugar content. However, the contrast C₆ showed reduction of 0.89 grams in MF and reduction of 4.90 mm in the DTF.

In contrast, C₄ (Colômbia dose 0 vs. Colômbia dose 200), the longitudinal and transverse diameters differentiated the populations, and were increased in 2.99 mm (DTF) and 4.90 mm (DLT). Both additions are promising and important for selection. Thus, the mutation was effective at causing positive variations and providing satisfactory genetic gains for the obtainment of physalis fruits with larger diameter.

It was evident that the mutation induction also produced non-beneficial changes, as observed in the contrasts C₁, C₂ and C₅ due to reduced fruit transverse diameter. On the other hand, it resulted in positive changes demonstrated in the contrasts C₄, C₆ and C₁₂ for the diameter and content of soluble solids. The increased longitudinal and transverse diameters obtained in the Colômbia02 population at the dose of 200 Gy (contrast C₄) allow the selection of this population. In addition to the direct use as a new cultivar, promising populations can also be selected for the recombination of contrasting traits and the obtainment of segregating populations, such as Colômbia02, whose fruits present larger diameter, and CAV (dose of 200 Gy), whose average fruits present 14.8º Brix. Therefore, the hybridization of these populations and the assessment of the segregant populations in the recombination of the traits would be a further step for the achievement of physalis populations with fruits of superior quality.

As already said, the cultivation of physalis is incipient in Brazil. There are no physalis varieties recorded nor standards for the marketing of the fruit. In Colombia, the standardization, classification and packaging of physalis fruits are regulated by the Colombian Technical Standard - NTC 4580, connected to the Instituto Colombiano de Normas Técnicas (Colombian Institute of Technical Standards) (ICONTEC, 1999), which establishes the basic requirements for the marketing of fresh fruit and fruit processing. For such, fresh fruits should have diameters between 21.00 and 23.00 mm, mass between 5 and 8 grams and average sugar content of 15º brix. These values, compared to the results obtained, demonstrate the lower quality of the promising mutant populations. The Colômbia02 population (dose 200 Gy), for example, presented average fruit mass of 3.8 grams;
DTF of 19.00 mm; and DLF of 18.5 mm. Therefore, the direct selection and/or obtainment of segregating populations from hybridizations in mutant populations may not lead to satisfactory gains for higher quality fruit, as established by the Colombian Standards.

Fruit size is the most attractive trait and the most demanded by the consumer market (ADRIANO et al., 2011), especially in species marketed in the small fruit group. This quantitative trait is possibly governed by a high number of genes, which may have prevented the mutagenic agent from causing changes of remarkable magnitude. Therefore, breeding strategies can be adopted to improve the quality of physalis fruit. In addition to breeding via physical mutation induction, polyploidy induction may be a promising strategy for the achievement of larger fruits. Genome duplication is usually followed by changes in agronomically important traits (PARISOD et al., 2010). In Physalis isocarpa Brot., Robledo-Torres et al. (2011) obtained polyploid genotypes (2n = 4x = 48) with significantly higher values for fruit transverse diameter (6.5 mm increase) and content of total soluble solids (1.68°Brix increase).

Given the predominance of tetraploidy in P. peruviana (2n = 4x = 48) (LAGOS et al., 2008), genome duplication and the octoploid individuals obtained may provide satisfactory results, with fruits of the desired size. It is worth noting that genome duplication and the obtaining of octoploid plants in P. peruviana may increase plant infertility, which is a negative result, due to irregular chromosome pairing during meiosis. An alternative to this biological barrier may be the asexual propagation of physalis via cutting, considering the ease of obtaining and multiplying seedlings.

Still with regard to ploidy in physalis, it should be pointed out that the sensitivity of diploid and polyploid plants treated with the mutagenic agent decreases when the ploidy level of plants increases (CHANDHANAMUTTA; FREY, 1974). This leads to the conclusion that the duplication of genes in the polyploids reduces the frequency of mutation. Thus, tetraploid species have four alleles per gene locus, while diploid species have two alleles. This could be verified in the species Musa acuminata Colla. DLsp was 20-25 Gy in diploid genotypes; 30-35 Gy in triploid genotypes and 35-40 Gy in tetraploid genotypes.

In physalis, there may be a positive relation between the degree of ploidy and the dose of irradiation used, which suggests that higher doses will possibly be effective at causing genetic changes of greater magnitude. If higher irradiation doses are effective at causing significant changes in fruit mass and content of soluble solids, hybridization between contrasting populations can ensure a successful selection. Therefore, the results obtained provide new perspectives to the physalis breeding program by combining the induction of mutation by gamma rays (increased doses of irradiation) and chromosome duplication (increased ploidy level). This gives farmers assurance regarding the superiority of the populations under cultivation conditions. The availability of more productive genotypes with good quality fruits is a goal to be achieved by the physalis breeding programs, so that the country can become self-sufficient in the production physalis fruits.

Conclusion

Physical irradiation with the use of the mutagenic agent Cobalt60 resulted in variation in populations of physalis, but was not efficient at selecting superior populations for fruit traits, including fruits of larger size, due to the magnitude of the values.

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References


