Agronomic potential of $\text{BC}_1F_2$ dwarf round tomato populations

Potencial agronômico de populações $\text{F}_2\text{RC}_1$ de tomateiro anão do tipo salada

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ABSTRACT

The use of dwarf lines to obtain mini-tomato hybrids has provided agronomic and economic benefits. In Brazil, round tomatoes predominate over other varieties. The benefits of using a dwarf parent in round tomato hybrids has yet to be explored, making it important to develop dwarf round tomato lines. Backcrossing is the most suitable method to develop these lines. Evaluation and selection of the dwarf populations can improve the development of such lines. Thus, the aim of this study was to select $\text{BC}_1F_2$ populations of dwarf round tomatoes with agronomic potential and high-quality fruit. The study was conducted at the Vegetable Experimental Station of the Federal University of Uberlândia (UFU). A randomized block design was used, with 15 treatments and three replicates. The genetic material analyzed consisted of 12 $\text{BC}_1F_2$ dwarf tomato populations, plus both parents (recurrent and donor) and a commercial hybrid. The characteristics assessed were: average fruit weight (g), total soluble solids (ºBrix), number of locules (locules per fruit$^1$), fruit shape, pulp thickness (cm), longitudinal (cm) and transverse fruit diameter (cm), internode length (cm) and plant height (cm). The data were submitted to mean testing, multivariate analyses and a selection index. In general, average fruit weight in the dwarf populations increased significantly after the first backcross, with some fruits exhibiting a similar shape to round tomatoes. Selection of the populations UFU-DTOM7, UFU-DTOM10, UFU-DTOM5, UFU-DTOM9, and UFU-DTOM3 resulted in an estimated 6% increase in the number of locules, transverse diameter, TD/LD ratio and average fruit weight. The $\text{BC}_1F_2$ dwarf populations UFU-DTOM7 and UFU-DTOM10 were the most promising for develop inbred lines with round fruits. Despite the considerable progress achieved in this study, we suggest a second backcross, in order to obtain lines and, posteriorly, hybrids with round fruits and compact plants.

Index terms: Backcrossing; dwarfism; Solanum lycopersicum.

RESUMO

Vantagens agronômicas e econômicas tem sido observadas com o uso de linhagens anãs para obtenção de híbridos em minitomate. No Brasil, predomina-se o cultivo de tomate do tipo salada. A exploração dos benefícios proporcionados pelo uso do parental anão em híbridos de tomateiro do tipo salada ainda não é uma realidade. A avaliação e seleção de populações de porte anão pode aumentar a eficiência do desenvolvimento de tais linhagens. Sendo assim, torna-se necessário o desenvolvimento de linhagens anãs do tipo salada. O retrocruzamento é o método mais adequado para se desenvolver tais linhagens. Portanto, o objetivo deste trabalho foi selecionar populações $\text{F}_2\text{RC}_1$, de tomateiro anão com potencial agronômico e qualidade de fruto. O experimento foi conduzido na Estação Experimental de Hortaliças da Universidade Federal de Uberlândia (UFU). Utilizou-se o delineamento de blocos casualizados com 15 tratamentos e três repetições. O material genético avaliado consistiu de 12 populações $\text{F}_2\text{RC}_1$, de tomateiro anão, mais ambos os genitores (recorrente e doador) e um híbrido comercial. As características avaliadas foram: massa média (g), teor de sólidos solúveis (ºBrix), número de lóculos (lóculos fruto$^1$), formato, espessura da polpa (cm), diâmetro longitudinal (cm) e transversal do fruto (cm); comprimento dos internódios (cm) e altura das plantas (cm). Os dados foram analisados por meio de teste de médias, análises multivariadas e índice de seleção. De maneira geral, a massa média dos frutos das populações anãs aumentou significativamente após o primeiro retrocruzamento e algumas destas apresentaram o formato de fruto próximo ao do segmento salada. Ao selecionar as populações UFU-DTOM7, UFU-DTOM10, UFU-DTOM5, UFU-DTOM9 e UFU-DTOM3 foi estimado um incremento de até 6% no número de lóculos, diâmetro transversal, relação DT/DL e peso médio do fruto. As populações anãs $\text{F}_2\text{RC}_1$, UFU-DTOM7 e UFU-DTOM10 se destacaram sendo promissoras para obtenção de linhagens anãs. Apesar do avanço considerável obtido na presente pesquisa, sugere-se a realização do segundo retrocruzamento e posterior obtenção de linhagens para formar híbridos.

Termos para indexação: Retrocruzamento; nanismo; Solanum lycopersicum.
INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the important vegetables cultivated worldwide, Brazil being a major producer. In 2018, the country produced an estimated 4.0 million metric tons of tomato over a cultivated area of approximately 60,000 hectares (Instituto Brasileiro de Geografia e Estatística - IBGE, 2019). In Brazil, tomatoes are classified into four groups: “Minitomate, Salada, Caqui, Santa Cruz and Saladete” (Alvarenga, 2013). Out of Brazil, this tomato groups are known as cherry or grape (mini-tomatoes), round, beefsteak, chonto, and saladette or roma, respectively. Among these, the round tomato is widely grown for fresh-market in Brazil and is the most economically important.

These tomatoes are grown primarily as field crops, involving high costs per hectare and significant financial risk. As a result, several studies have been conducted aimed at increasing crop yield by modifying the plant spacing (Wamser et al., 2012), the number of stems per plant (Matos; Shirahige; Melo, 2012; Wamser et al., 2012) and fertilization (Mueller et al., 2013). Nevertheless, few studies have focused on a vital aspect capable of improving yield and fruit quality without increasing crop management and production costs: breed for short internodes trait.

Short internodes result in more compact tomato plants (Gardner; Panthee, 2012; Panthee; Gardner, 2013a, 2013b), which facilitates pruning, training (Figueiredo et al., 2015) and harvesting, apart from increase the number of clusters per linear meter of stem. Hybrids capable of producing a larger number of clusters in a same space are economically viable due to their superior yield, increasing the profit per plant. In a one-hectare plot containing 18,000 plants, one extra cluster per stem would represent a significant increase in yield. The current production cost of one hectare in Brazil is more than BRL 100,000 (~USD 25,015) (18º42'43.19"S, 47º29'55.8"W and 873 m.a.s.l.). Plants were grown in a 4-meter-high greenhouse with an arched roof, covered in clear UV-resistant polyethylene film (150 micra) and white anti-insect net on the sides.

The genetic material analyzed consisted of 12 dwarf tomato populations obtained from the first backcross (BC$_F_1$) after hybridization of a pre-commercial inbred line with round-type fruit (recurrent parent) versus the dwarf line UFU MC TOM1 (Maciel; Silva; Fernandes, 2015), plus both parents and a commercial hybrid (Paronset®), totaling 15 treatments. The BC$_F_2$ populations and inbred lines belong to the UFU tomato germplasm. The recurrent parent and commercial hybrid have an indeterminate growth habit and red round fruit, while the UFU MC TOM1 dwarf line used as the donor parent, have an indeterminate growth habit and grape-type fruits (Finzi et al., 2017b; Maciel; Silva; Fernandes, 2015). Since the dwarf phenotype is recessive and monogenic (Maciel; Silva; Fernandes, 2015), backcrosses were performed in way to transfer at recessive allele.

Seeds were sowed in 200-cell polystyrene trays, on January 10, 2019. Twenty-eight days after sowing (DAS), the seedlings were transplanted into 5-liter plastic pots. A commercial coconut fiber substrate was used in both the trays and pots. The crop treatments applied during the study were in line with recommendations for tomatoes grown in greenhouses (Alvarenga, 2013). The recurrent parent and commercial hybrid were grown vertically by training or trailing them onto two stakes strung with twine.

A randomized block design was used, with 15 treatments and three replicates. The experimental plots consisted of six plants spaced 0.3 cm apart and distributed across two rows, with 0.8 m between plots, totaling 270 plants in the greenhouse.

Harvesting was carried out once a week from April 11 to June 19, 2019, totaling nine harvests. Fully ripe fruits
were collected from each plot and the following agronomic traits assessed:

- **Average fruit weight (g) (AFW):** ratio between weight and the total number of fruits harvested from the plot.

- **Total soluble solids (ºBrix) (TSS):** determined as the average of all the fruits harvested from the plot. After harvest, the tomatoes were blended in a food processor and total soluble solids content was measured with a digital refractometer (Atago PAL-1 3810).

- **Transverse fruit diameter (cm) (TD):** the fruit was cut in half horizontally and the horizontal length measured using a ruler, considering the average of three measurements.

- **Longitudinal fruit diameter (cm) (LD):** the fruit was cut in half vertically and the vertical length measured using a ruler, considering the average of three measurements.

- **Fruit shape:** obtained based on the ratio between the transverse and longitudinal diameters (TD/LD). The recurrent parent and commercial hybrid were used as references for round tomatoes shape, in order to classify the fruits.

- **Pulp thickness (cm) (PT):** length between the peel and the beginning of the locule measured with a ruler after cutting the fruit vertically, considering the average of all the fruits collected from the plot.

- **Number of locules (locules per fruit -1) (NL):** counted after cutting the fruit in half horizontally, considering the average count for all the fruits collected from the plot.

- **Internode length (cm) (I):** determined using the following equation: [(plant height/number of nodes)-1], in the two center plants from each plot, considering the average of the two measurements.

- **Height (cm) (HT):** the vertical length of the entire plant, measured with a ruler in the two center plants from each plot and considering the average of the two measurements.

After verification of the assumptions via homogeneity of variance (Levene’s test), normality (Kolmogorov-Smirnov test) and additivity analyses (Tukey’s test of nonadditivity), data were transformed for AFW, LD and HT and data for internode length, tabulating the real values for these variables.

For AFW, the relative superiority (RS%) of each BC$_1$F$_2$ population in relation to the donor parent was calculated using the equation: \( \text{RS\%} = \left( \frac{C1}{C2} - 1 \right) \times 100 \), where RS% is the percentage difference between BC$_1$F$_2$ populations and the donor parent, C1 the AFW of the dwarf population to be calculated, and C2 the AFW of the donor parent.

Additionally, the data obtained were analyzed by three independent models: mean test, multivariate analyses and a selection index. In the mean test, data were submitted to analysis of variance (F test) and means compared using Tukey’s test (p<0.05).

Multivariate analyses were performed solely to determine the genetic dissimilarity among the dwarf plants (BC$_1$F$_2$ populations and the donor parent), obtaining the dissimilarity matrix based on the Mahalanobis generalized distance. Genetic divergence was represented by a dendrogram produced via the unweighted pair-group method with arithmetic mean (UPGMA) and Tocher optimization method. Clustering via the UPGMA method was validated using the cophenetic correlation coefficient (CCC), calculated by the Mantel test (Mantel, 1967).

The selection index was calculated based on the sum of ranks (Mulamba; Mock, 1978), using only the dwarf populations and recurrent parent. In order to estimate selection gains, 33% of the populations were selected. The selection criteria used were shortening the internodes and increasing the other variables. The economic weight adopted was the coefficient of genetic variation of each variable, as recommended by Cruz, Regazzi and Carneiro (2012). All analyses were performed using Genes software (Cruz, 2016).

**RESULTS AND DISCUSSION**

The BC$_1$F$_2$ dwarf tomato populations differed from the parents and commercial hybrid in all the traits assessed (Table 1). As expected, the recurrent parent and commercial hybrid were superior, particularly for fruit weight, internode distance and plant height. Since the primary objective of this study was to assess the agronomic gain provided by the first backcross, greater emphasis will be given to comparisons between dwarf populations and the donor parent.

In general, substantial increases in the fruit weight of dwarf populations were obtained after only one backcross (Figure 1). The fruit weight values of UFU-DTOM9, UFU-DTOM7, UFU-DTOM4 and UFU-DTOM10 differed statistically from those of the donor parent, with relative superiority of 341, 282, 280 and 268%, respectively (Figure 2). This indicates that the first backcross was successful to transfer loci related to fruit weight from the recurrent parent to the dwarf populations.
Table 1: Agronomic traits assessed in BC₁F₂ dwarf tomato populations, the recurrent and donor parents and commercial hybrid.

| Treatments            | AFW* | TSS  | TD  | LD  | TD/LD | PT  | NL  | I     | HT  |
|-----------------------|------|------|-----|-----|-------|-----|-----|-------|-----|
| UFU-DTOM1             | 9.3  | 9.7  | 2.5 | 2.9 | 0.9   | 0.4 | 2.1 | 0.9   | 24.4|
| UFU-DTOM2             | 7.9  | 9.4  | 2.3 | 2.4 | 0.9   | 0.4 | 2.0 | 0.9   | 22.7|
| UFU-DTOM3             | 7.9  | 10.0 | 2.4 | 2.4 | 1.0   | 0.4 | 2.7 | 0.9   | 24.8|
| UFU-DTOM4             | 11.3 | 9.7  | 2.2 | 2.4 | 0.9   | 0.4 | 2.7 | 0.9   | 26.2|
| UFU-DTOM5             | 9.9  | 9.3  | 2.6 | 2.4 | 1.1   | 0.3 | 2.9 | 1.1   | 28.2|
| UFU-DTOM6             | 7.7  | 9.5  | 2.4 | 2.4 | 1.0   | 0.3 | 2.3 | 0.9   | 28.8|
| UFU-DTOM7             | 11.3 | 8.4  | 2.8 | 2.5 | 1.1   | 0.4 | 3.0 | 0.9   | 24.2|
| UFU-DTOM8             | 8.5  | 9.2  | 2.3 | 2.5 | 0.9   | 0.3 | 2.0 | 0.9   | 30.5|
| UFU-DTOM9             | 13.1 | 8.8  | 2.6 | 3.1 | 0.8   | 0.4 | 2.1 | 1.1   | 34.1|
| UFU-DTOM10            | 10.9 | 8.8  | 2.6 | 2.5 | 1.1abc| 0.4 | 2.5 | 0.9   | 26.3|
| UFU-DTOM11            | 8.7  | 9.4  | 2.3 | 2.2 | 1.1abc| 0.3 | 2.0 | 1.0   | 31.0|
| UFU-DTOM12            | 8.4  | 9.3a | 2.5 | 2.6 | 1.0   | 0.4 | 2.3 | 1.2   | 30.4|
| Commercial hybrid     | 82.3 | 5.2  | 6.1 | 4.8 | 1.3abc| 0.9 | 3.4 | 6.7   | 205.2|
| Donor parent          | 3.0  | 9.0  | 1.6 | 2.6 | 0.6   | 0.3 | 2.0 | 0.8   | 24.6|
| Recurrent parent      | 50.4 | 6.7  | 5.2 | 4.1 | 1.3abc| 0.6 | 4.9 | 5.4   | 198.0|
| KS²                   | 0.8  | 0.9  | 0.8 | 0.9 | 1.0   | 0.1 | 0.1 | 0.8   | 0.7 |
| F (Levene)³           | 1.4  | 1.5  | 1.3 | 2.7 | 3.1   | 2.8 | 4.3 | 2.6   | 2.4 |
| F (Additivity)⁴       | 0.9  | 1.1  | 0.4 | 0.6 | 1.4   | 0.1 | 2.5 | 2.3   | 1.1 |
| CV (%)                | 13.8 | 12.9 | 12.3| 6.3 | 7.2   | 18.6| 16.9| 4.2   | 8.4 |

*AFW: average fruit weight (g), TSS: total soluble solids (ºBrix), TD: transverse diameter (cm); LD: longitudinal diameter (cm); TD/LD: ratio between the transverse and longitudinal diameters; PT: pulp thickness (cm); NL: number of locules (locules per fruit-1); I: internode length (cm); HT: plant height (cm). ¹Means followed by different letters in the column do not differ according to Tukey's test at 0.05 significance; ²KS, F (Levene's test)³, F (Tukey's test)⁴: statistics of the Kolmogorov-Smirnov, Levene's and Tukey's tests, respectively; amounts in bold indicate normal distribution, homogeneous variances and additivity at 0.01 significance. Paronset®: control/commercial hybrid.

Individual assessment of the transverse and longitudinal diameters of the dwarf tomatoes show no significant differences from the donor parent. However, based on the TD/LD ratios, the UFU-DTOM5, UFU-DTOM7, UFU-DTOM10 and UFU-DTOM11 populations were statistically similar to the recurrent parent and commercial hybrid. This confirms that the fruits of these populations have similar shape as round tomatoes (Figure 3).

Total soluble solids, pulp thickness, number of locules, plant height and internode length did not differ between dwarf populations and the donor parent, with average values of 9.3 ºBrix, 0.4 cm, 2.4 locules per fruit¹, 27.6 cm and 1.2 cm, respectively. The high soluble solid content is a desirable trait due to its association with sweeter flavored tomatoes, which are preferred by consumers (Maciel et al., 2015; Schwarz et al., 2013). Moreover, the short internodes of the dwarf populations are also beneficial because they result in more compact plants in hybrid combinations, with more clusters per linear meter of stem (Finzi et al., 2017a).

It is important to highlight that the dwarf tomato populations are part of the first backcross with the recurrent parent. For the next phase of this study, we suggest performing another backcross and subsequently obtaining hybrid round tomatoes from dwarf lines similar to those breed by Finzi et al. (2017a) in mini-tomatoes. This makes it important to use different selection strategies to obtain superior dwarf populations, with genetic dissimilarity measures and selection indices proving to be promising alternatives.
Figure 1: Comparison between the phenotype of the donor parent and a plant from the population UFU-DTOM9.

Figure 2: Relative superiority of the average fruit weight of dwarf tomato populations (BC₁F₂) in relation to the donor parent.

Dissimilarity between dwarf plants, estimated by the generalized Mahalanobis distance, varied from 3.37 (UFU-DTOM10 and UFU-DTOM11) to 99.64 (UFU-DTOM7 and UFU-DTOM13), demonstrating genetic diversity (data not shown). To better visualize dissimilarity, a dendrogram was produced using the UPGMA technique (Figure 4) and a graph based on Tocher’s optimization method (Figure 5).

The dendrogram exhibited a cophenetic correlation coefficient of 0.88 and significance according to the t test (p<0.01), making it a satisfactory tool to represent the information in the matrix and subsequent group formation. In other studies on tomato crops, the UPGMA method was also efficient at discriminate groups (Araujo et al., 2016; Maciel et al., 2018a; Peixoto et al., 2018). With the 30% cutoff point in the dendrogram, the populations formed three distinct groups. Group I contained most of the dwarf populations, group II UFU-DTOM9 and group III the donor parent. All the dwarf populations differed from the donor parent, confirming that the first backcross improved the agronomic performance of the former. The fact that UFU-DTOM9 was in a separate cluster from the others is likely because this population exhibits higher fruit weight. The dissimilarity graph based on the Tocher method (Figure 3) demonstrated that UFU-DTOM7, UFU-DTOM5 and UFU-DTOM10 differed most from the donor parent. This visualization of genetic divergence among individuals is important in identifying potential genotypes in a breeding program (Maciel et al., 2018b).

In addition to dissimilarity measures, the selection index is simultaneously based on several different traits (Cruz; Regazzi; Carneiro, 2012; Rosado et al., 2012) and has the advantage of estimating genetic gains for the largest possible number of characteristics (Rezende et al., 2014; Vasconcelos et al., 2010). The use of genetic gain can significantly reduce the time and resources needed to select potential plants for a breeding program (Heffner; Jannink; Sorrells, 2011). Thus, the greatest genetic gains, evenly distributed across all the traits analyzed, were obtained by selecting five populations, namely UFU-DTOM7, UFU-DTOM10, UFU-DTOM5, UFU-DTOM9 and UFU-DTOM3. Selection of these populations resulted in an estimated 6% increase in the number of locules, transverse diameter, TD/LD ratio and average fruit weight (Figure 6). The selection gain for the remaining characteristics was
deemed less significant due to the low coefficient of genetic variation (data not shown). The selection index used here proved satisfactory in alfalfa (Vasconcelos et al., 2010), acai (Teixeira et al., 2012), passion fruit (Rosado et al., 2012), popcorn (Freitas et al., 2013), potato (Terres et al., 2015), soybean (Bizari et al., 2017) and tomato (Nick et al., 2013).

Figure 3: Fruit shape comparison: from left to right, respectively - donor parent, hybrid (F1 - donor versus recurrent parent), UFU-DTOM7 (BC$_1$F$_2$) and the recurrent parent.

Figure 4: Dendrogram of genetic divergence obtained the UPGMA method as a measure of dissimilarity. The letters DP indicate the donor parent.

Figure 5: Graphic representation of dissimilarity in BC$_1$F$_2$ dwarf tomato populations based on the Mahalanobis distance. The numbers indicate the UFU-DTOM BC$_1$F$_2$ dwarf tomato genotypes and DP the donor parent. The colors in the graph symbolize variability from 0 to 1, with 1 indicating the greatest genetic divergence.
The UFU-DTOM7 and UFU-DTOM10 populations stood out in both univariate analysis and estimated genetic gain. Additionally, dissimilarity measures (UPGMA and Tocher methods) confirmed their genetic dissimilarity in relation to the donor parent, confirming their agronomic potential. A second backcross is suggested in these populations to subsequently obtain hybrids from dwarf lines with round fruits.

**CONCLUSION**

The BC$_1$F$_2$ dwarf populations UFU-DTOM7 and UFU-DTOM10 were the most promising for developing inbred lines and, posteriorly, hybrids with round fruits and compact plants.

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