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Agronomic potential, pest resistance, and fruit quality in BC_1F_3 dwarf round tomato populationss

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Abstract

Combining broad pest resistance, high yield, and nutritional quality of fruit in the same hybrid is a challenge for round tomato breeders. Thus, development of a dwarf round tomato line for obtaining hybrids may be an excellent alternative for achieving such objectives. The aim of this study was to evaluate the agronomic potential, fruit quality, and pest resistance of BC_1F_3 dwarf round tomato populations. We evaluated 13 treatments and 4 replications. Treatments consisted of 10 BC_1F_3 dwarf populations originating from self-fertilization of dwarf populations selected in BC_1F_2, plus both parents (recurrent and donor) and a commercial hybrid. The traits evaluated were yield, number of fruit, mean weight, number of locules, shape, pulp thickness, longitudinal and transverse diameter of the fruit, precocity index, and soluble solids, β-carotene, lycopene, and acylsugar content. The data were analyzed through the means test. In general, there was an increase of up to 1035% in average fruit weight of the dwarf populations in relation to the donor parent. In addition, some dwarf populations had fruit with 49% more lycopene compared to the commercial hybrid, as well as greater acylsugar content in the leaves (273%). The UFU-DW22 dwarf population stood out with good agronomic potential, higher lycopene content in the fruit, and high acylsugar content, which provides pest resistance. To continue this research, a second backcross is suggested in order to obtain lines and, posteriorly, hybrids with round fruits and compact plants, pest resistance and nutritional quality of fruit.

Keywords: acylsugar, dwarf, lycopene, *Solanum lycopersicum*

Introduction

In Brazil, tomatoes are classified into five groups: "Minitomate, Salada, Caqui, Santa Cruz and Saladete" (Alvarenga, 2013). Out of Brazil, this tomato groups are known as cherry or grape, round, beefsteak, chonto, and saladette or roma, respectivelly. Among them, tomato from the round group type is the most sold for *in natura* consumption in Brazil. Guided by the interest of producers and consumers, breeding programs have invested in new technologies to boost the round tomato production.

In the last decades, the nutritional quality of tomato fruit was impaired through development of hybrids with fruit of uniform maturation and longer shelf life (Powell et al., 2012; Nguyen et al., 2014; Zsögön et al., 2017; Lupi et al., 2019). It has become essential to improve the flavor quality and also the content of antioxidant compounds in tomato fruit, such as lycopene and β-carotene, widely known for their activity in preventing cancer and heart diseases and protection of the immune system (Tian et al., 2016).

The development of genotypes with greater pest resistance is also fundamental. This can be achieved through introgression of genes belonging to wild species rich in allelochemicals, such as acylsugars. Acylsugar is mainly found in the glandular trichomes and promotes resistance to various pests by the mechanism of antixenosis (Maciel et al., 2018a, b; Peixoto et al., 2020). Combining broad resistance to pests and diseases, greater nutritional quality, and high yield in the same genetic material is the main challenge of breeders.

In this context, Finzi et al. (2017a) developed mini-tomato hybrids arising from the cross between the parent of a normal phenotype and a dwarf plant variety. The results were hybrids of normal phenotype with reduced internodes, a greater number of bunches per linear meter of stem, and, consequently, higher yield. In spite of the

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success obtained by the authors, the dwarf parent used in obtaining the hybrids has small fruit with an oblong shape (Maciel et al., 2015), which made its direct use in obtaining hybrids for the round segment unviable.

Thus, Finzi et al. (2020) performed the first backcross (BC_1F_2), aiming at the development of dwarf lines with round fruit. The authors observed an increase in average fruit weight and also modification in fruit shape. However, it is important to evaluate the progress in new BC_1F_3 populations originating from self-fertilization and selection in BC_1F_2. In addition, it is essential to check the nutritional quality of the fruit, resistance to pests, and the agronomic performance of BC_1F_3 populations that will be directed to the development of dwarf plant lines and, subsequently, hybrids of the round type fruit. Therefore, the aim of this study was to evaluate the agronomic potential, fruit quality, and pest resistance of BC_1F_3 dwarf round tomato populations.

Materials and Methods

The experiment was conducted from October 2019 to April 2020 at the Vegetable Crop Experimental Station of the Universidade Federal de Uberlândia (UFU), Campus Monte Carmelo, MG (18°42'43.19"S, 47°29'55.8"W, and altitude of 873 m). The plants were grown in an arch type greenhouse (7 × 21 m) covered with 150-micra transparent polyethylene film with an ultraviolet radiation inhibitor additive and lateral curtains of a white anti-aphid screen.

The genetic material evaluated consisted of 10 BC_1F_3 dwarf populations that were originated from self-fertilization of dwarf populations selected in BC_1F_2 by Finzi et al. (2020). The BC_1F_2 populations were obtained by backcrossing after hybridization of a pre-commercial homozygote line of round fruit with a dwarf line, UFU MC TOM1 (Maciel et al., 2015). Thus, in addition to the 10 BC_1F_3 dwarf populations, both parents (recurrent and donor) and a commercial hybrid (Paronset®) were used, totalizing 13 treatments. The BC_1F_3 populations and the parents belonged to the tomato germplasm bank of UFU. The recurrent parent and the commercial hybrid are characterized by their indeterminate growth habit and red round fruit. In contrast, UFU MC TOM1 is a homozygote line of dwarf size with indeterminate growth habit (Finzi et al., 2017b) and oblong mini-tomato type fruit (Maciel et al., 2015), which was used as a donor parent. Since expression of the dwarf phenotype is of recessive and monogenic origin (Maciel et al., 2015), the backcrosses were carried out for transfer of the recessive allele.

Seeds were sown in polystyrene trays (200 cell) on October 16, 2019. Seedlings were transplanted 58 days after sowing in a 5-liter capacity plastic pots. Commercial substrate of a coconut fiber base was used both in the trays and in the pots. Throughout the time of the experiment, crop treatments were performed as recommended for the tomato crop grown in a protected environment (Alvarenga, 2013). The recurrent parent and the commercial hybrid were oriented vertically with one stem in a cord training system.

A randomized block experimental design was used, with 13 treatments and four replications. The experimental plots consisted of six plants, distributed in double rows at a spacing of 0.3 × 0.3 m. A spacing of 0.8 m was used between the double rows (access lanes), for a total of 312 plants, in a greenhouse.

Tomatoes were harvested weekly from February 13 to April 23, 2020, for a total of nine harvests. The fruit from each experimental plot was harvested in the full maturity stage, and the following agronomic traits were evaluated:

Yield (kg plant^{-1}) (Y): ratio between the weight of fruit collected and the number of plants of the plot.

Number of fruits (tomatoes plant^{-1}) (NF): ratio between the total number of tomatoes of the plot and the number of plants of the plot.

Average fruit weight (Kg) (AFW): ratio between the weight of the tomato fruit collected from the plot and the number of tomatoes from the plot.

Fruit transverse diameter (cm) (TD): obtained with the assistance of a ruler after cutting the fruit horizontally in the middle, measuring its horizontal length. Then the mean of the diameter of all the tomatoes collected from the plot was calculated.

Fruit longitudinal diameter (cm) (LD): obtained with the aid of a ruler after cutting the fruit vertically in the middle, measuring its vertical length. Then the mean of the diameter of all the tomatoes collected from the plot was calculated.

Fruit shape (TD/LD): obtained by the ratio between the transverse diameter and longitudinal diameter (TD/LD). The recurrent parent and the commercial hybrid were used as references of the round segment to allow classification of the fruit.

Pulp thickness (cm) (PT): obtained with the assistance of a ruler after cutting the fruit vertically in the middle, measuring the length between the fruit peel and the beginning of the locule. Then the mean was calculated, considering all the tomatoes collected from the plot.

Number of locules (locules fruit^{-1}) (NL): obtained after cutting the fruit horizontally in the middle, counting the number of locules. Then the mean was calculated, considering all the tomatoes collected from the plot.

Total soluble solids content (°Brix) (TSS): obtained by the mean of all the fruit collected in the plot, using a portable digital refractometer (Atago PAL-1 3810).

Precocity index (%) (PI): ratio between the sum of the weight of all the fruit produced in the first five harvests and the total weight of all the fruit, multiplied by 100.

Lycopene (LC) and β-carotene (bCC) content (mg 100 mg^{-1}): Extraction of the fruit pigments was performed according to the methodology proposed by Nagata & Yamashita (1992), with adaptations. For evaluation of the carotenoids (β-carotene and lycopene), 3 ml of 100% acetone was added to tubes containing 1 g of the ground and homogenized fruit. To avoid oxidation of the carotenoids, the samples were incubated at 4°C in the absence of light for 48 hours. Two phases were separated, and an aliquot was removed from the upper phase for estimation of optical density at 450 nm and 470 nm in a spectrophotometer. The β-carotene content (βCC) and lycopene content (LC) were calculated according to Rodrigues-Amaya (2001) and Rodrigues-Amaya & Kimura (2004).

Acylsugar content (nmol cm^{-2} of leaf area): obtained at 69 days after transplanting, using a sample composed of eight leaf disks (equivalent to 4.2 cm^2) obtained from the upper third of each plant of the plot. Extraction and quantification followed the methodology proposed by Resende et al. (2002) and adapted by Maciel & Silva (2014). The tomato *Solanum pennellii* Correll (accession LA-716), rich in acylsugars (Maluf et al., 2010), was used only as a parameter for comparison.

After verification of assumptions with Levene's test for homogeneity of variances, Kolmogorov-Smirnov's test for residual normality, and Tukey's additivity test for block additivity), analysis of variance (F=0.05) was performed for the data, and the mean values were compared by the Scott-Knott test (p=0.05). The analyses and graphics were performed using the Sisvar software (Ferreira, 2011) and the R version 3.6.3 software (R Core Team, 2020).

Results and Discussion

In general, the genotypes differed in all the traits evaluated, except for the β-carotene content of the fruit. The yield of the commercial hybrid was ten times greater than the yield of the dwarf plants. This occurred, as expected, due to the phenotype of the dwarf plants being totally different from the normal plants, which can easily be seen in Figure 1 through the contrast between the size of the recurrent parent and the dwarf populations. Therefore, in this study, it will be more appropriate to emphasize the differences between dwarf populations and the dwarf parent. Furthermore, the main objective of the study was to determine the agronomic performance, the nutritional quality of the fruit, and the indirect resistance to pests of BC_1F_3 dwarf populations arising from self-fertilization of the BC_1F_2 populations that were selected by Finzi et al. (2020) for development of dwarf lines and, subsequently, hybrids from dwarf lines with round fruits.

The mean yield of the BC_1F_3 populations was 0.2 kg $plant^{-1}$, and they did not differ from each other (Table 1). However, they differed in regard to number of fruits and their average weight. The dwarf populations produced around 6 tomatoes $plant^{-1}$, except for the UFU-DW17 population, with an average of 16.9 tomatoes $plant^{-1}$. However, UFU-DW17 had lower values for average fruit weight, which explains its similar yield.

In general, the average fruit weight of the BC_1F_3 populations was at least three times greater than the average fruit weight of the donor parent. The UFU-DW22 population stood out, obtaining fruit with 1035% more weight. In the BC_1F_2 populations observed by Finzi et al. (2020), the greatest increase in average fruit weight was 341%. Although the experiments were performed in different environments, such differences between the performance of the BC_1F_3 and BC_1F_2 populations can be explained by advancement in the cycle of self-fertilizations, which change the genetic constitution, reducing the number of loci in heterozygosity and, consequently, the expressions of their traits.

The transverse diameter of the fruit of the BC_1F_3 populations was greater than that of the donor parent, exhibiting values from 3.1 cm to 4.4 cm, while the longitudinal diameter of the fruit in all the plants ranged from 3.0 cm to 5.2 cm. The relation between the diameters allows the classification of the fruit regarding its shape, determined in this study by the variable TD/LD. The UFU-DW12 and UFU-DW19 populations exhibited an oblong shape visually similar to the donor parent (Figure 1). In contrast, based on the statistical test of TD/LD, it is important to note that the UFU-DW4, UFU-DW8, UFU-DW18, and UFU-DW25 populations had fruit with the same shape as the recurrent parent. It should be emphasized that the fruit illustrated in Figure 1 is the fruit most representative of each treatment, and the diameter values might not coincide with their respective mean values shown in Table 1.

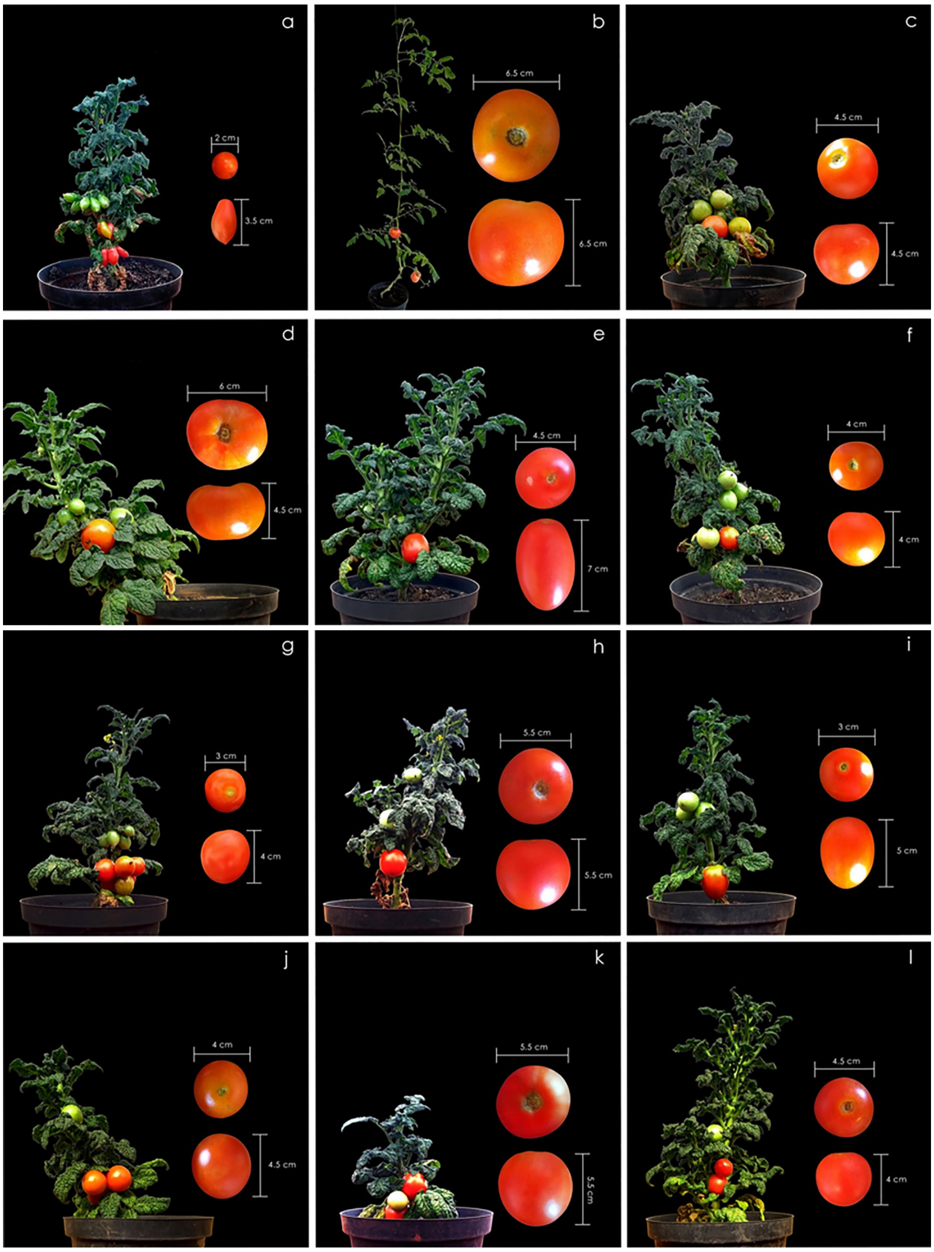

Figure 1. Comparison between the phenotype of the parents (donor and recurrent) and the BC_1F_3 dwarf populations. The fruit that is shown best represents each treatment. a: donor parent, b: recurrent parent, c: UFU-DW4, d: UFU-DW8. e: UFU-DW12, f: UFU-DW16, g: UFU-DW17, h: UFU-DW18, i: UFU-DW19, j: UFU-DW21, k: UFU-DW22, l: UFU-DW25.

Table 1. Agronomic traits evaluated in the BC_1F_3 dwarf tomato populations, recurrent parent, donor parent, and commercial hybrid.

Treatments	Y[1]	NF	AFW	TD	LD	DT/DL	PT	NL	PI
UFU-DW4	0.2 c	7.4 c	27.0 e	3.8 d	3.5 e	1.1 b	0.6 c	3.1 c	51.5 a
UFU-DW8	0.2 c	7.0 c	34.1 d	4.2 c	3.7 d	1.1 b	0.6 c	4.0 b	38.1 b
UFU-DW12	0.3 c	9.9 c	32.6 d	3.5 e	4.7 b	0.8 e	0.6 c	2.1 d	43.0 b
UFU-DW16	0.1 c	5.9 c	18.2 f	3.2 e	3.4 e	0.9 c	0.5 d	2.5 d	34.5 b
UFU-DW17	0.3 c	16.9 b	15.6 f	3.1 e	3.5 e	0.9 d	0.4 d	2.1 d	47.3 a
UFU-DW18	0.2 c	5.2 c	31.3 d	4.1 c	3.8 d	1.1 b	0.7 c	3.3 c	37.8 b
UFU-DW19	0.2 c	8.0 c	24.0 e	3.4 e	4.2 c	0.8 e	0.6 c	2.2 d	33.9 b
UFU-DW21	0.1 c	4.9 c	24.7 e	3.5 e	3.8 d	0.9 c	0.6 c	2.9 d	34.7 b
UFU-DW22	0.3 c	6.1 c	55.6 c	4.4 c	5.2 a	0.9 d	0.8 b	3.1 c	40.3 b
UFU-DW25	0.1 c	6.1 c	20.3 f	3.2 e	3.0 e	1.1 b	0.4 d	2.1 d	56.0 a
Donor parent	0.2 c	39.8 a	4.9 g	1.8 f	3.2 e	0.6 f	0.3 e	2.0 d	50.5 a
Recurrent parent	0.7 b	7.4 c	90.0 b	5.5 b	5.1 a	1.1 b	0.7 b	4.7 a	63.1 a
Commercial hybrid	2.1 a	20.2 b	103.3 a	6.1 a	4.9 b	1.2 a	0.9 a	3.3 c	53.6 a
KS[2]	0.1*	0.1*	0.1*	0.6*	0.1*	0.8*	0.1*	0.1*	0 .1*
F (Levene)	4.7	2.4*	3.6	0.9*	2.1*	1.4*	1.6*	3.3	2.3*
F (Additivity)	1.8*	0.3*	1.8*	5.2*	0.0*	0.6*	0.0*	0.0*	8.1
F (Anova)[3]	76.7	51.6	136.7	59.9	30.7	48.1	16.7	14.7	2.8
CV (%)	31.6	24.4	13.4	7.3	6.7	5.5	14.5	15.6	25.1

Y: yield (kg plant^{-1}), NF: number of fruits (tomatoes plant^{-1}), AFW: average fruit weight (g), 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}), PI: precocity index (%). [1]Mean values followed by different letters in the column differ from each other by the Scott-Knott test at 0.01 significance; [2]KS, F (Levene), F (Additivity): statistics of the Kolmogorov, Levene, and Tukey tests, respectively; values with asterisk (*) indicate residues with normal distribution, homogeneous variances, and additivity at 0.01 significance; [3] F (Anova): statistics of the Anova test.

In addition to shape, the pericarp thickness and the number of locules are important components that have an impact on the quality of tomato fruit. These traits are related to firmness of the fruit, such that those with thicker pericarps and with a smaller number of locules can be firmer and better support long-distance transport (Siddiqui et al., 2015). All the dwarf plants had fruit with greater pericarp thickness than the fruit of the donor parent. The increase of 167% observed in pericarp thickness of UFU-DW22 in relation to the donor parent made the fruit of UFU-DW22 comparable to the fruit of the recurrent parent. In addition, the fruit of most of the dwarf plants had few locules, in a manner similar to the donor parent, except for UFU-DW4, UFU-DW8, UFU-DW18, and UFU-DW22.

The earlier harvest trait also varied among genotypes. From the first to fifth harvest of the fruit, the donor parent, recurrent parent, and commercial hybrid had already achieved more than half of their total production, with precocity indices of 50.5%, 63.1%, and 53.6%, respectively. The UFU-DW4, UFU-DW17, and UFU-DW25 populations also had precocity indices statistically similar to those already cited. However, the precocity of the other dwarf plants in the same period was 38%, on average. High yield and earlier harvest are two of the main traits sought after by tomato producers (Maciel et al., 2018).

In addition to agronomic performance, the nutritional quality of the fruit is fundamental. The lycopene and β-carotene content constitute a large part of the nutritional value of the tomato fruit (Siddiqui et al., 2015). The concentration of β-carotene in the fruit did not vary among the genotypes, with a mean value of 2.53 mg.100 mg^{-1}(Figure 2). In contrast, the lycopene concentration of the fruit ranged from 1.91 to 2.94 mg.100 mg^{-1}. The populations UFU-DW8, UFU-DW16, UFU-DW17, UFU-DW21, UFU-DW22, and UFU-DW25 exhibited the highest values of lycopene, with values 49%, 44%, 30%, 42%, 49%, and 41% superior to the commercial hybrid, respectively. The concentrations of lycopene in the fruit may depend on the genotype, the stage of maturity, and the interaction of the genotype with the environment (Londoño-Giraldo et al., 2020). In this study, the difference in the lycopene concentrations is explained by the genotype.

Another trait very important for the nutritional quality of the fruit is the soluble solids content. This is directly related to flavor because the higher the content, the greater the expression of sweet flavor in the fruit, which is preferred by consumers (Maciel et al., 2015). The fruit in this study had soluble solids greater than or equal to 4.0 °Brix, and the BC_1F_3 populations had values that ranged from 4.9 to 5.7, with an average difference of 1.3° Brix between the fruit of the commercial hybrid and the recurrent parent (Figure 2).

It should be emphasized that, in recent decades, researchers have sought to develop genotypes with fruit of greater nutritional quality and flavor in relation to the genotypes on the market. In this context, Zsögön et al. (2018) modified the morphology of the wild tomato *Solanum pimpinellifolium* L., as well as the size, number, and nutritional quality of tomatoes (fruit) through gene editing by the CRISPR-Cas9 technique. With these new genotypes, the authors reported an increase of 500% in the lycopene content of the fruit compared to the *S. lycopersicum* that is widely grown. In addition, Lupi et al. (2019) created a genetically modified (transgenic) tomato line with overexpression of the gene *SlGLK2*, which impedes uniform maturation but increases the nutritional quality of the fruit. The authors obtained an increase in the level of tocopherol (vitamin E) in ripe fruit and higher soluble solids content, possibly through regulation of the genes that codify enzymes of sugar metabolism. In the present study, achieving higher lycopene and soluble solids content was made possible by the genetic variation of the genotypes, without requiring more complex biotechnological techniques that involve gene manipulation.

Figure 2. β-carotene (bCC), Lycopene (LC) and Total soluble solids (TSS) content in the BC_1F_3 dwarf tomato populations, recurrent parent, donor parent, and commercial hybrid. Mean values followed by different letters differ from each other by the Scott-Knott test at 0.05 significance.

In addition to the challenge of improving the nutritional quality of the fruit, another considerable challenge faced by breeders is the development of genotypes with pest resistance. Acylsugar is an allelochemical present in the glandular trichomes that promotes resistance to various pests by the antixenosis mechanism (Lucini et al., 2015; Gruber, 2017; Maciel et al., 2018a, b; Peixoto et al., 2020). The allelochemical has high correlation with resistance of the plants to red spider mite, silverleaf whitefly, leafminer fly and tomato leafminer (Lucini et al., 2015; Rakha et al., 2017; Maciel et al., 2018a; Peixoto et al., 2019; Peixoto et al., 2020). In this study, the wild tomato *S.pennellii* had high contents of the acylsugar allelochemical (mean of 36.66 nmol cm^{-2} of leaf area), as was expected (Figure 3). However, a novel and very important result was observed for the tomato crop: the donor parent also obtained high acylsugar content in the leaves (mean of 32.61 nmol cm^{-2} of leaf area), which did not differ statistically from *S. pennellii*. This shows that the donor parent of this study also has potential to transfer resistance genes to its progeny, just as *S.pennellii*, which is used in breeding programs to develop tomato genotypes with pest resistance. That is why high amounts of the allelochemical were also found in the dwarf populations UFU-DW21 and UFU-DW22 (29.89 and 30.18 nmol cm^{-2} of leaf area, respectively). In addition, even not exhibiting high content of the allelochemical as in the plants cited above, the other dwarf populations were still superior to the commercial hybrid and the recurrent parent. The increase in the acylsugar content of the dwarf populations ranged from 101% (UFU-DW18) to 273% (UFU-DW22) in comparison with the mean of the commercial hybrid and recurrent parent.

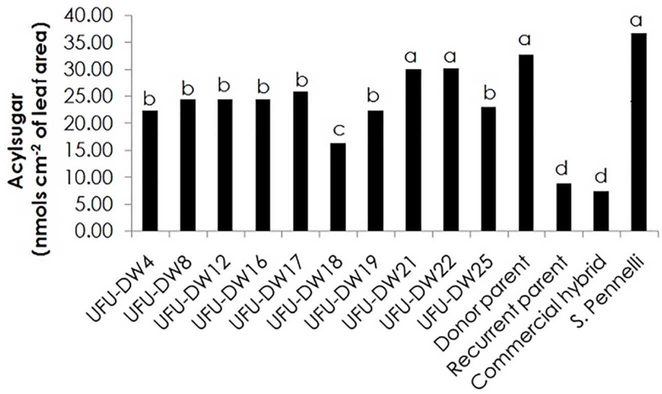

Figure 3. Acylsugar content (nmol cm^{-2} of leaf area) in BC_1F_3 dwarf tomato populations, *S.pennellii* (LA-716 accession), recurrent and donor parents, and the commercial hybrid. Means followed by different letters differ according to the Scott-Knott test at 0.05 significance.

In general, the BC_1F_3 dwarf populations exhibited agronomic potential and considerable progress in relation to the BC_1F_2 generation (Finzi et al., 2020), as well as higher content of lycopene in the fruit and indirect resistance to pests compared to the hybrids currently on the market. The UFU-DW22 population stood out, with greater average fruit weight, as well as lycopene and acylsugar content. Results show that the use of dwarf lines with round-type fruit coming from the dwarf parent of this study provides various advantages for obtaining hybrids beyond the increase in yield reported by Finzi et al. (2017a). To proceed in future studies with the BC_1F_3 dwarf plants, a second backcross is suggested to obtain lines and, subsequently, hybrids from dwarf lines with round fruits.

Conclusions

The dwarf population UFU-DW22 has agronomic potential, higher lycopene content in the fruit, and high acylsugar content, which confers pest resistance.

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