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Scientific Article

Introgression of dwarfing genes into tomato fruit through backcrossing aiming at salad-type background

Introgressão de genes de nanismo em tomateiro por retrocruzamento visando fruto do tipo salada

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ABSTRACT - This study investigated the introgression of dwarfing genes via tomato populations through successive backcrosses, aiming to improve salad-type fruit quality and agronomic traits. Hybridization between the recurrent parent (UFU-57) and the dwarf donor parent (UFU MC TOM 1) was followed by three generations of backcrossing (BC1, BC2, BC3). The methodological steps included hybridization, selection of dwarf plants, and evaluation of agronomic traits and fruit quality. Significant improvements were observed in agronomic traits and nutritional quality of fruits, with UFU-DTOM 4#4-11-1 (BC3) showing the most substantial increase in fruit mass, achieving an 816% increase compared to the dwarf donor parent. The results indicate the effectiveness of backcrossing in restoring salad-type fruit traits and enhancing fruit weight, pulp thickness, and nutritional content such as β-carotene and lycopene. The conclusion highlights the importance of the third backcrossing generation in developing dwarf tomato lines with superior traits for the salad fruit market segment.

Keywords: Backcross. Tomato. Plant Dwarfism.

RESUMO - Este estudo investiga a introgressão de genes de nanismo em populações de tomate por meio de retrocruzamentos sucessivos, visando melhorar a qualidade dos frutos do tipo salada e as características agronômicas. A hibridização entre o genitor recorrente (UFU-57) e o genitor doador anão (UFU MC TOM 1) foi seguida por três gerações de retrocruzamento (BC1, BC2, BC3). As etapas metodológicas incluíram hibridização, seleção de plantas anãs e avaliação das características agronômicas e da qualidade dos frutos. Foram observadas melhorias significativas nas características agronômicas e na qualidade nutricional dos frutos, com UFU-DTOM 4#4-11-1 (BC3) apresentando o aumento mais substancial na massa do fruto, alcançando um aumento de 816% em comparação com o genitor doador anão. Os resultados indicam a eficácia do retrocruzamento na restauração das características dos frutos do tipo salada e no aumento da massa do fruto, espessura da polpa e conteúdo nutricional, como β-caroteno e licopeno. A conclusão destaca a importância da terceira geração de retrocruzamento no desenvolvimento de linhas de tomate anão com características superiores para o mercado de frutos do tipo salada.

Palavras-chave: Retrocruzamento. Tomateiro. Nanismo em Plantas.

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INTRODUCTION

Tomato (*Solanum lycopersicum*) is a cornerstone of global agriculture, celebrated for its high yield and widespread consumption (FAOSTAT, 2024). Their versatility, evident in their consumption across diverse culinary forms from sauces and preserves to fresh consumption underscores the need for cultivars tailored to meet varied market demands. The growth habits of tomato plants, whether determinate, semi-determinate, or indeterminate, delineate distinct production chains, each catering to specific end uses (ZHU et al., 2023).

The quest for enhancing tomato yield has led to the emergence of hybrid utilization as a promising strategy, facilitating increased yield potentials (INGALLINA et al., 2020). Currently, a predominant trend is observed in developing tomato hybrids characterized by reduced internode lengths, thus enabling maximal fruit production within constrained spatial parameters (ZSÖGÖN et al., 2017). This reduction in internode length can be orchestrated by strategically integrating dwarfism genes utilizing advanced genetic improvement techniques (PANTHEE; GARDNER, 2013).

The utilization of dwarf plants through plant breeding represents a key strategy in various crops, aiming to develop more compact and productive plant varieties (WANG et al., 2023). Plant height, a pivotal trait, is intricately regulated by phytohormone interactions and internode length (LIU et al., 2020). Genetic factors such as gene D, integral to brassinosteroid synthesis, and mutations at loci such as d play key roles in modulating plant height by influencing internode length (MARTI et al., 2006). Additionally, mutants like Elongated Internode (EI) and tomato Internode elongated-1 (tie-1) contribute to internode elongation, while others like procera (pro), short internode (si), and dwarf (d) influence internode length through diverse mechanisms (KWON et al., 2020).

The exploration of dwarfism in tomatoes remains relatively nascent (SUN et al., 2019). However, the feasibility of incorporating dwarfism genes into this crop using the dwarf UFU MC TOM1 lineage represents a promising avenue, offering the potential for internode reduction (MACIEL; SILVA; FERNANDES, 2015). Hybrids resulting from crosses between this mini-tomato lineage (♂ dwarf) and conventional counterparts (♀ normal size) have demonstrated notable agronomic advantages (FINZI et al., 2017). Leveraging backcrossing techniques facilitates the selection of desired genetic backgrounds, which is essential for optimizing traits (GOMES et al., 2021).

Given the scarcity of tomato dwarf germplasm, developing dwarf lines bearing Salad-type fruits holds significant importance, enabling the exploration of this technology within this market segment. Consequently, acquiring and characterizing a Salad-type dwarf tomato germplasm exhibiting high agronomic potential, fruit quality, and pest resistance lay the foundation for cultivating promising genotypes, thereby bolstering tomato breeding programs.

The primary objective of this study is to evaluate the agronomic potential, fruit quality, and acylsugar levels of dwarf populations derived from the first, second, and third backcrosses with conventional tomatoes. Additionally, the study aims to identify the most promising candidates for further line development of salad-type tomatoes.

MATERIAL AND METHODS

Study area and plant material

The hybridization and backcrossing stages were conducted between January 2019 and August 2022 at the Vegetable Experimental Station of the Federal University of Uberlândia (UFU), Campus of Monte Carmelo, MG (18° 42'43.19" S, 47°29'55.8" W, altitude of 873 m). The dwarf tomato plant populations utilized in this study belong to the germplasm bank of the Federal University of Uberlândia.

Cultivation conditions

The seeds were sown in polyethylene trays (200 cells) filled with a commercial substrate based on coconut fiber. Seedling production occurred in a hoop-style greenhouse (7 x 21 m), enclosed with white anti-aphid mesh on the sides and 150-micron UV-resistant transparent polyethylene film on the roof.

Transplanting was conducted into 5-liter plastic pots containing the same substrate as the sowing after 30 days after sowing (DAS). The experiment was conducted in a twin-arch roof-type greenhouse (14 x 48 m), 4 m high, with side screens made of anti-aphid mesh and a transparent polyethylene cover of 200 microns, UV resistant. Cultural practices were conducted according to recommendations for tomato cultivation.

Hybridization and backcrossing

Initially, hybridization was performed between UFU-57♀ and UFU MC TOM 1♂. UFU-57 was employed as the female parent, characterized by homozygosity, pre-commercial status, normal size (dwarf gene, DD), indeterminate growth habit (SPSP), and salad-type agronomic traits (recurrent parent = UFU-57). UFU MC TOM 1 was used as the male parent, characterized by dwarf stature (dd), indeterminate growth habit (SPSP), and mini-tomato-type fruits (donor parent) (FINZI et al., 2017).

Following the F1 generation, the first backcrossing (BC1) was conducted, and subsequently, the self-pollination (BC1F2 generation). Dwarf plants were selected from the BC1F2 generation, and the second backcrossing, BC2F1, was conducted, followed by self-pollination BC2F2 (BC2). Dwarf plants from the BC2F2 generation were selected, and the third backcrossing, BC3F1, was conducted, followed by self-pollination BC3F2 (BC3). In the BC1F2, BC2F2, and BC3F2 generations, only dwarf plants with salad-type genetic backgrounds were selected.

Experimental Design

For the experiment, five dwarf BC3F2 populations with salad-type backgrounds (UFU-DTOM 21#2-1-1, UFU-DTOM 19#1-3-1, UFU-DTOM 19#1-4-1, UFU-DTOM 19#1-4-2, UFU-DTOM 4#4-11-1), five dwarf BC2F2 populations with salad-type backgrounds (UFU-DTOM 21#2-1, UFU-DTOM 19#1-3, UFU-DTOM 19#1-4, UFU-DTOM 19#1-5, UFU-DTOM 4#4-11), and three dwarf BC1F2 populations with salad-type backgrounds (UFU-DTOM 19#1, UFU-DTOM 4#4, UFU-DTOM 21#2) were utilized. Additionally, the recurrent parent (UFU-57), donor parent (UFU MC TOM 1), and a commercial control (Paronset® hybrid) were included, totaling sixteen treatments. For a comparison of variables related to indirect pest resistance, the wild accession *Solanum pennellii* was selected. Moreover, the cultivar Santa Clara and the wild accession *Solanum pennellii* were used as standards for lower and higher levels of acylsugars in leaflets compared to the backcrossing populations (Figure 1).

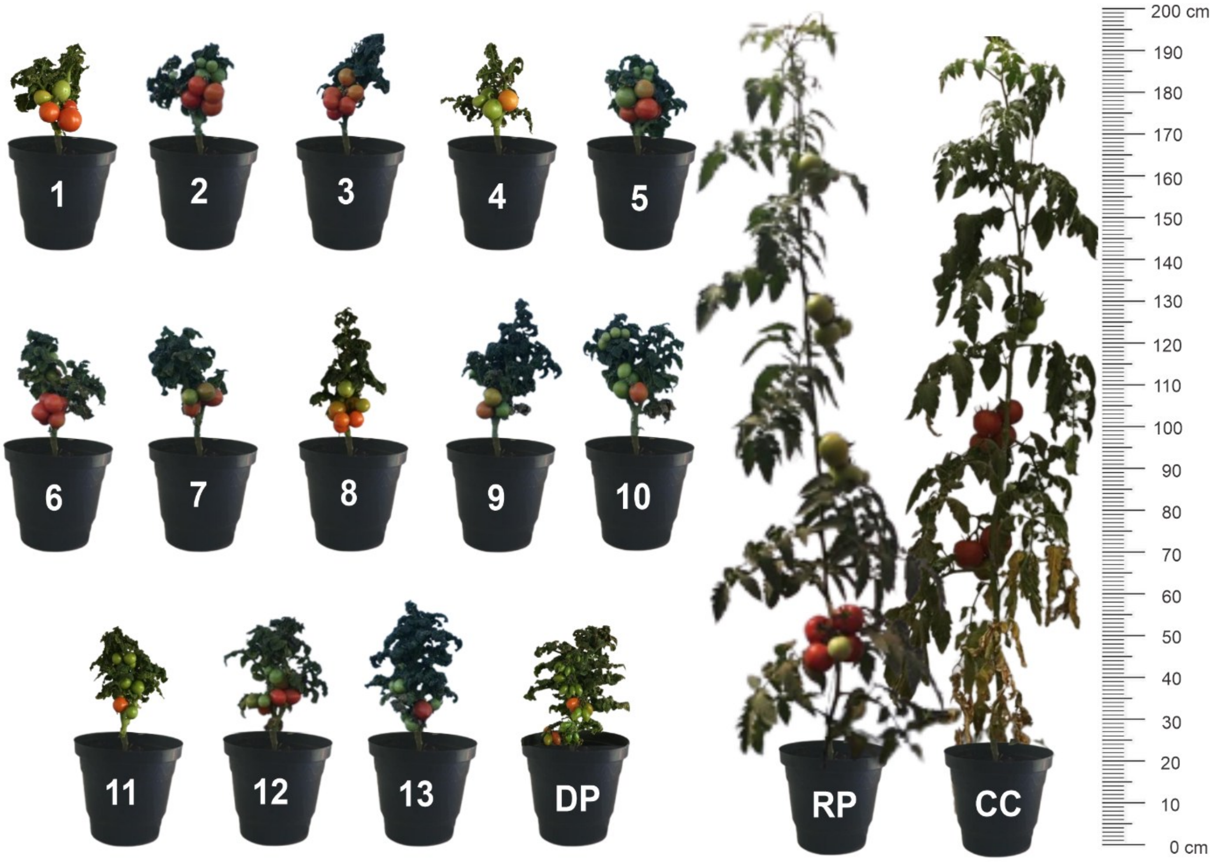

Figure 1. BC3 generation (1: UFU-DTOM 21#2-1-1; 2: UFU-DTOM 19#1-3-1; 3: UFU-DTOM 19#1-4-1; 4: UFU-DTOM 19#1-4-2; 5: UFU-DTOM 4#4-11-1); BC2 generation (6: UFU-DTOM 21#2-1; 7: UFU-DTOM 19#1-3; 8: UFU-DTOM 19#1-4: 9: UFU-DTOM 19#1-5; 10: UFU -DTOM 4#4-11); BC1 generation (11: UFU-DTOM 19#1; 12: UFU-DTOM 4#4; 13: UFU-DTOM 21#2); recurrent parent (RP); donor parent (DP); commercial control (CC).

Experimental design

The experiment was conducted in a randomized complete block design (RCBD) with four replications. Each experimental plot consisted of six plants arranged in double rows with a spacing of 0.3 x 0.3 m and 0.8 m between double rows. Treatments were evaluated for agronomic traits, fruit quality, and quantification of acylsugar content in leaflets (pest resistance).

Agronomic trait evaluation

Harvests were performed after the onset of fruit ripening. Fruits from each experimental plot were harvested at full maturity to evaluate agronomic traits. The harvested fruits from each plot were counted and weighed, and the average fruit weight (FW) was determined in grams. Subsequently, fifteen fruits from each plot were sampled and analyzed for the following parameters: fruit length (FL) - measured from the peduncle scar to the blossom end of the fruit; fruit diameter (FD) - measured in the transverse direction of the cut fruit; fruit shape (FS) - determined by the ratio between fruit length and fruit diameter; pulp thickness (PT) - determined as the greatest distance of the fruit mesocarp; number of locules (NL) - determined by directly counting of locules in the fruit. Plant height (PH) was determined based on the shoot apical meristem. Internode length (IL) was determined as the ratio between plant height and the number of nodes on the plant measured at the end of the crop cycle.

Regarding fruit quality, the following traits were evaluated: pH, titratable acidity, soluble solids content, and carotenoids (β-carotene and lycopene).

The soluble solids content, expressed in °Brix, was measured with a portable digital refractometer (Atago PAL-13810).

Pigments were extracted from the fruits. Carotenoids (β-carotene and lycopene) were evaluated by adding 1.0 g of homogenized tomato pulp and peel to 3 mL of 80% acetone solvent, stored in test tubes. To prevent carotenoid oxidation, the samples were placed in a refrigerator at 4°C for 48 hours in the dark. Two phases formed, and the supernatant was removed, forming an aliquot for measuring optical density at 450 and 470 nm wavelengths using a spectrophotometer. The concentrations of β-carotene (βC) and lycopene (LC) were estimated.

The quantification of acylsugars in the leaflets for indirect determination of pest resistance was conducted 90 days after sowing through a sample composed of eight leaf disks (corresponding to 4.2 cm^2). Leaflets from the upper third of the plants were collected and placed in test tubes, followed by extraction and determination.

Statistical analysis

In this study, the data were submitted to the normality test of residuals using the Shapiro-Wilk test ($p < 0.01$). The homogeneity of variances was analyzed by the Oneill Mathew test ($p < 0.01$), and for block additivity, the Tukey test was performed ($p < 0.01$). Subsequently, an analysis of variance was conducted using the F test ($\alpha = 0.05$). Means were grouped using the Scott-Knott test ($\alpha = 0.05$) and compared using the Dunnett test ($\alpha = 0.05$), where the donor parent (UFU MC TOM 1, dwarf plant) was considered the control to highlight improvements after each backcross. Additionally, genetic parameters were evaluated: genotypic determination coefficients (h^2) and the ratio between genetic and environmental coefficient of variation (CV_g/CV_e). Genetic dissimilarity among populations was obtained from the generalized Mahalanobis distance matrix. Genetic diversity was represented by a heat-map and dendrogram generated from the minimum and maximum distances, analyzed using R software (2022).

Selection Gain Estimation

Only dwarf phenotype traits were analyzed for selection gain estimation. For selection gain estimates, 30.7% of the population was selected. The selection criterion was based on the magnitude of genetic distance through an ideotype. For the genotype-ideotype distance index, ideal values were established as the highest means among the evaluated traits except for height and internode length with lower means. Dwarf plant populations were classified according to Mahalanobis distance (D2) concerning the ideotype, with those showing the smallest distances to this ideotype considered more favorable. All analyses were performed using the GENES software integrated with R and Matlab software (CRUZ, 2016).

RESULTS AND DISCUSSION

The dwarf Salad-type tomato populations, the commercial control (Paronset® hybrid), the recurrent parent UFU-57, and the donor parent (UFU MC TOM1) differed statistically in all agronomic traits, indicating variability among treatments (F-test, $\alpha = 0.05$). As expected, the recurrent parent and the commercial hybrid Paronset® showed higher means for most agronomic traits (Table 1).

Comparing the dwarf donor parent (UFU MC TOM1) to the dwarf plants from the first, second, and third backcrosses, there was an increase in fruit mass by 390%, 539%, and 816%, respectively. It should be considered that mini-tomato fruits are small, unlike salad-type tomatoes, which can weigh up to 500 g, making this increase in size essential to make the fruits suitable for the salad market segment.

Table 1. Mean values of agronomic traits evaluated in dwarf tomato populations across the first, second, and third backcross generations.

Genotypes	Generation	FW	FL	FD	FS	PF	NL	PH	IL
UFU-DTOM 21#2-1-1	BC3	31.62 c*	3.90 b*	3.70 e*	1.06 b*	2.59 c	5.16 c*	35.13 c	1.83 c*
UFU-DTOM 19#1-3-1	BC3	34.79 c*	3.90 b*	4.00 d*	0.98 c*	3.07 b*	5.14 c*	36.88 c	1.73 c*
UFU-DTOM 19#1-4-1	BC3	29.43 d*	3.79 b*	3.48 f*	1.09 b*	2.69 c*	3.49 e*	37.00 c	1.60 c
UFU-DTOM 19#1-4-2	BC3	34.06 c*	4.12 b*	3.76 e*	1.10 b*	2.38 c	4.48 d*	33.75 c	1.53 d
UFU-DTOM 4#4-11-1	BC3	43.34 b*	3.95 b*	4.29 c*	0.93 c*	3.44 b*	5.17 c*	31.38 c	1.50 d
UFU-DTOM 21#2-1	BC2	28.82 d*	3.99 b*	3.67 e*	1.09 b*	2.57 c	4.88 c*	32.88 c	1.68 c*
UFU-DTOM 19#1-3	BC2	30.26 d*	4.19 b*	3.59 e*	1.17 b*	2.91 b*	4.47 d*	33.75 c	1.80 c*
UFU-DTOM 19#1-4	BC2	26.85 d*	3.83 b*	3.54 f*	1.09 b*	2.91 b*	4.34 d*	32.75 c	1.58 c
UFU-DTOM 19#1-5	BC2	19.86 e*	3.52 c	3.32 f*	1.06 b*	2.78 c*	3.49 e*	33.25 c	1.70 c*
UFU-DTOM 4#4-11	BC2	28.77 d*	3.82 b*	4.05 d*	0.95 c*	3.07 b*	4.59 d*	26.00 d	1.43 d
UFU-DTOM 19#1	BC1	17.85 e*	3.66 c	3.50 f*	1.05 b*	3.32 b*	4.28 d*	25.50 d	1.35 d
UFU-DTOM 4#4	BC1	23.22 e*	3.61 c	3.63 e*	1.00 c*	2.97 b*	3.98 d*	30.88 c	1.68 c*
UFU-DTOM 21#2	BC1	20.31 e*	3.73 c	3.41 f*	1.09 b*	2.10 d	4.96 c*	34.63 c	1.48 d
UFU-57	RP	93.34 a*	5.02 a*	5.69 b*	0.88 d*	4.35 a*	6.51 b*	208.38 a*	6.43 a*
CHECK	HB	94.82 a*	4.92 a*	6.22 a*	0.79 e*	3.13 b*	7.46 a*	170.75 b*	6.05b*
UFU MC TOM1	DP	4.73 f	3.35 c	1.92 g	1.75 a	2.00 d	2.24 f	27.88 d	1.28 d
Mean		35.12	3.95	3.86	1.06	2.89	4.66	51.92	2.16
CV (%)		7.70	5.34	4.52	5.51	10.50	8.70	8.42	8.48
DMS Dunnett		5.65	0.44	0.36	0.12	0.63	0.84	9.14	0.38
h^2		99.69	94.84	99.18	97.97	92.60	97.12	99.83	99.67
CVg/Cve		9.06	2.06	5.51	3.47	1.76	2.90	12.39	8.70

FW: fruit weight (g); FL: fruit length (cm); FD: fruit diameter (cm); FS: fruit shape; PF: pulp thickness (cm); NL: number of locules (locule per fruit); PH: plant height (cm); IL: internode length (cm); BC1: first backcross; BC2: second backcross; BC3: third backcross; RP: recurrent parent; DP: donor parent; HB: commercial control (Paronset® hybrid); CV (%): coefficient of variation; h^2: genotypic determination coefficients; CVg/CVe: ratio between the genetic and environmental coefficients of variation. Means followed by different letters in the column belong to different groups by the Scott-Knott test at 0.05. The asterisk in the column indicates the difference from the dwarf donor parent UFU MC TOM 1 by the Dunnett test at the 0.05 probability level.

The increase in fruit mass became more pronounced with each successive backcross. Therefore, the third backcross showed superior results, with 80% of the populations (BC3) outperforming those from the second backcross (BC2). The population UFU-DTOM 4#4-11-1 stood out, with fruits averaging over 43 g.

Finzi et al. (2020) achieved a 341% increase in fruit mass in populations of dwarf salad-type tomatoes with just one backcross. In our present study, it reached 390%. The increase reached 816% from the third backcross, indicating that performing three backcrosses is interesting in recovering the desired traits. In this study, the main goal is to transfer genes related to fruit mass between the recurrent parent and dwarf populations. The dwarf plant populations from the third backcross showed greater fruit mass increases, enabling larger fruit production (Table 1). After transferring genes of interest, backcrossing allowed the restoration of agronomic traits, mainly related to yield. In the first (BC1), second (BC2), and third (BC3) generations of backcrosses, populations on average retained 75%, 87.5%, and 93.75% of the recurrent parent's genome, respectively.

The superiority of the (BC1), (BC2), and (BC3) dwarf tomato populations over the donor parent regarding average fruit weight, fruit length, fruit diameter, and pulp thickness, which are traits related to fruit mass (VAZQUEZ et al., 2022), agrees with the ability to restore the salad segment fruit pattern from the recurrent parent (UFU-57) in the dwarf populations. Studies by Finzi et al. (2020) and Gomes et al. (2021) evaluating tomatoes from the salad, Santa Cruz, and saladette segments, respectively, showed similar results, demonstrating the backcrossing procedure's efficiency in increasing fruit weight.

The fruit shape, defined by the ratio between transversal and longitudinal diameters, is a critical trait in tomato breeding and can impact consumer preference. Our study observed distinct fruit shapes among the parental lines. The donor parent (UFU MC TOM 1), belonging to the cherry tomato segment, exhibited an average fruit shape index of 1.75, indicative of an elongated fruit shape a characteristic known to be governed by recessive monogenic inheritance. Conversely, the other dwarf plant populations, including the recurrent parent (UFU-57) and the commercial control, displayed a fruit shape index close to 1, typical of the salad segment. This discrepancy was statistically significant compared to the donor parent.

In a recent study by Zhu et al. (2023), the cloning and functional characterization of fs8.1 were reported. This gene controls the elongated fruit shape and crush resistance of machine-harvestable processing tomatoes. The mutation in fs8.1 reduces the inhibitory effects on cell proliferation in the ovary wall, leading to elongated fruits with improved compression resistance. This research serves as an attempt to redesign the tomato fruit shape for mechanized production, offering a potential avenue for introducing the beneficial allele into fresh-market tomatoes without compromising quality, thus facilitating mechanical harvesting. While our study did not directly investigate the fs8.1 gene, understanding its role in fruit shape and resistance traits highlights the broader context of genetic improvements in tomatoes and underscores the potential of targeted breeding strategies to enhance fruit quality and agronomic performance.

Despite identifying the gene, our study, which did not aim to identify genes, observed the effect of introgression through backcrossing on altering fruit shape, bringing it closer to the current market standards.

On average, the donor parent (UFU MC TOM 1) produces cherry tomato-type fruits with two locules; only the (BC1) population UFU-DTOM 21#2 maintained these traits. The other populations were statistically different, supporting the hypothesis that backcrossing increases the number of locules, reaching the salad-type fruit standard traits represented by the recurrent parent (UFU-57) and commercial control.

The dwarf plants resulting from backcrossing showed greater pulp thickness than the donor parent (UFU MC TOM 1). Three dwarf populations stood out: UFU-DTOM 21#2-1-1, UFU-DTOM 19#1-3-1, and UFU-DTOM 4#4-11-1, belonging to generation BC3; UFU-DTOM 21#2-1 from generation BC2; and UFU-DTOM 21#2. Therefore, it can be observed that the third backcrossing stood out regarding the increase in pulp thickness, reaching an average increase of 130% compared to the donor parent, with an average thickness of 5.17 mm in the UFU-DTOM 4#4-11-1 population. Fruits with greater pulp thickness (PF) exhibit higher firmness, thus better handling and transportation resistance (SIDDIQUI; AYALA-ZAVALA; DHUA, 2015). Regarding plant height (PH) and internode length (IL), all populations of dwarf plants were inferior to the recurrent parent (UFU-57) and commercial control (Hybrid Paronset®). The reduction in internode length directly influences the reduction in plant height (LIU et al., 2020). Breeding programs aiming for plants with compact architecture, through short internodes, enable higher yield and better cultural management (RAJENDRAN et al., 2022). Thus, developing dwarf lines with Salad-type fruits is essential, as the germplasm of dwarf plants is scarce, presenting an obstacle to obtaining future hybrids with more productive and compact plants using this technology. Finzi et al. (2017) reported a higher yield of tomatoes with compact architecture.

In the present study, significant differences were observed for fruit quality-related traits, such as fruit acidity (pH), titratable acidity (TTA), soluble solids content (SS - ° Brix), β-carotene (βC), and lycopene (LI) content (F-test, $p \leq 0.05$) (Table 2). Currently, tomato breeding programs aim to develop materials that are both productive and have superior fruit quality compared to those available in the market (LONDOÑO-GIRALDO et al., 2021).

In fruit acidity (pH), the recurrent parent (UFU-57) exhibited a higher pH, with an average value of 4.45. All samples showed a pH below 4.5, which is desirable to ensure that pathogenic and spoilage microorganisms face greater difficulty in developing. pH and TTA are quality parameters related to the taste of the fruits (WATI; PAHLAWAN; MASITHOH, 2021). Titratable acidity expresses the amount of organic acid in the fruits and astringency, directly influencing the flavor of the fruit, allowing for a choice between more acidic or sweeter fruits according to the cultural traits and preferences of each region. The variability among dwarf tomato populations concerning TTA allows for selecting plants with sweeter or more acidic fruits according to preference.

Table 2. Means of fruit quality traits evaluated in populations of dwarf tomato plants in the first, second, and third backcross generations.

Genotypes	Generation	pH	TTA	SS (°Brix)	βC	LI
UFU-DTOM 21#2-1-1	BC3	4.05 c*	1.32 a*	6.48 c*	12.34 a	10.48 b
UFU-DTOM 19#1-3-1	BC3	4.07 c*	1.10 b*	6.30 d*	10.76 b	10.04 b
UFU-DTOM 19#1-4-1	BC3	4.20 b	0.96 d	6.55 c*	13.25 a*	11.70 a*
UFU-DTOM 19#1-4-2	BC3	4.05 c*	1.03 c*	6.55 c*	10.92 b	8.90 c
UFU-DTOM 4#4-11-1	BC3	4.05 c*	0.94 d	6.00 d*	9.80 c	7.38 d
UFU-DTOM 21#2-1	BC2	4.14 c	1.15 b*	6.50 c*	9.77 c	7.33 d*
UFU-DTOM 19#1-3	BC2	4.10 c	1.09 b*	6.53 c*	8.97 c*	7.76 d
UFU-DTOM 19#1-4	BC2	4.03 c*	0.83 e*	6.18 d*	9.49 c*	10.77 b
UFU-DTOM 19#1-5	BC2	4.21 b	0.85 e	6.50 c*	9.88 c	11.92 a*
UFU-DTOM 4#4-11	BC2	4.22 b	0.91 d	6.15 d*	9.48 c*	6.47 d*
UFU-DTOM 19#1	BC1	4.06 c*	1.03 c*	6.50 c*	9.64 c*	10.36 b
UFU-DTOM 4#4	BC1	3.95 d*	0.86 e	6.28 d*	9.41 c*	8.38 c
UFU-DTOM 21#2	BC1	4.07 c*	1.07 c*	6.43 c*	9.15 c*	7.56 d
UFU-57	RP	4.45 a*	0.88 e	6.53 c*	12.84 a*	12.86 a*
Control	HB	4.08 c*	0.89 e	6.95 b*	10.80 b	8.79 c
UFU MC TOM1	DP	4.19 b	0.93 d	7.85 a	11.05 b	9.33 c
Average		4.12	0.99	6.51	10.41	9.37
CV (%)		1.11	4.42	3.11	6.03	10.03
DMS Dunnett		0.09	0.09	1.32	1.96	0.42
h^2		96.02	97.26	94.06	94.37	93.70
CV_g/Cv_e		2.45	2.98	1.99	2.04	1.92

pH: fruit acidity; TTA: titratable acidity; SS: soluble solids content (°Brix); βC: β-carotene content (mg/100mg); LI: lycopene content (mg/100mg); BC1: first backcross; BC2: second backcross; BC3: third backcross; RP: recurrent parent; DP: donor parent; HB: commercial control (Paronset® hybrid); CV (%): coefficient of variation; h2: genotypic determination coefficient; CV_g/CV_e: ratio between genetic and environmental variation coefficients. Means followed by different letters in the column belong to different groups by the Scott-Knott test at 0.05. The asterisk in the column indicates the difference from the dwarf donor parent UFU MC TOM 1 by the Dunnett test at the 0.05 probability level.

Notably, populations derived from backcrossing endeavors exhibited soluble solids content averaging between 6 and 6.53 °Brix. Although our study did not delve into the intricate interplay of citric acid with the sugar/organic acid ratio, recent research has shed light on its potential dominance in shaping this aspect of tomato fruit chemistry. Li et al. (2021) underscored the pivotal role of citric acid, alongside L-malic acid, in influencing fruit Brix, emphasizing the contributions of genes implicated in carbohydrate (CHO) and tricarboxylic acid (TCA) metabolism to this phenomenon. This trait can potentially enhance the attractiveness of existing tomato cultivars by meeting the increasing consumer preference for sweeter fruits. The soluble solids content of fruits is intricately linked to their taste profile; higher concentrations are associated with sweeter flavors, rendering them more desirable to consumers (CAMMARERI et al., 2023). Soluble solids content exceeding 3 °Brix is conventionally deemed acceptable for fresh tomato consumption (SCHWARZ et al., 2013).

Regarding β-carotene (βC) and lycopene (LI) content, peak values were concurrently observed in both traits for the recurrent parent UFU-57 and the population UFU-DTOM 19#1-4-1 (BC3). Additionally, UFU-DTOM 21#2-1-1 (BC3) exhibited superior levels of β-carotene, while UFU-DTOM 19#1-5 (BC2) demonstrated heightened lycopene content.

We can observe that the recurrent parent, through backcrossing, not only recovered agronomic traits of interest but also contributed to improving the nutritional quality of the fruits. The levels of β-carotene and lycopene are directly related to the nutritional value of tomato fruits (SIDDIQUI; AYALA-ZAVALA; DHUA, 2015). Thus, developing the dwarf tomato population UFU-DTOM 19#1-4-1 through backcrossing is interesting for obtaining biofortified fruits in Salad-type hybrids.

The agronomic and fruit quality traits evaluated showed high coefficients of genotypic determination (h^2), considered high (BHERING et al., 2012), indicating that there is genetic variability among the evaluated populations, allowing for gains through the selection of the best populations (Table 1). Among the evaluated traits, the ratio CV_g/CV_e was greater than 1. The CVg/CVe ratio is valuable information for breeding; when the value reaches 1 or higher, the situation is beneficial for selection (LEITE et al., 2016). Therefore, phenotypic selection based on these traits is favorable. Improving the nutritional quality of fruits while simultaneously developing genotypes that are more productive and resistant to pests is a significant challenge. Acylsugars are allelochemicals found in glandular trichomes and promote resistance to various pests through the mechanism of antixenosis (DIAS et al., 2021). The wild accession *Solanum pennellii* has high levels of acylsugars and is used as a comparison parameter. The donor parents UFU MC TOM 1 and *S. pennellii* expressed higher averages concerning acylsugar levels (Figure 2). All dwarf tomato populations (BC1) and (BC2), as well as 40% of the populations (BC3), were superior to the recurrent parent UFU -57, the commercial control, and the Santa Clara cultivar in terms of acylsugar content. According to Dias et al. (2021), segregation regarding acylsugar content is observed in advanced tomato populations. Gomes et al. (2021) observed dwarf tomato populations with higher acylsugar levels.

In addition to genetic strategies aimed at enhancing tomato plant traits such as compactness and yield, the focus on enhancing resistance to pests is pivotal. Compounds like acylsugars have emerged as crucial elements in this pursuit. Acylsugars are specialized metabolites found in the glandular trichomes of tomato plants, known for their insect-repellent properties (GASPARINI et al., 2023). These compounds act as a natural defense mechanism, deterring pests such as aphids, whiteflies, and spider mites (DIAS et al., 2021). Therefore, integrating genetic traits associated with increased acylsugar production into tomato breeding programs could bolster plant resilience against insect infestations, contributing to sustainable crop management practices. This approach aligns with the overarching goal of developing robust tomato cultivars capable of withstanding biotic stresses while maintaining high yield and fruit quality. While our study has not directly assessed the damage caused by insects, the presence of high acylsugars indicates a likely enhancement of resistance to insects.

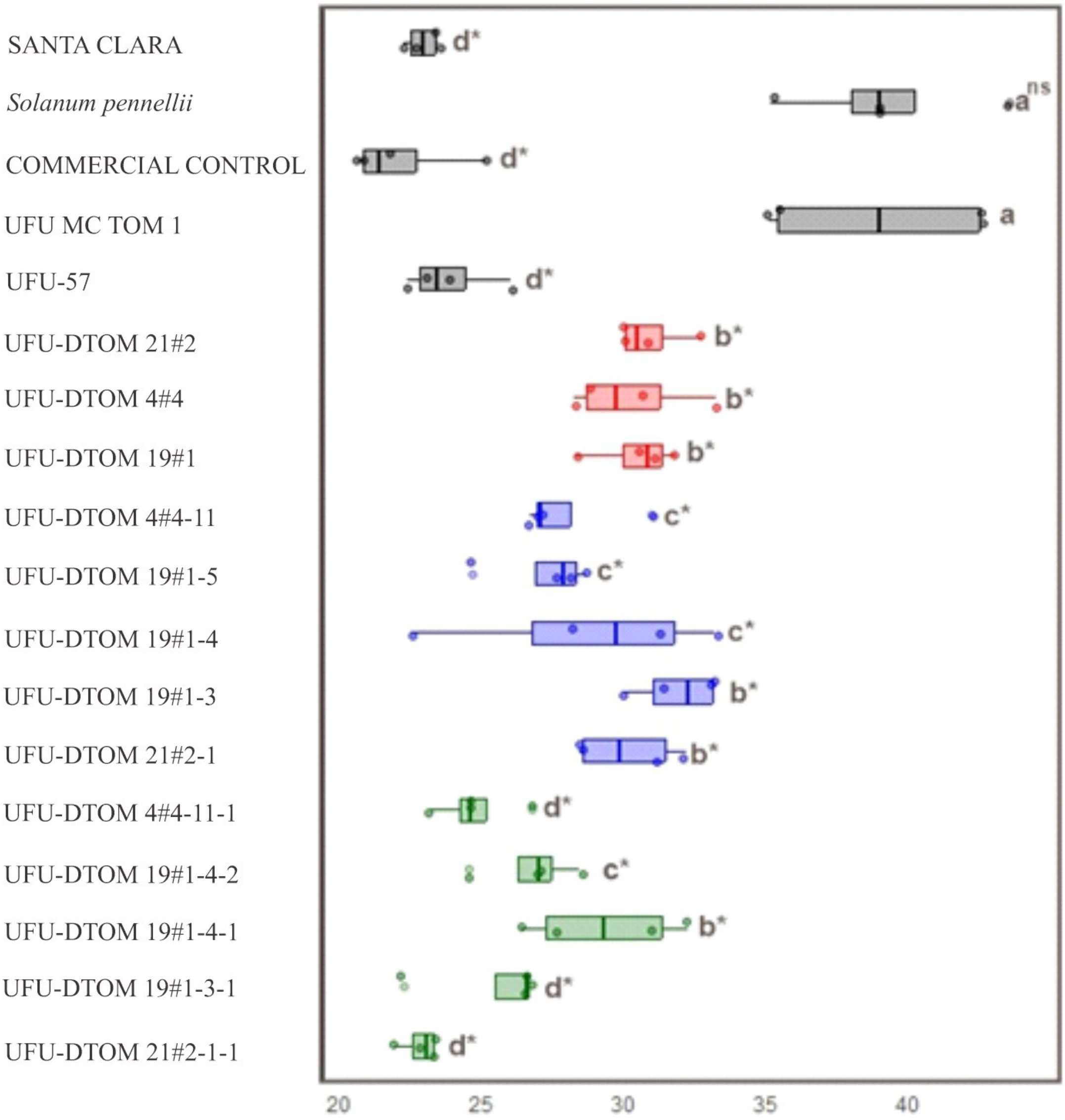

Figure 2. Comparative boxplot regarding the mean values of acylsugar levels. Means followed by different letters in the column belong to different groups by the Scott-Knott test at 0.05 significance level. Legend: Green: third backcrossing (BC3); Blue: second backcrossing (BC2); Red: first backcrossing (BC1); Black (Control).

The visualization of genetic dissimilarity using a heat map with a dendrogram obtained by the UPGMA method from the generalized Mahalanobis matrix contributed to the selection of superior dwarf tomato populations. Through visual analysis of the dendrogram, a cutoff line was established at 22.31%, a criterion defined considering the abrupt change in level (CRUZ, 2016), forming seven distinct groups due to genetic variability (Figure 3).

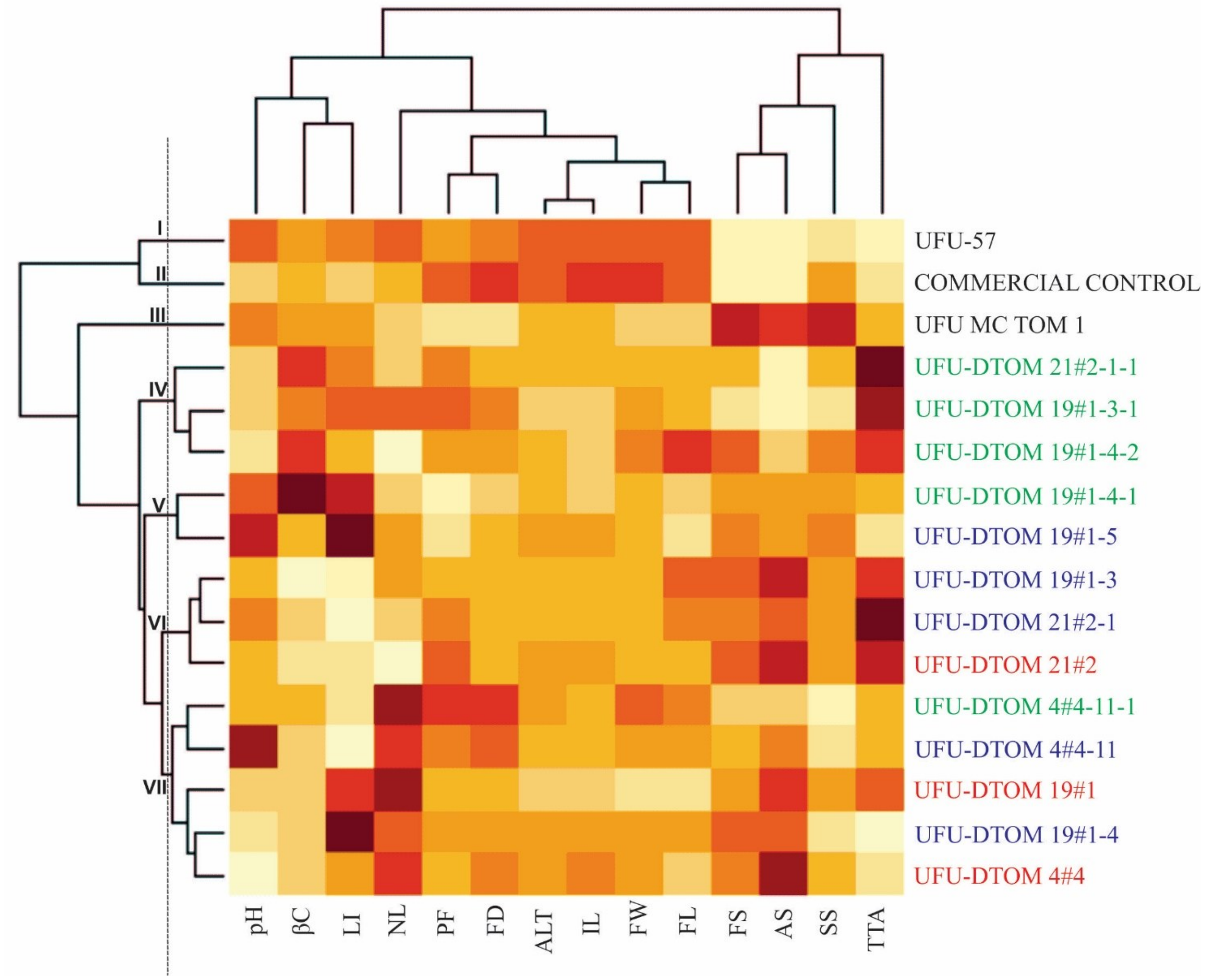

Figure 3. Dendrogram and Heat Map of genotypes regarding the traits of average fruit weight (FW), fruit length (FL), fruit diameter (FD), fruit shape (FS), pulp thickness (PF), number of locules (NL), titratable acidity (TTA), soluble solids content (SS), β-carotene (βC), Lycopene (LI), and acylsugar (AS). Legend: Green: third backcross (BC3); Blue: second backcross (BC2); Red: first backcross (BC1).

Group I was represented by the recurrent parent (UFU-57). Group II by commercial control. Group III was formed by the donor parent (UFU MC TOM 1), Group IV by populations UFU-DTOM 21#2-1-1 (BC3), UFU-DTOM 19#1-3-1 (BC3), and UFU-DTOM 19#1-4-2 (BC3). Group V by populations UFU-DTOM 19#1-4-1 (BC3) and UFU-DTOM 19#1-5 (BC2). Populations UFU-DTOM 19#1-3 (BC2), UFU-DTOM 21#2-1 (BC2), and UFU-DTOM 21#2 formed Group VI. Group VII was constituted by populations UFU-DTOM 4#4-11-1 (BC3), UFU-DTOM 4#4-11 (BC2), UFU-DTOM 19#1 (BC1), UFU-DTOM 19#1-4 (BC2), and UFU-DTOM 4#4 (BC1). The fact that the donor parent (UFU MC TOM 1) did not group with the dwarf tomato populations from the salad segment demonstrates that the backcrossing provided increments in the dwarf populations, linked with their differentiation.

From the dendrogram, it was observed that Group IV was formed only by populations from the third backcrossing, and this group spans populations (BC3) with smaller Mahalanobis distances. Group VII presented only population UFU-DTOM 4#4-11-1 from the third backcrossing among the others. However, it can be verified in the heat map that the average fruit weight of UFU-DTOM 4#4-11-1 stands out compared to the other populations from two and three backcrossings. Thus, it is observed that the population UFU-DTOM 4#4-11-1 (BC3) in another group presents genetic variability compared to the other populations (BC3). UFU-DTOM 4#4-11-1 (BC3) presents a higher average fruit weight in Group VII and among all the dwarf populations; therefore, this population (BC3) in another group presenting superior traits allows for greater options for selecting superior populations.

The UPGMA clustering method efficiently represented the genetic dissimilarity among genotypes, with a cophenetic correlation coefficient of 0.96. This method also evaluated the variability among tomato genotypes in other studies (OLIVEIRA et al., 2022). Dissimilarity measures and the genotype-ideotype index allow identifying and selection individuals with desirable traits. Based on the genotype-ideotype distance (Figure 4), superior dwarf tomato populations were selected.

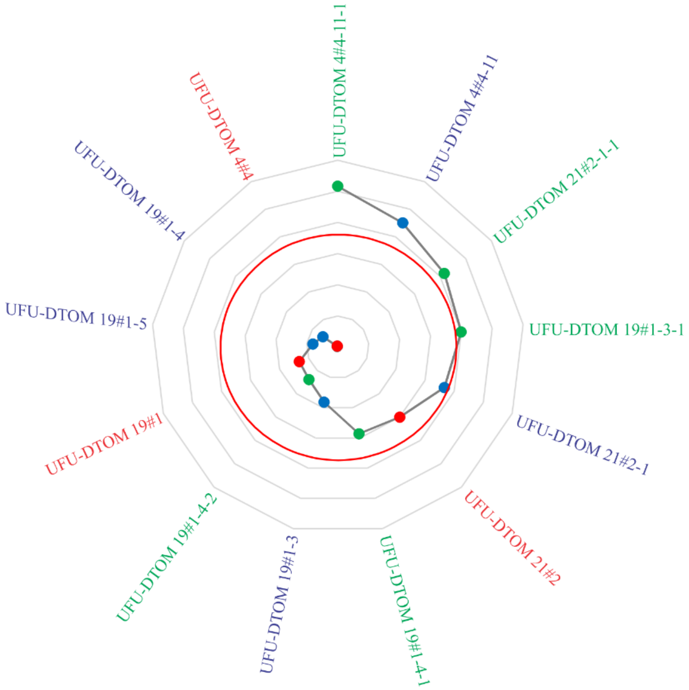

Figure 4. Genotype-ideotype distance for selection of superior dwarf populations after backcrossing. Legend: Green: third backcrossing (BC3); Blue: second backcrossing (BC2); Red: first backcrossing (BC1).

According to the genotype-ideotype distance index, populations UFU-DTOM 4#4-11-1 (BC3), UFU-DTOM 4#4-11 (BC2), UFU-DTOM 21#2-1-1 (BC3), and UFU-DTOM 19#1-3-1 (BC3) were selected. Thus, the ideotype-genotype index defined predominantly selected populations from the third backcrossing. The other populations (BC3), (BC2), and (BC1) showed greater distances and intermediate values. This methodology has been used in breeding studies to select genotypes for higher yields, waterlogging tolerance, and biotic stress (KLEIN et al., 2023).

Oliveira et al. (2022) evaluated dwarf tomato populations up to the second backcrossing and selected populations obtained through the second backcrossing. This study verified that conducting one more backcrossing can be efficient, as most of the selected populations are from the third backcrossing. The selected populations are important for tomato genetic improvement, leading to an enhanced dwarf tomato germplasm, being promising for the development of introgression lines and, subsequently, hybrids with additional advantages, compactness, yield, and pest resistance (FINZI et al., 2017), and belonging to the Salad segment.

CONCLUSION

The backcrossing method proved to be effective in obtaining improvements in agronomic traits and fruit nutritional quality. Populations UFU-DTOM 4#4-11-1 (BC3), UFU-DTOM 21#2-1-1 (BC3), UFU-DTOM 19#1-3-1 (BC3), and UFU-DTOM 4#4-11 (BC2) emerged as promising. Among them, UFU-DTOM 4#4-11-1 (BC3) stood out, as it was not only selected according to the genotype-ideotype index but also showed the highest increase in fruit mass, one of the most important traits for bringing the fruit closer to the Salad segment standard. Additionally, these populations exhibited higher levels of acylsugars, contributing to enhanced pest resistance. The predominant selection of Salad-type dwarf tomato populations from the third backcrossing (BC3) underscores the importance of conducting the third backcrossing to obtain introgression lines of dwarf tomatoes with Salad-type fruit traits.

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