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Dwarf Tomato Plants Allow for Managing Agronomic Yield Gains with Fruit Quality and Pest Resistance through Backcrossing

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Abstract: Increased productivity, nutritional quality, and pest resistance have been primary breeding goals. However, managing such increases in a genotype is challenging. In this context, gene introgression using dwarf plants is an alternative; however, there are no dwarf Santa Cruz tomato varieties for direct use in breeding programs. Therefore, the objective of this study was to improve fruit quality and pest resistance through successive backcrossing of dwarf Santa Cruz tomato populations with agronomic potential. Six and 13 dwarf tomato populations obtained from the first and second backcrossing, respectively, the donor parent, and the commercial cultivar 'Santa Clara' as the check, totalling 21 treatments, were evaluated. Univariate analysis and computational intelligence were used to evaluate the best genotypes. All agronomic variables showed significant and progressive increases after the first and second backcrossing. The highlighted BC_2 populations were Sci#16.1-2, Sci#25.1,1-2, Sci#25.1,2-2, Sci#3.1,1-2, Sci#3.1,2-2, Sci#8.3,1-2, and Sci#8.3,2-2, with significant increases in mean fruit weight, pulp thickness, fruit length and diameter, and acyl sugar content. The selected BC_2 populations can be used as male parents to obtain normal hybrids to achieve increased productivity, nutritional quality, and a broader spectrum of pest resistance owing to the presence of acyl sugars in the leaflets.

Keywords: *Solanum lycopersicum* L.; computational intelligence; dwarf plants; allelochemicals; nutritional quality

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1. Introduction

Tomatoes (*Solanum lycopersicum* L.) are of great socioeconomic relevance, being one of the most cultivated vegetables worldwide. In 2020, Brazil produced approximately 4.0 million tons of tomatoes, accounting for 2.1% of global production [1]. Tomatoes exhibit great diversity and have therefore been classified in the Brazilian market into the following groups: Minitomato, Salad, Caqui, Santa Cruz, and Saladete [2]. Of these, Santa Cruz tomatoes stand out for their greater postharvest durability, higher yield potential, and superior organoleptic characteristics than conventional long-shelf-life tomatoes grown in the country [3].

Field-grown tomato is considered a great financial risk, as the production cost is substantial because of the high susceptibility of this crop to biotic and abiotic stresses [4]. Therefore, higher yield [5] and better adaptation to diverse consumption demands have been the basis of tomato improvement programs.

Increasing tomato productivity involves the enhancement of quantitative inheritance [6,7]. However, these enhancements become limited in terms of the type of gene

action for productivity. In Minitomato, the use of a dwarf plant as the male parent has been demonstrated as a very successful alternative to enhance the productivity and nutritional quality of hybrids [8]. This can be achieved by crossing a dwarf parent with a normal-sized parent [9]. Hybrids from a dwarf genitor possess a short internode, resulting in more compact plants with more clusters per linear meter of stem and, consequently, greater productivity and fruit quality [8,10]. Additionally, dwarf plants are rich in allelochemical acyl sugars [11], which are secondary compounds that provide a broad spectrum of pest resistance [12].

Despite the potential of this strategy, there are no dwarf Santa Cruz populations for immediate hybrid production. Therefore, backcrossing is essential. The objective of this breeding method is to introduce a target trait, called the recurrent genitor, into highly adapted elite genotypes [13]. One of the major challenges in this approach is the management of yield increase while maintaining fruit quality and pest resistance. In this context, the use of dwarf plants in breeding can be an important alternative, as reported in Minitomato [8]; however, there has been limited research on Santa Cruz tomatoes. To this end, the objective of the present study was to achieve fruit quality and pest resistance by successively backcrossing dwarf Santa Cruz tomato populations with agronomic potential.

2. Materials and Methods

2.1. Materials and Experimental Design

The experiment was conducted from October 2019 to March 2020 at the Experimental Vegetable Station of the Federal University of Uberlândia (UFU), Monte Carmelo Campus, MG, Brazil (18°42′43.19″ S, 47°29′55.8″ W; 873 m a.s.l.). The plants were grown in an arch-type greenhouse (7 × 21 m^2; 4 m ceiling height), covered with a 150-micron transparent polyethylene film with additives against ultraviolet rays and lateral anti-insect white mesh curtains.

The genetic material evaluated included six dwarf tomato populations obtained from the first backcross (BC_1), 13 dwarf tomato populations obtained from the second backcross (BC_2), the donor parent (DP), and the commercial cultivar 'Santa Clara' as the check, totalling 21 treatments. BC_1 and BC_2 populations were obtained after hybridization between a homozygous pre-commercial strain of Santa Cruz fruits (UFU-TOM-Mother-2) and the dwarf strain UFU MC TOM1 [9]. The wild *Solanum pennellii* accession used in the present study was included to compare resistance to pest arthropods (acyl sugar content). The commercial check is characterized by indeterminate growth habits and Santa Cruz-type red fruits. UFU MC TOM1 used as the DP is a homozygous dwarf strain with indeterminate growth habits and oblong Minitomato-type fruits [8,9]. As the expression of the dwarf phenotype is recessive and monogenic [9], backcrossing was performed to transfer the recessive allele.

Sowing was performed in polystyrene trays (200 cells) on 3 October 2019. The seedlings were transplanted to 5 L plastic pots 36 days after sowing (DAS). Commercial coconut fiber substrate was used both in the trays and pots. Conventional treatments were applied throughout the experiment, as recommended for tomato crops in a protected environment [2].

The experiment followed a randomized block design with 21 treatments and four replicates. The experimental plots comprised six plants distributed in double rows with a spacing of 0.3 × 0.3 m. The spacing between the double rows was 0.8 m, totaling 504 plants.

2.2. Sample Collection and Evaluation

Harvests were performed weekly from 3 January to 6 March 2020, totaling 10 harvests. Fruits from each experimental plot were harvested at the full maturity stage, and the following agronomic traits were evaluated:

Mean fruit weight (g) (MFW): MFW was calculated as the ratio of the mass in grams and the number of fruits harvested from a plot.

Total soluble solids (°Brix) (TSS): After harvest, pulp juice from the fruits were analyzed for TSS content using a digital portable refractometer (Atago PAL-1 3810).

Fruit diameter (cm) (FD): The fruit was cut in half vertically and measured along the horizontal axis with a ruler.

Fruit length (cm) (FL): The fruit was cut in half vertically and measured along the vertical axis with a ruler.

Fruit shape (FS): FS was calculated as the ratio of transversal and longitudinal diameters (TD/LD). The commercial check was used as the reference of the Santa Cruz segment for fruit classification.

Pulp thickness (cm) (PT): The fruit was cut in half vertically, and the length between its skin and the beginning of the lobule was measured with a ruler.

Number of locules (locules fruit^{-1}) (NL): The fruit was cut in half horizontally and the locules were counted.

Internode length (cm) (IL): IL was calculated as [(plant height/number of nodes)] on two central plants of the plot.

Acyl sugar (AS) content (nmols·cm^{-2} of leaflet): At 75 days after sowing, AS content was measured using a sample comprising eight leaf disks (equivalent to 4.2 cm^2) from each plant in the plot, in triplicate. The disks were collected from leaflets in the upper third of the plants and placed in test tubes. Extraction and quantification followed a previously described protocol with minor modifications [14].

Regarding nutritional characteristics, β-carotene (CC) and lycopene (LC) were extracted and quantified in triplicate according to previously described procedures [15–17]. Briefly, fruit pulp was ground and 1 g was subsequently conditioned in a glass flask containing 3 mL of 100% acetone (Danâmida Ltd.a, Indaiatuba, SP, Brazil). The samples were protected from light and maintained at 8 °C for 48 h. The supernatant was then evaluated spectrophotometrically (Tecnal Ltd., Piracicaba, SP, Brazil). The absorbance of CC and LC was recorded at 450 and 470 nm, respectively.

2.3. Statistical Analyses

Statistical assumptions were verified by analyses for normality (Kolmogorov–Smirnov test), homogeneity (O'Neill and Mathews test), and additivity (Tukey's test for nonadditivity). Analysis of variance was performed using the F-test ($\alpha = 0.05$), and mean values were compared using the Scott–Knott test ($\alpha = 0.05$). Computational intelligence was applied to analyze genetic similarity using Kohonen self-organizing maps (SOMs).

Typically, SOM learning is achieved in three stages. Initially, synaptic weights are assigned to different neurons, followed by a competition process. The set of genetic values of each genotype is allocated to the neuron that best represents it (winning neuron). This allocation begins the comparison phase, with the winning neuron determining the approximation of the other neurons from similarity. Finally, the neurons establish the neighboring neurons and move on to the adaptation stage, characterized by adjustment of the weight of each variable.

Network training included 5000 epochs per iteration. The adopted model was validated using different configurations for the number of neurons. The combinations were tested with varying numbers of rows (2–5) and columns (2–5). Thus, the combination that best represented the genetic similarity of the analyzed genotypes was the one with four rows and four columns (16 organizational neurons) with a radius pattern equal to one, hexagonal neighborhood topology, feedforward network architecture with one input layer (medium) and one output layer, and Euclidean distance activation function. All analyses were performed using GENES integrated with R and MATLAB [18].

3. Results

Significant increases in fruit traits were observed in the backcross generations indicating the effectiveness of the backcrossing method in obtaining dwarf tomato genotypes with fruits belonging to the Santa Cruz segment (Figure 1).

Figure 1. Phenotypic comparison between donor parent (DP) and backcross (BC_1 and BC_2) populations. 1, donor parent; 2, Sci#6.1,1-2; 3, Sci#6.1,2-2; 4, Sci#6.1,3-2; 5, Sci#16.2-2; 6, Sci#16.1-2; 7, Sci#25.1,1-2; 8, Sci#25.1,2-2; 9, Sci#3.1,1-2; 10, Sci#3.1,2-2; 11, Sci#8.2-2; 12, Sci#20.4-2; 13, Sci#8.3,1-2; 14, Sci#8.3,2-2; 15, Sci#6.1.2,5; 16, Sci#16.2.1,3; 17, Sci#3.1.1; 18, Sci#8.2.1; 19, Sci#20.4.1; 20, Sci#8.3,1.2.

3.1. Agronomic Performance of Different Backcross Generations

Both BC_2 and BC_1 populations differed from the DP population in terms of all agronomic traits evaluated (Table 1) and presented a marked increase in all traits after the successive backcrossing cycles.

Dwarf populations of both backcross generations (BC_1 and BC_2) were superior to the DP population in terms of MFW, PT, and FD. Regarding MFW, the highlighted populations included Sci#6.1,2-2, Sci#16.1-2, Sci#25.1,1-2, Sci#25.1,2-2, Sci#3.1,1-2, Sci#3.1,2-2, Sci#8.2-2, Sci#20.4-2, Sci#8.3,1-2, and Sci#8.3,2-2 from BC_2 and population Sci#8.2.1 from BC_1, with mean weight exceeding 25 g. Regarding PT—an important characteristic for fruit quality—the highlighted populations included Sci#16.1-2, Sci#25.1,1-2, Sci#25.1,2-2, Sci#3.1,1-2, Sci#3.1,2-2, Sci#20.4-2, Sci#8.3,1-2, and Sci#8.3,2-2 from BC_2 and Sci#6.1.2,5 and Sci#8.2.1 from BC_1, with mean thickness between 0.50 (Sci#16.1-2) and 0.56 (Sci#8.3,2-2) cm.

Regarding FD, the highlighted populations included Sci#6.1,2-2, Sci#16.1-2, Sci#25.1,1-2, Sci#25.1,2-2, Sci#3.1,1-2, Sci#3.1,2-2, Sci#8.3,1-2, and Sci#8.3,2-2 from BC_2 and Sci#8.2.1 from BC_1. Regarding FL, which together with FD represents fruit size, only BC_2 populations Sci#16.1-2, Sci#25.1,1-2, Sci#25.1,2-2, Sci#3.1,1-2, Sci#3.1,2-2, Sci#8.2-2, Sci#20.4-2, Sci#8.3,1-2, and Sci#8.3,2-2 registered significant increases. Fruits belonging to the Santa Cruz segment are characterized by an FL/FD value close to 1 [19]. Accordingly, all BC_1 populations were classified as belonging to the Santa Cruz segment. Moreover, the BC_2 populations (Sci#6.1,1-2, Sci#6.1,2-2, Sci#16.1-2, Sci#20.4-2, Sci#8.3,1-2, and Sci#8.3,2-2) produced fruits characteristic to the Santa Cruz segment, whereas the remaining populations produced intermediate, slightly oblong fruits. All populations studied showed reduced NL ($\leq$3.0), characterizing firmer fruits.

Table 1. Morphological and agronomic characteristics and pest resistance (acyl sugar content) evaluated in backcross populations BC_2 and BC_1 of dwarf tomato, donor parent, check' Santa Clara', and wild *Solanum pennellii* genotypes.

Genotype	Generation	MFW	PT	FL	FD	FS	NL	IL	AS
Donor parent	-	3.61 ^{c}	0.20 ^{d}	2.95 ^{b}	1.61 ^{c}	1.83 ^{d}	2.00 ^{a}	1.26 ^{a}	36.28 ^{b}
Santa Clara	-	26.59 ^{a}	0.55 ^{a}	3.84 ^{b}	3.62 ^{a}	1.05 ^{a}	2.05 ^{a}	7.27 ^{b}	25.25 ^{b}
Sci#6.1,1-2	BC_2	20.32 ^{b}	0.47 ^{b}	3.77 ^{b}	3.26 ^{b}	1.15 ^{a}	2.75 ^{c}	1.99 ^{a}	36.63 ^{b}
Sci#6.1,2-2	BC_2	25.73 ^{a}	0.49 ^{b}	3.90 ^{b}	3.51 ^{a}	1.10 ^{a}	2.91 ^{c}	1.70 ^{a}	37.83 ^{b}
Sci#6.1,3-2	BC_2	20.36 ^{b}	0.48 ^{b}	3.98 ^{b}	3.09 ^{b}	1.29 ^{b}	2.44 ^{b}	2.33 ^{a}	47.81 ^{a}
Sci#16.2-2	BC_2	17.41 ^{b}	0.40 ^{c}	3.71 ^{b}	2.87 ^{b}	1.29 ^{b}	2.97 ^{c}	1.84 ^{a}	41.80 ^{a}
Sci#16.1-2	BC_2	24.11 ^{a}	0.50 ^{a}	4.16 ^{a}	3.37 ^{a}	1.23 ^{a}	2.86 ^{c}	1.95 ^{a}	36.55 ^{b}
Sci#25.1,1-2	BC_2	30.81 ^{a}	0.54 ^{a}	4.67 ^{a}	3.45 ^{a}	1.35 ^{b}	3.00 ^{c}	1.70 ^{a}	35.30 ^{b}
Sci#25.1,2-2	BC_2	28.82 ^{a}	0.52 ^{a}	4.64 ^{a}	3.48 ^{a}	1.33 ^{b}	2.66 ^{c}	1.54 ^{a}	37.35 ^{b}
Sci#3.1,1-2	BC_2	26.12 ^{a}	0.51 ^{a}	4.62 ^{a}	3.35 ^{a}	1.39 ^{b}	2.67 ^{c}	2.03 ^{a}	32.19 ^{b}
Sci#3.1,2-2	BC_2	31.59 ^{a}	0.54 ^{a}	4.81 ^{a}	3.81 ^{a}	1.28 ^{b}	2.80 ^{c}	1.60 ^{a}	41.10 ^{a}
Sci#8.2-2	BC_2	25.01 ^{a}	0.47 ^{b}	4.63 ^{a}	2.92 ^{b}	1.58 ^{c}	2.60 ^{c}	1.97 ^{a}	43.16 ^{a}
Sci#20.4-2	BC_2	32.72 ^{a}	0.54 ^{a}	4.76 ^{a}	3.31 ^{b}	1.44 ^{b}	2.77 ^{c}	1.65 ^{a}	47.45 ^{a}
Sci#8.3,1-2	BC_2	29.61 ^{a}	0.50 ^{a}	4.08 ^{a}	3.71 ^{a}	1.10 ^{a}	3.00 ^{c}	1.76 ^{a}	40.40 ^{a}
Sci#8.3,2-2	BC_2	32.56 ^{a}	0.56 ^{a}	4.69 ^{a}	3.76 ^{a}	1.24 ^{a}	2.73 ^{c}	1.94 ^{a}	41.86 ^{a}
Sci#6.1.2,5	BC_1	19.77 ^{b}	0.52 ^{a}	3.36 ^{b}	3.28 ^{b}	1.02 ^{a}	2.41 ^{b}	1.71 ^{a}	39.53 ^{b}
Sci#16.2.1,3	BC_1	17.93 ^{b}	0.47 ^{b}	3.60 ^{b}	3.05 ^{b}	1.18 ^{a}	2.22 ^{a}	1.57 ^{a}	39.26 ^{b}
Sci#3.1.1	BC_1	14.93 ^{b}	0.38 ^{c}	3.27 ^{b}	3.05 ^{b}	1.08 ^{a}	2.92 ^{c}	1.83 ^{a}	42.81 ^{a}
Sci#8.2.1	BC_1	25.13 ^{a}	0.52 ^{a}	3.46 ^{b}	3.62 ^{a}	0.95 ^{a}	2.75 ^{c}	1.74 ^{a}	39.91 ^{b}
Sci#20.4.1	BC_1	18.64 ^{b}	0.47 ^{b}	3.50 ^{b}	3.12 ^{b}	1.12 ^{a}	2.33 ^{b}	1.82 ^{a}	31.91 ^{b}
Sci#8.3,1.2	BC_1	17.98 ^{b}	0.45 ^{b}	3.53 ^{b}	3.02 ^{b}	1.18 ^{a}	2.49 ^{b}	2.02 ^{a}	44.21 ^{a}
Solanum pennellii	-	-	-	-	-	-	-	-	50.57 ^{a}
KS [1]	-	0.043	0.646	0.011	0.040	0.010	0.329	0.037	0.839
OM [2]	-	0.014	0.658	0.056	0.014	0.184	0.021	0.782	0.414
F (Tukey) [3]	-	0.9380	0.443	0.827	0.528	0.878	0.986	0.982	0.123

MFW, mean fruit weight (g); PT, pulp thickness (cm); FL, fruit length (cm); FD, fruit diameter (cm); FS, fruit shape; NL, number of locules (locules fruit^{-1}); IL, internode length (cm); AS, acyl sugar content (nmols·cm^{-2} of leaflet). Means followed by different letters in the column are significantly different according to the Scott–Knott test at a significance level of 0.01. [1–3]: Kolmogorov–Smirnov, O'Neill and Mathews, and Tukey's tests, respectively; Santa Clara, check/commercial cultivar with low acyl sugar content; *Solanum pennellii*, check with high acyl sugar.

Improvements aimed at reduced IL in tomato varieties, and consequently, superior plant architecture are an emerging trend in the market. Thus, the present study used the cultivar 'Santa Clara', which bears fruits belonging to the Santa Cruz segment, as a reference for inferences on the architecture of plants grown in the field. Specifically, IL was 7.27 cm in the cultivar 'Santa Clara', compared with 1.54 and 2.33 cm in Sci#25.1,2-2 and Sci#6.1,3-2, respectively.

The wild *S. pennellii* genotype showed the highest AS content (50.57 nmols·cm^{-2}). Notably, the BC_2 populations (Sci#6.1,3-2, Sci#16.2-2, Sci#3.1,2-2, Sci#8.2-2, Sci#20.4-2, Sci#8.3,1-2, and Sci#8.3,2-2) and the BC_1 populations (Sci#3.1.1 and Sci#8.3,1.2) did not differ from the wild genotype in terms of AS content.

3.2. Relative Superiority of BC_1 and BC_2 Generations

Overall, compared with the DP, both backcross generations (BC_1 and BC_2) showed significantly increased MFW, PT, FL, and FD (Table 1, Figure 2). In the BC_1 generation, MFW, PT, and FD were higher than those in DP, with a relative superiority of 428.07%, 134.17%, and 98.13%, respectively (Figure 2). For the same characters, BC_2 generations showed a relative superiority of 635.49%, 150.76%, and 109.70%, respectively. Moreover, for FL, compared with DP, the BC_2 generations achieved a relative superiority of 47.11%.

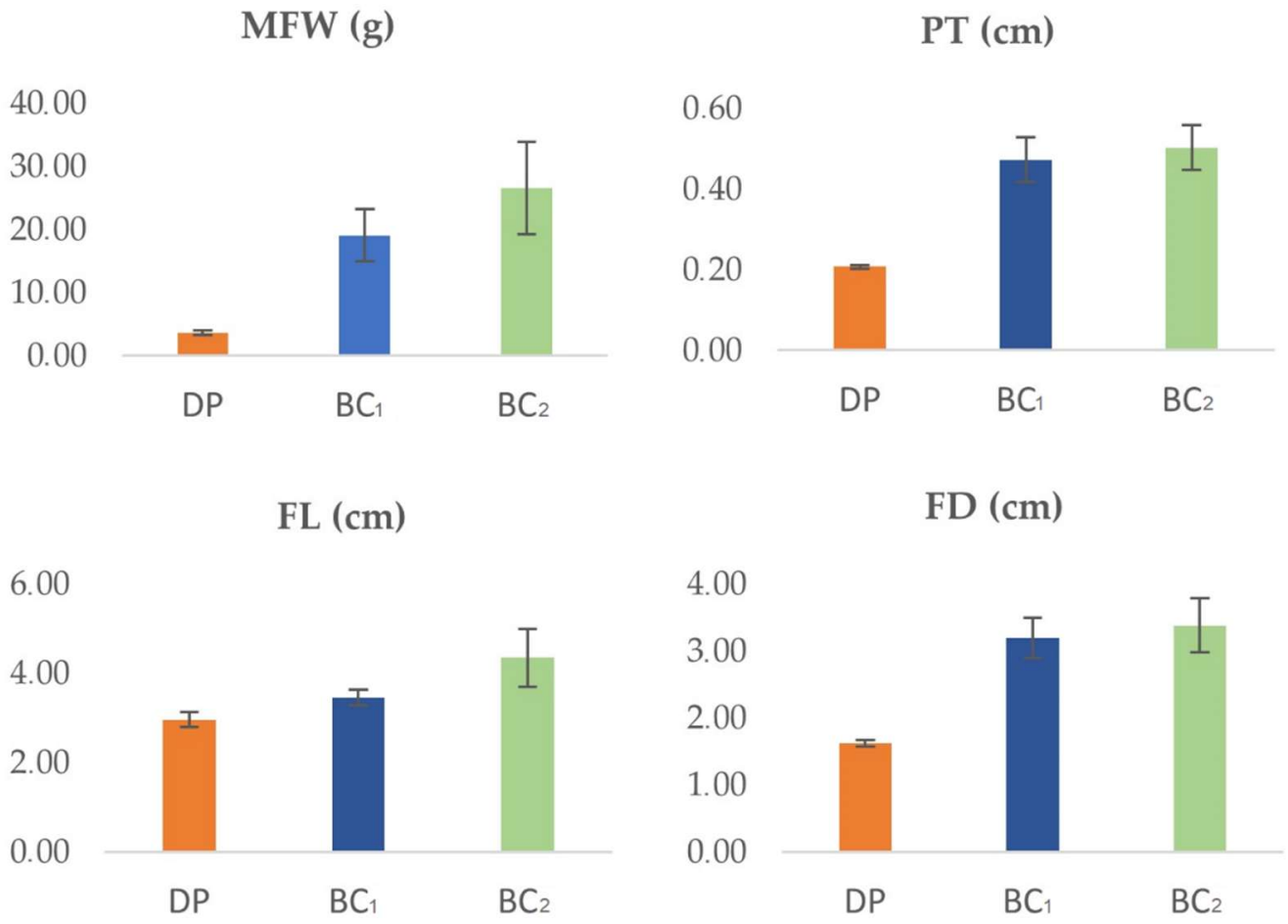

Figure 2. Relative superiority of backcross populations BC_1 and BC_2 for MFW, PT, FL, and FD. Values are presented as mean ± standard deviation for BC_2, BC_1, and DP (donor parent).

3.3. Fruit Quality Traits

Both BC_1 and BC_2 populations showed significant differences ($p < 0.05$) in terms of all fruit quality characteristics, as evaluated using the F-test (Table 2).

All backcross populations and Santa Clara showed lower TSS than the DP population (6.93 °Brix). The dwarf populations highlighted were Sci#16.2-2 (5.74 °Brix), Sci#25.1,2-2 (5.51 °Brix), and Sci#8.2-2 (5.72 °Brix) from BC_2 and Sci#3.1.1 (5.46 °Brix) from BC_1.

Regarding total CC, the highest content was recorded for the DP population; BC_2 populations Sci#6.1,1-2, Sci#6.1,3-2, Sci#16.1-2, Sci#25.1,2-2, Sci#8.2-2, and Sci#8.3,1-2; and BC_1 populations Sci#6.1.2,5, Sci#3.1.1, and Sci#8.3,1.2. Regarding LC, the highest content was recorded for the cultivar Santa Clara (5.07 mg·100 mg^{-1}). The dwarf populations highlighted were Sci#16.2-2, Sci#25.1,1-2, Sci#3.1,1-2, Sci#3.1,2-2, Sci#8.2-2, and Sci#8.3,2-2 from BC_2 and Sci#16.2.1,3, Sci#3.1.1, Sci#8.2.1, and Sci#8.3,1.2 from BC_1.

Table 2. Fruit quality characteristics evaluated in backcross populations BC_2 and BC_1 of dwarf tomato, donor parent, and check 'Santa Clara'.

Genotype [1]	Generation	TSS	CC	LC
Donor parent	Donor parent	6.93 [a]	1.74 [a]	2.94 [c]
Santa Clara	Check	5.37 [b]	0.87 [b]	5.07 [a]
Sci#6.1,1-2	BC_2	4.90 [c]	1.34 [a]	2.89 [c]
Sci#6.1,2-2	BC_2	4.80 [c]	0.88 [b]	3.08 [c]
Sci#6.1,3-2	BC_2	4.74 [c]	1.37 [a]	2.72 [c]
Sci#16.2-2	BC_2	5.74 [b]	0.48 [b]	4.04 [b]
Sci#16.1-2	BC_2	4.37 [c]	1.40 [a]	2.72 [c]
Sci#25.1,1-2	BC_2	4.77 [c]	1.10 [b]	2.42 [d]
Sci#25.1,2-2	BC_2	5.51 [b]	1.27 [a]	2.55 [c]
Sci#3.1,1-2	BC_2	5.03 [c]	0.87 [b]	2.40 [d]
Sci#3.1,2-2	BC_2	5.02 [c]	0.91 [b]	2.00 [d]
Sci#8.2-2	BC_2	5.72 [b]	1.31 [a]	2.42 [d]
Sci#20.4-2	BC_2	4.99 [c]	0.96 [b]	3.73 [b]
Sci#8.3,1-2	BC_2	4.58 [c]	1.20 [a]	2.67 [c]
Sci#8.3,2-2	BC_2	4.42 [c]	1.38 [a]	3.36 [b]
Sci#6.1.2,5	BC_1	4.68 [c]	1.31 [a]	2.57 [c]
Sci#16.2.1,3	BC_1	4.15 [c]	1.11 [b]	1.81 [d]
Sci#3.1.1	BC_1	5.46 [b]	1.41 [a]	2.15 [d]
Sci#8.2.1	BC_1	4.88 [c]	1.11 [b]	2.15 [d]
Sci#20.4.1	BC_1	5.03 [c]	1.06 [b]	2.75 [c]
Sci#8.3,1.2	BC_1	5.03 [c]	1.24 [a]	2.05 [d]
KS [2]	-	0.079	0.025	0.046
OM [3]	-	0.101	0.353	0.480
F (Tukey) [4]	-	0.487	0.460	0.323

TSS, total soluble solids (°Brix); CC, carotenoid content (mg·100 mg^{-1}); LC, lycopene content (mg·100 mg^{-1}). [1] Means followed by different letters in the column are significantly different from each other according to the Scott–Knott test at a significance level of 0.05. [2–4]: Kolmogorov–Smirnov, O'Neill and Mathews, and Tukey's tests, respectively; Santa Clara, check/commercial cultivar.

3.4. Genetic Dissimilarity between the Tested Genotypes

In a Kohonen SOM, most similar genotypes are grouped within the same neuron. In contrast, genotypes clustered in different neurons show genetic dissimilarity. Neurons consist of individuals that have some similarity with the neighboring class, and the most divergent and intermediate classes constitute the extreme and central regions of the map, respectively. An output layer comprising 4 rows × 4 columns was obtained from the Kohonen SOM. The genotypes evaluated in this study were grouped into 11 distinct neurons (Figure 3). Each hexagon represents a neuron and the amount of area filled within a hexagon indicates the concentration of grouped genotypes in that neuron. Thus, the greater the number of genotypes grouped in a neuron, the greater the filled area.

Neurons I, II, II, and XI included three populations each; neurons V and VIII included two populations each; and neurons VII, X, and XXI included one population each. Neurons IX and XIII included the donor parent and check 'Santa Clara', respectively. No genotype was allocated to neurons IV, VI, XIV, XV, and XVI (Figure 3).

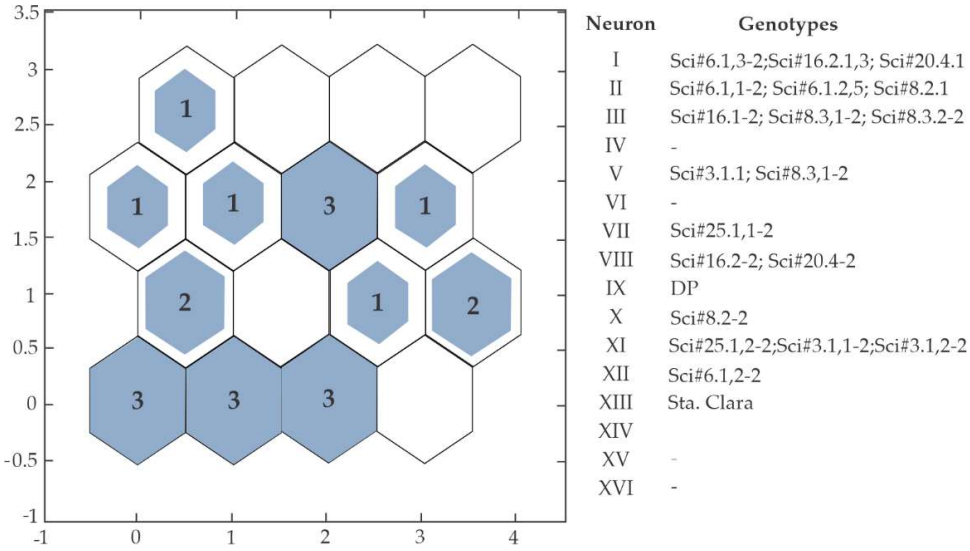

Figure 3. Topological Kohonen self-organizing network and genotype classification in the respective network neurons. DP, donor parent; Sta. Clara, check 'Santa Clara'.

4. Discussion

4.1. Feasibility of the Method

The presence of reduced internodes resulting in compact tomato plants is a trend for new hybrids [8,20]. A dwarf parent was used in the combination for obtaining Minitomato hybrids, which resulted in hybrids with reduced internodes, and consequently, an increased number of clusters per linear meter of stem and enhanced productivity. The morphology of the leaves and leaflets (Figure 1) was very different when compared to the findings of recent research carried out with dwarf plants [21].

The marked increases observed in BC_2 populations indicate the effectiveness of backcrossing cycles in the development of dwarf tomato genotypes with Santa Cruz-type fruits. Such increases in favorable alleles or complementary gene blocks in the development of segregating populations allow for the selection of superior genotypes based on improved traits of interest [22].

At this stage of the breeding program, populations derived from the second backcross generation (BC_2) should present 87.5% of the genome of the recurrent parent on average [22]. Melo et al. [23] and García-Fortea et al. [24] have reported satisfactory backcrossing results in passion fruit, corroborating our findings.

4.2. Effect of Backcrossing on Agronomic Traits, Resistance, and Nutritional Quality of Tomato

In addition to characteristics directly related to tomato fruits and plant architecture, breeding programs have aimed to introgress alleles linked to insect resistance. With this objective, several wild tomato species, such as *S. pennellii*, are used as a source of pathogen resistance. The major resistance trait of these species has been linked to the presence of allelochemicals such as ASs [25,26].

Acyl sugars are allelochemicals producing deleterious effects on the life cycle of pest arthropods, reducing their oviposition and altering their feeding preference [25–27]. Dias et al. [28] highlighted that F_2 genotypes selected for high AS content from the interspecific crosses between *S. pennellii* and a commercial cultivar were efficient in reducing the damage caused by tomato leaf miners, thereby being promising for the continuation of backcrossing cycles. Thus, dwarf populations with high AS levels used in the present study can be considered important sources of resistance to arthropod pests.

Current tomato breeding programs seek to develop cultivars that are not only productive but also tasty and rich in nutrients, vitamins, and antioxidants [29]. In this perspective, tomato TSS content is an important trait that is directly linked to the quality of fruit

taste [30,31]. In the present study, DP fruits presented the highest TSS level. The lower TSS levels in BC_1 and BC_2 fruits than in DP fruits can be explained by the increased fruit size in backcross generations, which diluted the sugars and soluble acids and decreased their concentrations [32]. According to Schwarz et al. [31], a TSS content of 3.0 °Brix is ideal for tomatoes intended for fresh consumption. Accordingly, all dwarf populations in the present study exhibited promising genotypes for the development of Santa Cruz strains with high TSS content and short IL.

4.3. Genetic Similarity Using Kohonen Self-Organizing Maps

Furthermore, to analyze the genetic dissimilarity between genotypes, we used Kohonen SOM, which is a computational intelligence strategy demonstrated to be efficient in identifying similarity patterns among genotypes and distinguishing and classifying them according to the distance between neurons in the network; as such, the shorter the distance, the greater is the similarity between genotypes contained in the respective neurons [33–36].

In the present study, all BC_1 populations were allocated to neighboring neurons (neurons I, II, and V), indicating similarity between them. Furthermore, the BC_2 populations (Sci#6.1,3-2 and Sci#6.1,1-2) were allocated to neurons I and II, respectively, indicating their similarity to BC_1 populations. Except for Sci#3.1.1 in neuron V, all other populations allocated to the respective neurons were characterized by small MFW increases. Moreover, all populations allocated to neurons I, II, and V showed reduced FL (Table 1, Figure 3).

BC_2 populations were distributed in neurons I, II, III, VII, VIII, X, XI, and XI, indicating moderate similarity between most populations of the respective generation (Figure 3). In the SOM analysis, all BC_2 and BC_1 populations were allocated to distinct DP neurons, validating the success of backcrossing cycles in rescuing part of the genetic constitution of the recurrent parent.

Multivariate techniques, machine learning, and SOMs were used by Sant'Anna et al. [37] to study genetic diversity in elite genotypes of rubber trees; they reported consistent results across these methods. Overall, the authors reported that the methods based on computational intelligence were highly efficient in detecting similarity among genotypes.

To obtain improved fruit traits, the BC_2 genotypes that stood out were selected for the third backcrossing cycle, obtaining dwarf tomato lines with Santa Cruz-type fruits.

In Minitomato, selected BC_2 genotypes should be used as the male parent to obtain normal hybrids with high yields [8]. Additionally, selected genotypes can provide hybrids with a broad spectrum of pest resistance [12] owing to high AS levels.

5. Conclusions

Significant and progressive increases were recorded in all agronomic response variables after the first and second backcrossing cycles. The BC_2 populations (Sci#16.1-2, Sci#25.1,1-2, Sci#25.1,2-2, Sci#3.1,1-2, Sci#3.1,2-2, Sci#8.3,1-2, and Sci#8.3,2-2) stood out with marked improvements in agronomic traits (MFW, PT, FL, and FD), the nutritional quality of fruits, and AS content of leaflets.

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