CROP SCIENCE

Morph-agronomic characterization of watermelon accessions with resistance to Fusarium Wilt

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Abstract: Fifty-five watermelon accessions were inoculated in pots using *Fusarium oxysporum* f. sp. *niveum* chlamydospores in a completely randomized design with five replications. Among the 42 accessions that were resistant, twelve accessions with a mean score ≤1 were selected for morpho-agronomic characterization. 'Sugar Baby' and 'Charleston Gray' were used as controls. Field research was conducted over two production cycles in a randomized block design with three replications and five plants per plot. For the characterization 13 quantitative agronomic traits were used. The dissimilarity measures were obtained by the sum of the matrices of standardized mean Euclidean distance. Coefficients of variation ranged from 5.33% (number of days until 50% of plants with at least one female flower) and 17.20% (mass of characterized fruit). In the second cycle, a reduction in days was observed for the flowering. For soluble solids content, the accession BGH-UNIVASF 40 was statistically equal to the commercial cultivars in the second cycle. Accessions were grouped similarly using two grouping methods, Tocher and the farthest neighbor method. The most promising accessions were BGH-UNIVASF 40, 169, 177 and 210 for use in future hybridizations. BGH-UNIVASF 76, 128 and 185 (*Citrullus lanatus* var. *citroides*) can be used as resistant Fusarium wilt rootstocks.

Key words: *Citrullus lanatus*, *Fusarium oxysporum* f. sp *niveum*, genetic divergence, genetic resistance, traditional agriculture.

INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai] is widely diffused among different groups of producers in several countries (Paris 2015, Pereira et al. 2017, Singh et al. 2017). The domestication of watermelon through the selection of desirable traits has led to narrow genetic diversity in the current elite watermelon cultivars (Levi et al. 2001). This narrowing of the genetic base has led to the loss of alleles conferring resistance to important watermelon diseases (Meru & McGregor 2016).

Fusarium wilt, caused by *Fusarium oxysporum* Schlechtend.: Fr. f. sp. *niveum* (E. F. Sm.) W. C. Snyder and H. N. Hans. [*Fon*], causes crop damage in several countries (Zhang et al. 2015). The disease can infect the plant at different developmental stages by causing vascular obstruction of the roots and stem which leads to the death of the plant. The pathogen is a soil-borne fungus and produces resistance structures, chlamydospores, which may persist in the soil for many years (Zhang et al. 2005, Lü et al. 2014) which makes disease control difficult. Genetic resistance is the most effective and

desirable form of disease control. Because of this fact, resistance to Fusarium wilt has been the subject of several studies (Meru & McGregor 2016, Branham et al. 2017, Costa et al. 2018).

One of the most promising alternatives for studies involving disease resistance is the search for sources of resistance in the germplasm. According to Davies & Allender (2017), the search for sources of resistance must include the wild relatives and the native individuals existing for each species. It must also include materials cultivated by farmers with differentiated systems of watermelon production. This occurs in the Brazilian northeastern region where, for several years, watermelon has been cultivated by traditional agriculture producers. In traditional farming, producers maintain a system of constant seed exchange between themselves which has promoted the conservation of variability for different characteristics of the crop (Romão 2000, Queiróz 2004).

Characterization of the germplasm allows for the identification of materials that associate variability to different characters of agronomic interest and resistance to diseases. Thus, the objectives of this study were to evaluate the reaction of watermelon accessions collected in traditional agriculture regarding resistance to Fusarium wilt, to perform the morph-agronomic characterization of the materials with the best levels of resistance and to identify the most promising genotypes for possible future uses.

MATERIALS AND METHODS

Evaluation of the reaction of watermelon accessions inoculated with Fon isolate

The experiment was conducted in laboratories and the greenhouse at the Federal University of the São Francisco Valley in Petrolina, Pernambuco, Brazil.

Initially the accessions have been tested for Fusarium wilt resistance in pots. Inoculums were obtained from a pure colony of the isolate of *Fon* (FON10) and inoculations were done using the method of chlamydospore infested soil as described by Costa et al. (2018).

Fifty-five accessions of watermelon from the traditional agriculture of the semi-arid region of Pernambuco conserved at Univasf Germplasm Bank (BGH-UNIVASF) were used for inoculation, plus the commercial cultivar Sugar Baby, which was used as a negative control (without inoculation) and a positive control (with inoculation), considering that it is a susceptibility standard to *Fon* (Zhou et al. 2010). Seeds were inoculated by sowing in 250 mL disposable cups containing chlamydospore-infested substrate. Five replications were used for each treatment. Sowing of the negative control was in substrate enriched only with PS medium and no inoculums.

After sowing, the plants were kept in a room with continuous illumination for four days. After this period the plants were taken to a greenhouse with a 50% shaded screen and irrigated daily. An evaluation was performed 22 days after inoculation using a grading scale according to Dias et al. (2002). Accessions with mean scores less than or equal to two were classified as resistant. Accessions with mean scores higher than two were classified as susceptible (Nascimento et al. 1995).

After the evaluation, 12 accessions with the lowest grades among the resistant ones, with mean scores of ≤1, were chosen for field morph-agronomic characterization.

Conduction of the field experiment

The field experiment was conducted in the agricultural production area of the Federal University of the São Francisco Valley (UNIVASF) in Petrolina, PE (9 ° 19 '10' 'S, 40 ° 33' 39 '' W,

mean altitude of 376 m). According to Köppen's classification, the climate of the region is characterized as semi-arid tropical, type BSh, characterized by scarce and irregular precipitations with rains in the summer and strong evaporation due to the occurrence of high temperatures.

Experiments were conducted in two production cycles between April and August 2016 and between October 2016 and January 2017. Climatic data (mean air temperature and relative humidity) were obtained from the UNIVASF / CCA automatic meteorological station (Figure S1) during the crop development.

The twelve accessions classified as resistant to the inoculations with *Fon* were selected after inoculation above described, including, nine accessions of *C. lanatus* var. *lanatus* from traditional agriculture producers and three forage watermelons (*C. lanatus* var. *citroides*). Besides these accessions were used as control two commercial cultivars (Sugar Baby which is susceptible to all races of *Fon* and Charleston Gray which is resistant to race 0 of *Fon*).

Plants were obtained through the production of seedlings in plastic trays. Commercial substrate for vegetables was used. Seedling transplantation occurred when the plants had two definitive leaves completely developed. A randomized block design with three replications and five plants was adopted for the two seasons. The spacing used was 3.00 m between plant rows and 0.80 meters between plants.

In the preparation of the area before each planting, manure was incorporated into the soil (2 L m^{-1}). Plants were irrigated using a localized irrigation method, a drip system. This system was used to perform the necessary fertigations during each cycle. For the management of pests and diseases, phytosanitary treatments required by the crop were applied as insecticides for the management of insects such as whitefly (*Bemisia tabaci* Genn.), aphids (*Aphis gossypii* Glover) and powdery mildew [*Podosphaera xantii* (Castagne) U. Braun & Shishkoff.

During the flowering period of the first cycle controlled pollinations occurred to perform self-fertilization which was aimed at the multiplication of seed of the accessions. The second cycle had open pollination. Fruits were harvested between 30 and 35 days after self-fertilization in the first cycle or after the opening of the first female flower in the second cycle. Subsequently, the fruit characterization was performed.

Morphological characterization of the accessions

Characterization of the accessions was performed using 13 agronomic traits, of which three were selected from those listed by the International Union for the Protection of New Varieties of Plants (UPOV 2005), number of days until 50% of plants had at least one female flower (HFF), mass of characterized fruit (MCF) and thickness of pericarp (TP). The others agronomic traits were: transverse fruit diameter (TFD), longitudinal fruit diameter of the fruit (LFD), diameter of the stalk insertion (DSI), basal region rind thickness (BRRT), apex region rind thickness (ARRT), soluble solids (°Bx), fruit production (PROD), yield (Y), branch length (BL) and weight of 100 seeds (W100) (Souza et al. 2005, Silva et al. 2006). Yield was estimated based on the value obtained in the plot.

Statistical analysis

Data were initially tested for variance homogeneity by the Bartlett test and for the normal distribution of residues by the Kolmogorov-Smirnov test. Variables that did not meet the ANOVA assumptions were transformed using log x. ANOVA was not performed for variables that did not meet the assumptions

even after data transformation. The analysis of variances was performed separately for each cycle and as a joint analysis. To determine the genetic divergence between the accessions, it obtained the dissimilarity matrix by the standardized Euclidean mean distance. Accessions were grouped using the farthest neighbor hierarchical method and the Tocher optimization method. For the latter method, the number of groups was determined according to the method of Mojena (1977) and by visual analysis (Milligan 1981).

Statistical analysis was performed using the GENES statistical programs (Cruz et al. 2013) and R Core Team (2016). The graphics were made using SigmaPlot® software (version 10.0, Systat Software, Inc., San Jose, CA).

RESULTS

Reaction of inoculated accessions

Among the inoculated accessions, 42 were classified as resistant to Fusarium Wilt according to the criteria adopted of the mean being equal to or below 2. Five of the resistant accessions obtained the lowest value of the observed mean score, which was 0.67. Of the 13 accessions classified as susceptible (Supplementary Material - Table SI), two obtained a mean score superior to the cultivar Sugar Baby, which is susceptible to the four known races of *Fon* (Zhou et al. 2010).

Morph-agronomic characterization of resistant accessions

Values of the relationship between the maximum and the minimum root mean square error (RMSE$^+$ / RMSE$^-$) of the individual analysis ranged from 1.17 (thickness of pericarp) to 4.08 (branch length). The genotype-environment interaction was significant (p <0.05) for six variables (Table I). The coefficient of variation

(CV) values varied between 5.33% (number of days until 50% of plants with at least one female flower) and 17.20% (mass of characterized fruit) (Table I). Accuracy of selection (AS) of the experiments ranged from 0.91 to 0.99, where the variable number of days until the opening of the female flower at least 50% of the plants (HFF) obtained the lowest values (Table I).

Analysis of the number of days until 50% of plants had at least one female flower (HFF) and branch length (BL) that showed non-significant genotype-environment interaction were performed using the cycle means. There was a significant reduction in the number of days for HFF from the first to the second cycle, from 56.60 to 49.79 days, respectively. It was also observed for the length of the branches, that the average length in the first cycle was statistically higher than that observed in the second cycle, decreasing from 6.11 to 5.65 meters.

Mass of the characterized fruit (MCF) differed statistically between cycles for the accessions BGH-UNIVASF 40, 116, 128, 185 and for cultivars Charleston Gray (CG) and Sugar Baby (SB) (Table II). The accessions BGH-UNIVASF 128 and 185 were superior to the others in relation to MCF in the first cycle. In the second cycle, besides these accessions, the cultivars Charleston Gray and BGH-UNIVASF 76 were also grouped together. For both cycles, the accessions BGH-UNIVASF 169, 177 and 210 were grouped with intermediate values of fruit mass differing statistically from the group formed by accessions of forage watermelon with higher values and from the group of accessions that presented with lower means, where BGH-UNIVASF 321 and 398 were grouped in first cycle and BGH-UNIVASF 321 in the second cycle (Table II).

In the evaluation of the other variables related to the fruit, a difference was observed between cycles in the transverse fruit diameter (TFD) for the accessions BGH-UNIVASF 40, 185

Table I. ANOVA summary for agronomic traits evaluated: Mass of characterized fruit (MCF), transverse fruit diameter (TFD), longitudinal fruit diameter of the fruit (LFD), thickness of pericarp (TP), basal region rind thickness (BRRT), soluble solids (°Bx), branch length (BL), weight of 100 seeds (W100) and number of days until the opening of the female flower at least 50% of the plants (HFF). Genotypes root mean square error (RMSE$_G$). Minimum (Min) and maximum (Max) value for each variable. Ratio of maximum root mean square error by the minimum root mean square error (RMSE$^+$ / RMSE$^-$). Coefficient of variation (CV) of the characters on individual analysis (Ind.) and on joint analysis (Joint). F value estimated for the accessions. Accuracy selection (AS). Significance of the characters for accession on joint analysis of the experiments and significance of the genotype-environment interaction (GxE).

| Trait | Cycle | Value | | RMSE$_G$ | Genotype significance | RMSE$^+$ / RMSE$^-$ | F | AS | CV (%) Joint | GxE |
		Min	Max							
MCF (kg)[1]	1**	0.62	15.08	23.90	**	2.32	45.74	0.99	17.16	**
	2[1]**	0.83	12.87	10.70				0.96		
TFD (cm)	1**	9.70	24.90	16.91	**	1.22	22.79	0.97	6.34	*
	2**	10.50	24.30	13.72				0.96		
LFD (cm)[1]	1**	7.50	50.50	215.43	**	1.85	51.71	0.99	9.03	**
	2[1]**	13.50	48.30	167.80				0.97		
TP (cm)	1**	0.30	4.00	1.32	**	1.17	31.54	0.98	14.21	**
	2[1]**	0.40	3.00	0.26				0.93		
(BRRT) (cm)	1**	0.30	4.10	0.51	**	1.87	8.45	0.9	14.76	**
	2**	0.60	3.90	0.35				0.92		
°Bx[1]	1[1]**	1.90	10.50	11.59	**	1.56	79.96	0.99	9.06	**
	2**	2.10	11.90	14.14				0.99		
BL (m)	1**	1.55	14.48	12.19	**	4.08	24.18	0.95	14.65	ns
	2[1]**	1.89	10.53	7.01				0.98		
W100 (g)[1]	1**	1.95	18.40	34.82	**	1.92	54.61	0.98	10.71	**
	2[1]**	3.25	16.58	15.11				0.98		
HFF (days)	1**	42	66	64.80	**	2.61	13.77	0.91	5.30	ns
	2**	39	60	52.60				0.96		

*Statistically significance at 1% of probability. **Statistically significance at 5% of probability. [1]Data transformed by log x.

and Charleston Gray. For the longitudinal fruit diameter (LFD), BGH-UNIVASF 40, 116, 128, 185 and 'Charleston Gray' differed between cycles (Table II). Only the accessions BGH-UNIVASF 76, 128, 177 and 185 remained in the same grouping for the variable TFD when the observations were made within the cycle. For the longitudinal fruit diameter (LFD), only BGH-UNIVASF 128 and 185 were grouped in the same group in both cycles. For this variable, accessions BGH-UNIVASF 169 and 177 were grouped into groups with intermediate values, differing statistically from BGH-UNIVASF 40, 174 and Sugar Baby in the first cycle, and BGH-UNIVASF 174 in the following cycle, in which the lower values for the transverse diameter were observed in the two

cycles (Table II). For the thickness of pericarp (TP) there was an increase in the second cycle for the accessions BGH-UNIVASF 40, 116 and 'Charleston Gray', already for BGH-UNIVASF 128 and 185 occurred reduction for the variable (Table II). Accessions were grouped in three groups in cycle 1, and in two groups in the cycle

2, where only BGH-UNIVASF 185 was higher than the other accessions in the first cycle (Table II). Basal region rind thickness (BRRT) was different between the cycles for the BGH-UNIVASF 40, 76 and 185 accessions. BGH-UNIVASF 128 and 185 were grouped in cycle 1 with the highest values for this variable. As in the first cycle

Table II. Mean score of reaction of the accessions of watermelon plus the Sugar Baby cultivar inoculated with *Fusarium oxysporum* f. sp *niveum* (*Fon*). Averages of the 12 accessions plus the cultivars Sugar Baby (SB) and Charleston Gray (CG) for the variables that presented significant value for the genotype-environment interaction: mass of characterized fruit (MCF), transverse fruit diameter (TFD) and longitudinal fruit diameter (LFD), thickness pericarp (TP) and basal region rind thickness (BRRT), soluble solids (°Bx), weight of 100 seeds (W100) and branch length (BL).

Accessions	Mean Score of reaction	MCF (kg)		TFD (cm)		LFD (cm)		TP (cm)		BRRT (cm)		°Bx		W100 (g)	
		Cycle 1	Cycle 2	Cycle 1	Cycle 2	Cycle 1	Cycle 2	Cycle 1	Cycle 2	Cycle 1	Cycle 2	Cycle 1	Cycle 2	Cycle 1	Cycle 2
BGH 40[1]	0.67 [(±0,58)3]	2.76 Bd[4]	3.85 Ac	16.58 Bc	18.41 Aa	17.24 Be	21.51 Ab	1.24 Bc	1.63 Aa	1.99 Bb	2.51 Aa	7.09 Bb	8.28 Aa	3.73 Be	5.88 Ac
BGH 76[2]	0.67 [(±0.58)]	7.98 Ab	7.02 Aa	20.48 Aa	19.97 Aa	35.26 Ab	34.41 Aa	2.19 Ab	1.83 Ba	2.07 Ab	1.57 Bb	2.55 Ad	2.51 Ae	12.38 Aa	12.73 Aa
BGH 116[1]	1.00 [(±0.00)]	3.02 Bd	4.73 Ab	15.07 Ac	16.41 Ab	27.90 Bc	37.87 Aa	1.00 Ac	1.30 Ab	1.92 Ab	2.06 Aa	5.15 Bc	7.18 Ab	6.57 Bd	8.43 Ab
BGH 128[2]	1.00 [(±0.58)]	10.00 Aa	7.68 Ba	20.12 Aa	20.57 Aa	42.86 Aa	36.81 Ba	2.52 Ab	1.99 Ba	2.53 Aa	2.31 Aa	2.66 Bd	3.17 Ad	13.82 Aa	12.23 Aa
BGH 169[1]	0.67 [(±0.58)]	4.95 Ac	5.43 Ab	17.60 Ab	17.15 Aa	34.05 Ab	35.27 Aa	1.37 Ac	1.49 Ab	2.19 Ab	1.95 Ab	7.49 Ab	7.31 Ab	6.82 Bd	8.65 Ab
BGH 174[1]	0.75 [(±0.50)]	3.59 Ac	3.42 Ac	18.77 Ab	18.94 Aa	18.25 Ae	18.17 Ac	1.15 Ac	1.18 Ab	2.03 Ab	2.33 Aa	5.79 Ac	6.11 Ac	7.61 Ac	8.49 Ab
BGH 177[1]	1.25 [(±0.50)]	4.49 Ac	4.72 Ab	17.21 Ab	16.67 Ab	30.21 Ac	34.25 Aa	1.11 Ac	1.22 Ab	1.68 Ac	1.80 Ab	7.69 Ab	7.40 Ab	6.36 Bd	9.09 Ab
BGH 185[2]	1.00 [(±0.58)]	10.83 Aa	7.09 Ba	21.62 Aa	19.26 Ba	42.55 Aa	35.98 Ba	3.07 Aa	1.76 Ba	2.74 Aa	1.87 Bb	2.74 Ad	2.81 Ae	15.27 Aa	11.53 Ba
BGH 210[1]	0.67 [(±0.58)]	4.72 Ac	4.89 Ab	17.39 Ab	17.67 Aa	29.85 Ac	31.34 Aa	1.09 Ac	1.34 Ab	2.08 Ab	1.77 Ab	7.41 Ab	7.21 Ab	6.97 Bd	8.99 Ab
BGH 321[1]	1.00 [(±0.00)]	2.08 Ae	1.93 Ae	13.75 Ac	13.01 Ac	22.02 Ad	23.16 Ab	1.13 Ac	1.20 Ab	1.62 Ac	1.56 Ab	5.78 Ac	6.46 Ac	7.67 Ac	7.83 Ab
BGH 395[1]	1.00 [(±0.00)]	2.87 Ad	2.73 Ad	15.09 Ac	15.36 Ab	24.49 Ad	22.51 Ab	1.12 Ac	1.07 Ab	1.51 Ac	1.29 Ab	6.10 Ac	5.63 Ac	7.86 Ac	7.70 Ab
BGH 398[1]	1.00 [(±0.00)]	2.30 Ae	2.47 Ad	14.69 Ac	14.69 Ab	21.09 Ad	22.55 Ab	0.97 Ac	1.05 Ab	1.50 Ac	1.62 Ab	6.70 Ab	5.77 Bc	7.89 Ac	7.69 Ab
SB	2.75 [(±0.96)]	2.92 Bd	3.78 Ac	17.31 Ab	18.89 Aa	18.18 Ae	20.88 Ab	0.87 Ac	1.17 Ab	1.39 Ac	1.72 Ab	8.70 Aa	9.46 Aa	3.65 Be	4.55 Ad
CG	-	4.15 Bc	7.17 Aa	15.16 Bc	18.69 Aa	28.39 Bc	38.13 Aa	1.21 Ac	1.43 Ab	1.43 Ac	1.67 Ab	5.97 Bc	8.83 Aa	9.12 Ab	9.47 Ab

[1]Accessions of the species *Citrullus lanatus* var. *lanatus*; [2]Accessions of the species *Citrullus lanatus* var. *citroides*; [3]standard deviation of reaction of the accessions of watermelon to fusarium Wilt [4]Means followed by the same capital letter in the row and lowercase in the column do not differ statistically by the Scott-Knott test at 5% significance.

BGH-UNIVASF 128 was grouped in the cycle 2 along with the accessions of *C. lanatus* var. *lanatus*, BGH-UNIVASF 40, 116 and 174 in the group with the highest BRRT values. Besides the two commercial cultivars, BGH-UNIVASF 177, 321, 395 and 398 presented the lowest basal region rind thickness during the two cycles (Table II).

Genotype-environment interaction had a significant effect on soluble solids content (°Bx), changing this variable for three accessions and the 'Charleston Gray' cultivar (Table II). For BGH-UNIVASF 40, 116 and 128, as well as for 'Charleston Gray', the value of this variable was higher in the second cycle. For BGH-UNIVASF 398 there was a reduction of °Bx in relation to the first cycle. Commercial cultivar Sugar Baby obtained the highest value for this variable in cycle 1, and in cycle 2 also obtained the best index for this variable, although it did not differ statistically from 'Charleston Gray' and the BGH-UNIVASF 40 accession that had brix increase to 8.28 °Bx.

Seven accessions differed statistically between the cycles for the variable weight of 100 seeds (W100). BGH-UNIVASF 40 and 'Sugar Baby' had the smallest seed size in first cycle. Already in cycle 2 "Sugar Baby" was statistically different from the other treatments.

Diameter of the stalk insertion (DSI) ranged from 0.85 (± 0.08) cm to 2.10 (± 0.19) cm in the fruits of the first cycle, in which the BGH-UNIVASF 174 and 116 accessions obtained the highest and the lowest means for this variable, respectively. In evaluating the second experiment, these same accessions differed statistically from the others, with BGH-UNIVASF 174 having the highest mean for the variable (1.86 ± 0.02 cm) and BGH-UNIVASF 116 being the significantly lower access among the treatments tested (0.68 ± 0.10 cm).

The accessions *C. lanatus* var. *citroides*, BGH-UNIVASF 76 (31.50 Mg ha^{-1}), 128 (23.17 Mg ha^{-1}) and 185 (32.70 Mg ha^{-1}) presented the three highest yield in the first cycle. These accessions were also superior in yield with 14.46, 16.49 e 15.46 Mg ha^{-1}, in the second cycle with accessions listed in the same order as above. For *C. lanatus* var. *lanatus,* cultivar Sugar Baby presented the lowest yield values for both cycles with measurements of 4.95 and 4.85 Mg ha^{-1} in order of cycles. BGH-UNIVASF 169 accession was among the most productive, with 13.47 and 11.75 Mg ha^{-1} in the first and second cycle respectively, as well as BGH-UNIVASF 210, with 13.43 and 11.71 Mg ha^{-1}, respectively. Average yield of the accessions in the first cycle was higher than the second, 13.66 and 10.62 Mg ha^{-1}, respectively.

There was correlation significant between eight variables in the present study (Table III). Among the variables, significant positive and negative correlations were observed, most of them of the first type. Significant positive correlation was observed between MCF with TFD and LFD. Branch length had significant positive correlation with HFF, TP and BRRT. The last significant positive correlation was observed between yield (Y) and LFD, which in turn obtained significant negative correlation with DSI. Soluble solids also obtained significant negative correlation with W100 (Table III).

Analysis of genetic divergence

Two large groups were formed in the grouping by the farthest neighbor method, with the cut done at 0.87, according to the Mojena Method (Figure 1). The first group is formed by accessions BGH-UNIVASF 76, 128 and 185 which are the forage watermelon accessions. The second group is formed by 'Sugar Baby', 'Charleston Gray', BGH-UNIVASF 40, 174, 169, 177, 210, 116, 321, 398 and 395 accessions of *C. lanatus* var. *lanatus*. By using visual analysis (Milligan 1981) performing cutting at 0.40 two subgroups are formed between accessions of *C. lanatus* var. *lanatus*. The first group containing of accessions BGH-UNIVASF 40, 174 and 'Sugar Baby' and second group the

Table III. Pearson correlation coefficients of nine genotypes of *C. lanatus* var. *lanatus* plus the commercial cultivars Sugar Baby and Charleston Gray for 11 agronomic traits: number of days until 50% of plants had at least one female flower (HFF), mass of characterized fruit (MCF), the thickness of pericarp (TP), number of days until 50% of plants had at least one male flower (HMF), transverse fruit diameter (TFD), longitudinal fruit diameter of the fruit (LFD), diameter of the stalk insertion (DSI), basal region rind thickness (BRRT), apex region rind thickness (ARRT), soluble solids (°Bx), fruit production (PROD), yield (Y), branch length (BL) and weight of 100 seeds (W100).

	MCF	TFD	LFD	DSI	TP	BRRT	°Bx	Y	BL	W100
HFF	-0.02^{ns}	-0.28^{ns}	0.24^{ns}	0.06^{ns}	0.44^{ns}	0.33^{ns}	-0.42^{ns}	0.59^{ns}	0.75^{**}	0.41^{ns}
MCF		0.62^{*}	0.76^{**}	-0.25^{ns}	0.48^{ns}	0.48^{ns}	0.33^{ns}	0.43^{ns}	0.28^{ns}	0.14^{ns}
TFD			0.03^{ns}	0.19^{ns}	0.19^{ns}	0.55^{ns}	0.51^{ns}	0.05^{ns}	0.05^{ns}	-0.37^{ns}
LFD				-0.61^{*}	0.39^{ns}	0.29^{ns}	0.02^{ns}	0.6^{*}	0.27^{ns}	0.39^{ns}
DSI					0.19^{ns}	0.13^{ns}	-0.19^{ns}	-0.42^{ns}	0.15^{ns}	-0.02^{ns}
TP						0.54^{ns}	-0.14^{ns}	0.22^{ns}	0.72^{*}	0.22^{ns}
BRRT							-0.04^{ns}	0.21^{ns}	0.66^{*}	-0.14^{ns}
°Bx								0.23^{ns}	-0.25^{ns}	-0.63^{*}
Y									0.37^{ns}	0.34^{ns}
BL										0.16^{ns}

other accessions of the variety and 'Charleston Gray'. The cophenetic correlation value for the group was 0.94.

Considering the clustering generated by the Tocher Method, we observe the formation of four groups. The first group was formed by accessions BGH-UNIVASF 177, 210, 169, 116, 'Charleston Gray', 398, 395 and 321. The accessions BGH-UNIVASF 128, 185 and 76 made up the second group. The third group has formed by BGH-UNIVASF 40 and 174 and the last group by 'Sugar Baby' (Table IV).

DISCUSSION

Reaction of inoculated accessions

The majority of accessions analyzed in the present study were classified as resistant to Fusarium wilt, a reaction observed in 75% of the inoculated accessions (Table SI). This high rate of resistant material demonstrates the importance of small producers in the conservation of existing watermelon variability in the northeastern region of Brazil (Romão 2000). The seeds used in the present study were from collections of traditional agriculture and spontaneous plants. According to Romão et al. (2008), in this region of Brazil the production of watermelon has been done with the adoption of different agrosystems. The authors highlight among these systems the cultivation of subspontaneous watermelons, the watermelon grown for consumption and the cultivation of watermelon for commercial purposes. Also, according to these authors, the first two systems have favored the selection of plants for seed dormancy. Another characteristic of interest in this type of cultivation is the possibility of selecting materials resistant to diseases. According to Romão (2000), the set of agricultural practices adopted by traditional farmers where there is no use of chemicals has made possible the selection of plants resistant to some pathogens. The search for materials in germplasm aiming at the development of disease resistant cultivars is frequent in the

Table IV. Grouping obtained by the Tocher method based on the sum of the dissimilarities matrices obtained by the standardized Euclidean mean distance for the variables.

Groups	Genotypes							
1	BGH 177	BGH 210	BGH 169	BGH 116	Charleston Gray	BGH 398	BGH 395	BGH 321
2	BGH 128	BGH 185	BGH 76					
3	BGH 40	BGH 174						
4	Sugar Baby							

literature. Davies & Allender (2017), in a survey carried out on research using the accessions of the germplasm bank of the UK Vegetable Genebank (UKVGB), verified that resistance to pests and diseases are the main topics studied. Costa et al. (2018) identify eight resistant accessions from the Active Bank Germplasm of Cucurbitaceae in Brazilian northeast from Embrapa, which corresponds to 32 % of total accessions evaluated.

Resistant accessions identified in this study could be sources in studies for the development of resistant cultivars. Morph-agronomic characterization of the most promising accessions for Fusarium wilt resistance makes it possible to find among these materials accessions for direct use by the farmers, besides facilitating future research using some of these accessions as parents. Materials used as sources of resistance that have other interesting agronomic characteristics associated is desirable for the breeder (Yang et al. 2016). Thus, this can reduce the steps for transfer of the loci controlling the resistance to the disease for the elite material.

Morph-agronomic characterization

Of the variables evaluated for the 12 accessions and two commercial cultivars evaluated in the field, the genotype-environment interaction was significant for seven variables. In the morph-agronomic characterization of accessions it is desirable that the evaluated characters be stable in different environments so that the observed variability is of the genetic constitution. However, quantitative variables are usually controlled by several genes, thus the environmental effect is expected to be observed (Lynch & Walsh 1998).

Short cycle length is considered one of the advantages in the cultivation of watermelon, and the use of early cultivars is interesting to the producer because it provides a faster availability of fruit, and depending of price fluctuation, quick financial return. In addition, shorter cycles allow the plant to develop in periods unfavorable to pathogens, allowing to the farmer, diseases control through escape (McGregor et al. 2014). Among the evaluated accessions, there was a reduction in the number of days for flowering in 50% of plants with at least one female flower (HFF). The appearance of the first flower is a variable that is influenced by conditions of high temperatures and intense light according to Wehner (2008). Here, it was observed that the second cycle was earlier than the previous one for plant flowering (Figure S1). The results for precocity are in agreement with the ones proposed by Wehner (2008) since the

temperatures were higher during the second cycle period.

Difference between the length of the branches of the cultivars and the accessions exemplifies the importance of the domestication of the watermelon for some agronomic characteristics. Shorter branches are preferred because the reduced spacing requirements permit more plants per area. It was also possible to observe in the present study this variable correlates positively with the increase in the number of days for flowering, which may lead to a higher plant development (Table III). Otherwise, the selection of plants with shorter branches may lead to early plants, as this phenotype correlates positively with the reduction of days for middle flowering (HFF). Another observation about this variable was the significant correlation with thickness of the pericarp (TP), where it can be observed that the selection for short branch lengths may reduce the thickness of pericarp.

Effects of the interaction on the thickness of the pericarp (TP) and basal (BRRT) (Table II) showed increased thickness of BRRT and TP for BGH-UNIVASF 40 and reduced thickness for BGH-UNIVASF 76, 128 and 185 in the second cycle. According to Szamosi et al (2009), the thickness is one of the four characteristics of greater importance in distinction between watermelon cultivars. Cracking fruit is an important factor that cause significant losses in yield and quality fruits of watermelon. Different factors are involved in this physical failures, among them are aspects such as genetic composition, morphological, environmental and physiological aspects (Jiang et al. 2019). In genetic terms, it is known that fruit cracking in watermelon is controlled by the recessive gene "e – explosive" (Guner & Wehner 2004). Morphologically, from the producer's point of view, the thickness of the pericarp can also be related to the type of transportation

used after production, since fruits with less thick rind tend to be less resistant to bulking loads. In the present study, it was observed that the accession BGH-UNIVASF 174 was grouped between the accessions with smaller averages of thickness for the lateral and apex regions of the rind. Another pertinent observation for this accession was the more frequent occurrence of larger pistil scar size as well as the common presence of cracks in the fruits of this accession.

The effect of the genotype-environment interaction was observed in six of the evaluated genotypes for the variable mass of characterized fruit (MCF), in three accessions for transverse fruit diameter (TFD) and five for longitudinal fruit diameter (LFD) related to the fruit size characterized (Table II). During the conduction of the two cycles, the highest temperatures were observed in the second cycle. According to Wehner (2008), the optimal condition for the development of watermelon fruits involves the occurrence of high temperatures associated with high light intensity.

With the occurrence of higher temperatures in the second cycle, an increase in production would be expected (Figure S1). However, there was an increase over the first cycle for only BGH-UNIVASF 40, 116, 174, 398 and 'Charleston Gray'. Oliveira et al. (2015), who studied the influence of three planting dates on yield and fruit quality, found that in the semi-arid conditions of Mossoró - RN, planting and transplanting in August and September, respectively, had higher yield. Under the conditions of the present study, this would represent the conduction of the experiment with the plants exposed to high temperature conditions and with high relative humidity.

Soluble solids (°Bx) of the BGH-UNIVASF 40, 116, 128 and Charleston Gray cultivars was higher in the second cycle indicating the effect of the genotype-environment interaction. For
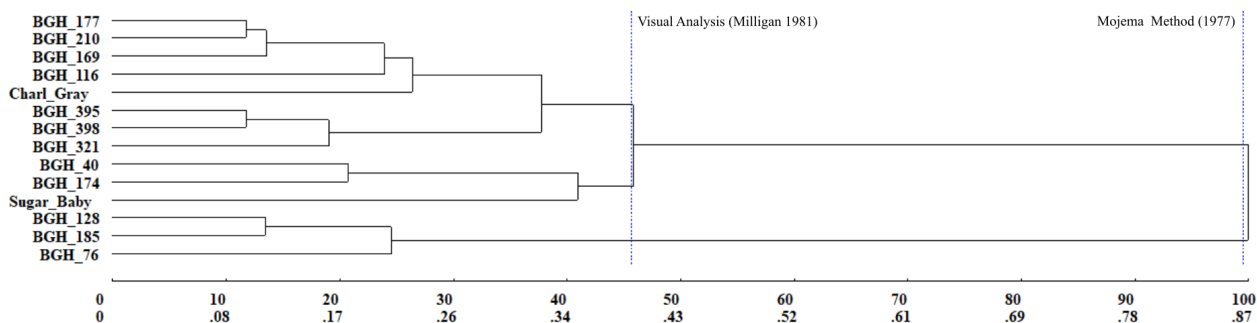
Figure 1. Dendrogram obtained by clustering analysis of the farthest neighbor, based on the sum of the dissimilarity matrices obtained by the standardized Euclidean mean distance for the variables of 12 accessions of watermelon plus the cultivars Sugar Baby and Charleston Gray.

BGH-UNIVASF 398, the effect was a reduction of soluble solids in relation to the first cycle (Table II). °Bx is a variable that is highly correlated to the percentage of sugar in watermelon pulp and is one of the main characteristics of commercial interest. In the present study it can observe the existence of significant negative correlation between weight of 100 seeds (W100) and soluble solids (°Bx). Thus, when selecting fruits with small seeds, selection can contribute to the increase of soluble solids content. For Sandlin et al. (2012), watermelon °Bx is considered as a polygenic character influenced by genetic and environmental factors and has no correlation with fruit size or weight. However, it does have a positive correlation with fruit maturity. The soluble solids of cultivars Sugar Baby and Charleston Gray were consistent with those found in literature (Queiróz et al. 2001). Leão et al. (2007) found a °Bx mean value of 7.33 in a study to evaluate eight accessions of watermelon for lycopene content, soluble solids and their correlations with the measurements of the chromatographer. Morais et al (2008), in a study on irrigation and nitrogen doses, obtained values between 8.4 and 10.7 °Bx for the same cultivar. Ramos et al. (2009), in a study on the effect of planting density on performance and fruit quality parameters of cultivars with small

fruit, obtained a value of 7.19 °Bx for the cultivar Sugar Baby.

The use of rootstocks for the management of Fusarium wilt in watermelon cultivation is practiced by farmers in some countries (Everts & Himmelstein 2015). Thus, BGH-UNIVASF 76, 128 and 185, being of the species *C. lanatus* var. *citroides*, have potential for use as rootstock because of their resistance to Fusarium wilt. However, it would be necessary to carry out subsequent studies to test the compatibility of these accessions as in the research conducted by Santos et al. (2014). These authors studied, among other variables, the compatibility of five accessions of this variety as rootstocks and found success with one accession.

Genetic divergence

Due to the large number of analyzed variables, cluster analysis was performed. In using the complete linkage method or the farthest neighbor method of grouping and performing the cut at 12.45, according to the method of Mojena (1977), two main groups were formed (Figure 1). Note that these two groups formed a similar division as to the subspecies division, with the first group consisting of accessions of *C. lanatus* var. *citroides* and the second group consisting of accessions of *C. lanatus* var. *lanatus* (Figure 1). When cutting was performed based on visual

analysis by observing dendrogram points where there was an abrupt branching change (Milligan 1981), were formed two groups between the accessions of the species *C. lanatus* var. *lanatus*. The accessions BGH 40 and BGH 174 plus 'Sugar Baby' were grouped in the first group and the other eight accessions in the second group.

The grouping performed using the optimization method (Tocher's method) grouped the accessions into four groups (Table IV), the same number of groups and similar distribution within the grouping obtained by the farthest neighbor method (Figure 1).

Considering the accessions evaluated in the morph-agronomic evaluation, the BGH-UNIVASF 40 and 174 were the most divergent between the twelve accessions on *Citrullus lanatus* var *lanatus* evaluated in the morph-agronomic characterization. Considering the groupings formed and the best performances obtained for variables of agronomic interest such as yield, precocity and °Brix BGH-UNIVASF 40, 169, 177 and 210 accessions were the most promising accessions for use in future hybridization for the development of new cultivars resistant to Fusarium Wilt. In addition, while for short-term use, BGH-UNIVASF 76, 128 and 185 accessions have potential for rootstock use.

Acknowledgments

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SUPPLEMENTARY MATERIAL

Table SI. Mean score, standard deviation of the reaction of the 55 accessions of watermelon plus the Sugar Baby cultivar inoculated with *Fusarium oxysporum* f. sp *niveum* (*Fon*). The accessions were classified as resistant (R) (mean score ≤2) and susceptible (S) (mean score >2) to Fusarium Wilt.

Figure S1. Data on mean temperature (°C) and mean relative humidity (%) recorded in the two cycles, the first between the months of May 2016 and August 2016 and the second from October 2016 to January

2017. SOURCE: LabMet – Federal University of the São Francisco Valley (Univasf).

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