ORIGINAL ARTICLE

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Genetic divergence between watermelon lines selected for resistance to *Pappaya ringspot virus*, type W

Divergência genética entre linhagens de melancia selecionadas para resistência ao Pappaya ringspot virus, estirpe melancia

ABSTRACT: *Papaya ringspot virus*, watermelon type (*Pappaya ringspot virus* - PRSV-W) is the main watermelon viral disease in Brazil. The work intended to evaluate the genetic divergence of watermelon lines type Crimson Sweet obtained from the cross of the resistant accession PI 595201 with Crimson Sweet and selected for to PRSV-W. Thirteen characteristics in 23 lines of the cross PI 595201 with Crimson Sweet and two control cultivars were evaluated. The genetic divergence was evaluated by multivariate procedures such as the Mahalanobis distance and by the Tocher's optimization grouping and nearest neighbor methods. The characteristics which contributed the most to the genetic divergence of the watermelon genotype were pulp coloration, total soluble solids, pH and titratable acidity. The Mahalanobis distance by the Tocher optimization grouping and nearest neighbor methods separated the lines into two distinct groups. The line WMX-001G-09-04-58-07pl#08 of group II was the most dissimilar in relation to the others of group I, which are WMX-001G-09-04-03-03pl#18, WMX-001G-09-04-03-03pl#21, WMX-001G-09-04-03-03pl#22, WMX-001G-14-02-55-01pl#07, WMX-001G-14-02-55-01pl#15 and Crimson Sweet – (Nova Crimson Sweet® - Agristar). Crosses between genotypes of different groups can result into superior hybrids.

RESUMO: *O vírus da mancha anelar do mamoeiro, estirpe melancia (Pappaya ringspot vírus - PRSV-W) é a principal virose da melancia no Brasil. O trabalho teve como objetivo avaliar a divergência genética de linhagens de melancia tipo Crimson Sweet obtidas do cruzamento do acesso PI 595201 com Crimson Sweet e selecionadas para resistência ao PRSV-W. Foram avaliadas 13 características em 23 linhagens do cruzamento de PI 595201 com Crimson Sweet e duas cultivares testemunhas. A divergência genética foi avaliada por procedimentos multivariados como a distância generalizada de Mahalanobis e pelos métodos de agrupamento de otimização de Tocher e vizinho mais próximo. As características que mais contribuíram para a divergência genética dos genótipos de melancia foram coloração da polpa, sólidos solúveis totais, pH e acidez titulável. A distância generalizada de Mahalanobis pelos métodos de agrupamento de otimização de Tocher e vizinho mais próximo separou as linhagens em dois grupos distintos. A linhagem WMX-001G-09-04-58-07pl#08 do grupo II foi a mais dissimilar em relação as demais do grupo I, que são WMX-001G-09-04-03-03pl#18, WMX-001G-09-04-03-03pl#21, WMX-001G-09-04-03-03pl#22, WMX-001G-14-02-55-01pl#07, WMX-001G-14-02-55-01pl#15 e Crimson Sweet – (Nova Crimson Sweet® - Agristar). Cruzamentos entre genótipos de grupos diferentes podem resultar em híbridos superiores.*

Rev. Cienc. Agrar., v. 57, n. 4, p. 367-372, out./dez. 2014

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1 Introduction

The watermelon [*Citrullus lanatus* (Thumb.) Matsum & Nakai] is subject to various diseases caused by viruses that can reduce both its yield and quality (Vieira et al., 2010). In Brazil, the viruses of the family *Potyviridae* has received more attention of the breeders for being factors limiting to watermelon growing.

Among the species of the genus *Potyvirus* affecting cucurbitaceae, *Papaya ringspot virus*, type W has been the most important in the watermelon crops and seeds of resistant cultivars are not available on the market up to now (Vieira et al., 2010).

About 38 plant species in 11 genera of *Cucurbitaceae* comprehend the host cycle of PRSV-W (Purcifull et al., 1984). The chemical control of vectors for the control of the virus is harmless due to the non-persistent transmission of the virus by the vector, which in the field is done by several aphid species, standing out the species *Myzus persicae* and *Aphis* spp. (Zambolin; Dusi, 1995). On the long term, the association of the genetic resistance with other alternative control measures has been recommended as the most effective way for the management and control of PRSV-W under field conditions (Vieira et al., 2010).

In a breeding program for resistance to viral infection, one of the most important issues is the choice of the parents to obtain populations where the selection will be carried out or they will be used for the obtaining of hybrids. Thus, the estimate of the divergence is one of the alternatives, since, more divergent the parents are, the greater the variability resulting in the segregating population, increasing the probability of selecting favorable combinations (Barbieri et al., 2005). Methodologies that allow grouping the parents together on the basis of the genetic divergence for a group of characteristics of interest may make the choice of the parents for these purposes easy (Bertini et al., 2010).

There are several methodologies that can be used for the evaluation of the genetic divergence among which the principal component analysis has been highlighted, in particular the generalized Mahalanobis distance when one can afford of experiments with replication. In relation to the other methods, the generalized Mahalanobis distance differs for taking into account the residual correlations among the evaluated traits (Cruz; Regazzi, 2001). When the goal is the segregation of genotypes in groups on the basis of the divergence for a number of characteristics, the Tocher optimization method has been preferred for allowing the formation of heterogeneous groups so that the largest average intragroup distance is shorter than the intergroup distance (Cruz; Regazzi, 1994), decreasing the effect of the groups originally formed by discrepant characteristics. There are available germplasms for the obtaining of resistant varieties through conventional breeding of watermelon for resistance to local isolates of PRSV-W. Of those, access PI 595201 used as a source of resistance by the United States Department of Agriculture (USDA), has proved one of the most promising (Maluf et al., 1997). With the purpose of transferring genes with resistance alleles to cultivar Crimson Sweet, Azevedo (2001) did the crossing of access PI 595201 with Crimson Sweet and found that the inheritance of the genetic resistance of that access

to isolates of PRSV-W is conferred by 2 to 3 loci. Beserra Júnior et al. (2006) evaluated the inheritance of resistance to WMV in PI 595201 and found that there are probably 3 to 5 loci involved in the control of resistance of the introduction PI 595201 to WMV, with the indicative of complete dominance in the sense of increased resistance to WMV. Under the climatic conditions of Tocantins, from the cross of access PI 595201, advanced lines of watermelon with characteristics similar to the commercial standard Crimson Sweet, which can be used in obtaining new hybrids with greater adaptation to the local growing conditions, were obtained. Accordingly, information on the genetic divergence of these lines is of great importance.

The work aimed to evaluate the genetic divergence between watermelon genotypes Crimson Sweet type selected for resistance to PRSV-W arising from the crossing of the resistant access PI 595201 with Crimson Sweet.

2 Materials and Methods

The experiments were conducted in the experimental area of the Universidade Federal do Tocantins – UFT, Campus Gurupi, located in the southern region of the state of Tocantins, in the agricultural year 2011. The annual average temperature of the period was 29.5°C, with annual average rainfall of 1804 mm.

The experimental design utilized was a randomized complete block with three replications. Each plot consisted of six plants (spaced 1.5 m between plants in the rows and 2.0 m between planting rows), the four central plants being regarded as the useful plot.

The seedlings were grown in a nursery with a screen frame and automated irrigation system by means of sowing in disposable 80 ml cups containing substrate obtained from the mixture of carbonized rice straw with commercial substrate. After 25 days from sowing, the transplanting of the seedlings to the final location was done. For watering, sprinkler in conventional production systems was used and as to the fertilizations, they were performed according to soil analysis and the requirement of the crop.

It was evaluated 25 genotypes, being 23 strains obtained from the cross of accession PI 595201 (non-recurrent parent) with a cultivar Crimson Sweet (recurrent parent) and selected for resistance to PRSV-W and WMV, which were: 1-WMX-001G-09-04-58-07pl#07; 2-WMX-001G-09-04-58-07pl#08; 3-WMX-001G-09-04-58-07pl#14; 4-WMX-001G-09-04-03-03pl#05; 5-WMX-001G-09-04-03-03pl#06; 6-WMX-001G-09-04-03-03pl#11; 7-WMX-001G-09-04-03-03pl#12; 8-WMX-001G-09-04-03-03pl#13; 9-WMX-001G-09-04-03-03pl#18; 10-WMX-001G-09-04-03-03pl#21; 11-WMX-001G-09-04-03-03pl#22; 12-WMX-001G-14-02-55-01pl#01; 13-WMX-001G-14-02-55-01pl#03; 14-WMX-001G-14-02-55-01pl#04; 15-WMX-001G-14-02-55-01pl#05; 16-WMX-001G-14-02-55-01pl#07; 17-WMX-001G-14-02-55-01pl#08; 18-WMX-001G-14-02-55-01pl#09; 19-WMX-001G-14-02-55-01pl#10; 20-WMX-001G-14-02-55-01pl#11; 21-WMX-001G-14-02-55-01pl#12; 22-WMX-001G-14-02-55-01pl#13; 23-WMX-001G-14-02-55-01pl#15 and two controls: 24-Crimson Sweet – (Nova Crimson Sweet® - Agristar); 25-Crimson Sweet – (Crimson Sweet® - Sakata).

The strains were obtained from controlled hand crosses of Crimson Sweet with PI 595201 according to Ferreira (2005)

and advanced up to the F_6 generation with among- and within-family selection in each generation for resistance to PRSV-W and desirable plant and fruit characteristics (especially average fruit weight, pulp color, fruit skin stripe pattern and fruit shape). The African access PI 595201 possesses totally commercially unsatisfactory characteristics, white and slightly sweet pulp and skin, but it is resistant to PRSV-W.

Two harvests were performed, the first 60 days after transplanting and the second five days after the first one. The characteristics evaluated per plot were total average yield in t ha^{-1} (yt); average fruit mass in kg (AMF); fruit shape (FS) obtained according to the index coming from the division of the crosswise diameter by the lengthwise diameter, in which values smaller than 0.5 were considered long fruits, fruits between 0.5 to 0.79 oval and 0.80 to 1.00 spherical fruits; stripe pattern (SP) obtained by score scale, in which: 1 - fruits without stripes, 2 - large fruits with stripes, 3 - fruit with narrow stripe, and 4 - mottled fruits; skin thickness in the peduncle region in cm (STP); skin thickness in the inflorescence region in cm (STI); skin thickness in the distal region in cm (STD); peduncle diameter in cm (PD); external color (EC) according to score scale in which: 1 - stands for dark green fruits, 2 - medium green fruits, 3 - light green fruits, 4 - yellow fruits; titratable acidity (TA); pH; soluble solids (SS); pulp color (PC) obtained by score scale, namely: 1 - red pulp, 2 - deep pink pulp, 3 - medium pink pulp, 4 - light pink pulp and 5 - white pulp according to Silva et al. (2006).

The multivariate analyses were performed from the means obtained after procedures of univariate analysis of variance of the characteristics. In the genotype clustering, the generalized Mahalanobis distance (D^2), as a measure of dissimilarity was adopted. The establishment of groups was performed by means of the hierarchical optimization clustering method proposed by Tocher, according to criteria proposed by Singh (1981), based on the generalized Mahalanobis distance and the nearest neighbor method taking into account the degree of dependence among the variables studied with standardization of the data through j variables ($j = 1, 2, ..., j$) and i genotypes ($i = 1, 2, ..., i$), according to the data matrix of the value of the i-th genotype and j-th variable denoted by X_{ij} and the standardized value represented by Z_{ij}, with mean 0 and constant variance 1 (Cruz; Regazzi, 2001). Analyses of variance, genetic divergence and relative contribution of the characteristics were done with the Genes program (Cruz, 2006).

3 Results and Discussion

On the basis of the importance of traits, it is possible to classify the variables according to their contribution to total genetic divergence and eliminate those with poorer contribution. The relative contribution of the characteristics to the clustering of the genotypes evaluated is shown in Table 1. The characteristics that contributed the most to the divergence among the lines evaluated were: pulp color (18.87%), soluble solids (17.04%), pH (11.55%) and titratable acidity (9.98%) and while that has contributed the least was overall average yield (0.04%). These characteristics are essential in watermelon breeding programs because they can be used as auxiliary selection criteria for fruit yield and quality.

Table 1. Relative contribution to the genetic divergence in 25 watermelon genotypes selected for resistance to PRSV-W in 13 characteristics evaluated by the method Singh (1981) based on the generalized Mahalanobis distance.

Tabela 1. Contribuição relativa para a divergência genética entre 25 genótipos de melancia seleccionados para resistência à PRSV-W em 13 características avaliadas pelo método de Singh (1981) com base na distância generalizada de Mahalanobis.

Characteristics	Relative contribution (%)
Pulp color	18.87
Soluble solids	17.04
pH	11.55
Titratable acidity	9.98
External color	8.92
Peduncle diameter	7.10
Skin thickness in the distal region	6.63
Skin thickness in the inflorescence region	5.81
Skin thickness in the peduncle region	5.32
Stripe pattern	4.33
Fruit shape	4.21
Average mass of the fruit	0.11
Total average yield	0.04

According to Nascimento et al. (2011), the inner pulp color varies from white, yellow or red; however, there is commercially a preference for fruits with deep red color. Carotenoids are the main photosynthetic pigments that accumulate in fruits, playing an important role in fighting free radicals, giving color to both fruits and flowers (Lewinsohn et al., 2005). In the red-pulped watermelon, the main carotenoid that gives it the red color is lycopene. The chemical indices most commonly used in determining the ripeness of the fruit are: pH, titratable acidity and soluble solids (Chitarra; Chitarra, 1990), the authors also claim that the maturity index (SS/TA) is one of the best ways to evaluate the flavor of the fruit, giving a good idea of the balance between these two variables. According to Dias and Lima (2010), soluble solids represent a measure of the concentration of sugars and other solids diluted in the juice or pulp of the fruit, the authors stress that the value of the minimum °Brix recommended by the European Union is 9%, values from 10 °Brix being commercially required, however, °Brix undergoes spatial variation in the pulp (Leão et al., 2006), its being greater in the central region, with a gradual reduction as it approaches the skin.

In a similar work, Souza et al. (2005) found that the traits that contributed the most to the divergence among watermelon lines were the soluble solids content and the longitudinal diameter of fruits. However, for Queiróz et al. (2001) the analysis of the genetic divergence demonstrated that the characteristics of greatest importance, in addition to the soluble solids content (12.67%) were skin thickness at the lower portion of the fruit (40 54%), prolificacy (25.12%) and skin thickness on the upper portion of the fruit (8.39%), it follows, therefore, that these traits accumulated 86.73% of the total variability, indicating that are the traits that still present genetic variability in the lines. There was the formation of nine

distinct groups by the Tocher method (Table 2). Groups I, III and V had three genotypes, group III six genotypes, group IV four genotypes and group VI two genotypes. Groups VII, VIII, IX and X presented one genotype each. It is expected that the promising hybrids can be generated by divergent lines. In this sense, when the objective is the obtaining of hybrids, it is recommended that in the crosses of the lines, the crossing of the groups formed by only one genotype is prioritized, with the others, for being more divergent. Within the same group, the

identification of the lines of interest from the estimates of the means of the most important characteristics is recommended to direct the crosses (Nunes et al., 2011).

The choice depends on the traits of the parents to be improved, the type of inheritance and the source of germplasm available (Machado et al., 2000), so that the crosses are done between phenotypically complementary parents and carrying the traits required to meet the objectives of the breeding program (Veiga et al., 2000). Thus, the crossing of lines of distinct groups with high yield characteristics and lines of higher physicochemical characteristics possesses the genetic potential for future selection strategies.

The literature on the classification of genotypes using the multivariate resources in watermelon crop is scarce. However, this methodology has provided effective contributions to discriminate potentially useful genotypes in the breeding of various crops, inclusive with the indication of the characteristics that potentially aid in the production of genetically divergent populations (Ceolin et al., 2006).

From the dendrogram representing the dissimilarity between 25 watermelon genotypes (Figure 1), with the thread cutting 622.75 (which indicates significant difference at 5%) were identified forming two separate groups. The first group conssited of six genotypes, which were: 10- (WMX-001G-09-04-03-03pl#21); 24- (Crimson Sweet – (Nova Crimson Sweet® - Agristar); 11- (WMX-001G-09-04-03-03pl#22); 9- (WMX-001G-09-04-03-03pl#18) ; 23- (WMX-001G-14-02-55-01pl#15) and 16- (WMX-001G-14-02-55-01pl#07). The second group ws formed from genotype 1, followed by the other genotypes. In relation to the first group,genotype 2- (WMX-001G-09-04-58-07pl#08) was the most divergent (Figure 1). By the Tocher method (Table 2), the divergence
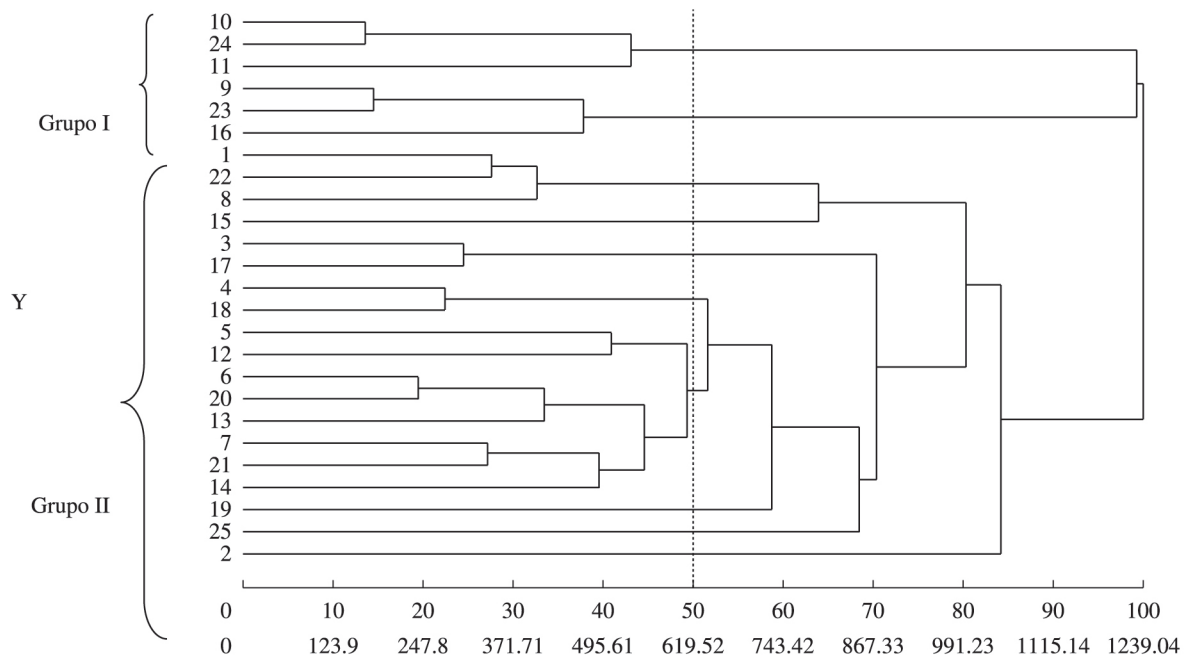
Table 2. Clustering among 25 watermelon genotypes by the Tocher's method on the basis of the generalized Mahalanobis distance (D^2), considering 13 agronomic characteristics in an assay conducted in Gurupi - TO.

Tabela 2. Agrupamento entre 25 genótipos de melancia pelo método de Tocher com base na distância generalizada de Mahalanobis (D2), considerando-se 13 características agronômicas em um ensaio realizado no município de Gurupi, TO.

Clustering	Number of lines	Lines
I	3	10, 24, 11
II	3	9, 23, 16
III	6	6, 20, 13, 7, 5, 12
IV	4	4, 18, 3, 17
V	3	1, 22, 8
VI	2	14, 21
VII	1	2
VIII	1	15
IX	1	19
X	1	25

Figure 1. Dissimilarity dendrogram among 25 watermelon genotypes selected for resistance reaction to PRSV-W for 13 characteristics by the nearest neighbor method by utilizing the generalized Mahalanobis distance in assays conducted in Gurupi - TO.

Figura 1. Dendrograma de dissimilaridade entre 25 genótipos de melancia, selecionados para reação de resistência à PRSV-W para 13 características, pelo método do vizinho mais próximo, utilizando a distância generalizada de Mahalanobis em ensaios realizados no município de Gurupi, TO.

of genotype 2- (WMX-001G-09-04-58-07pl#08) was also identified, which it lies isolated inside only one group. Agreement in the discrimination of groups between these two methods was also found wiht cassva cultivars by Vidigal et al. (1997) and with pumpkin accessions (*Cucurbita maxima*) by Amaral Júnior (1999).

Comparing the two methods, it is perceived that there was greater dissimilarity among the genotypes in the Tocher method, but as a method that evaluates the genetic divergence for hybridization purpose is sought, one can opt for the use of the nearest neighbor method by increasing thus the possibility of selecting lines that in hybridization results into hybrids with greater heterosis effect.

4 Conclusions

The characteristics that contribute the most to the genetic diversity of the watermelon lines obtained were pulp color, soluble solids, pH and titratable acidity. The nearest neighbor method separated the lines into two distinct groups. The line WMX-001G-09-04-58-07pl#08 of group II was the most dissimilar in relation to the others of group I, which are WMX-001G-09-04-03-03pl#18, WMX-001G-09-04-03-03pl#21, WMX-001G-09-04-03-03pl#22, WMX-001G-14-02-55-01pl#07, WMX-001G-14-02-55-01pl#15 and Crimson Sweet – (Nova Crimson Sweet® - Agristar-Nacional). Crosses between genotypes of different groups can result in superior hybrids.

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