

Morphological Variability and Phenotypic Association among Pairs of Characters of some *Watermelon chlorotic stunt virus* Resistant Lines of *Citrullus lanatus* Thunb.

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Abstract

Twelve lines of watermelon (*Citrullus lanatus* Thunb.) with resistance to *Watermelon chlorotic stunt virus* (WCSV) were morphologically characterized and association among pairs of characteristics was estimated. These watermelon lines were developed at the National Institute for Promotion of Horticultural Exports-University of Gezira-Sudan. Wide differences exist among these lines with respect to vegetative, inflorescence, fruit and seed characteristics. High variation coefficients were obtained for seed weight (51%), number of secondary branches (45.6%), fruit weight (41.7%), rind thickness (41.2%) and seeds/kg (40.3%). Plant canopy was positively associated with male flower (0.51) and ovary length (0.56). While female flower size and ovary length was positively associated with fruit weight (0.57; 0.63), the latter was also positively associated with total soluble solids (TSS) (0.51), seed size (0.73) and seed weight (0.63). Also, male flower size was positively associated with ovary length (0.6), fruit weight (0.64), TSS (0.54) and seed size (0.52). However, flower earliness (50%) was negatively associated with female flower size (-0.61) fruit weight (-0.56) and number of seeds/kg (-0.53). These lines are being used for the development of superior hybrid lines and genetic mapping.

Introduction

Watermelon is a valuable crop species, with wide broad phenotypic diversity in seed and fruit qualities (Wehner, 2007) and there is need for genetic research to identify genes affecting fruit quality and response to diseases. Watermelon is one of the most important vegetables produced in the Sudan, and is ranked the third after onion and tomato in irrigated sectors. Under rain fed, it is grown in vast areas for seeds production and as a

source of water reserve after rain fall season in drought areas in Western Sudan. Various pests and diseases attack the crop affecting both production and quality. Watermelon is prone to attacks by aphids, whiteflies or melon bugs transmitting viruses and causing direct damages. Aphids transmit cucurbit aphid-borne yellows virus (CABYV) and zucchini yellow mosaic virus (ZYMV) to watermelon. ZYMV can also be transmitted mechanically (Abass *et al.*, 2003). The whitefly-transmitted watermelon chlorotic stunt virus is the most destructive viral disease attacking the crop (Mohamed *et al.*, 2005). Watermelon chlorotic stunt virus (WCSV) belongs to the geminiviruses family with bipartite genome (DNA-a and DNA-b components). It was first reported in Yemen and Iran (Jones *et al.*, 1988; Walkely *et al.*, 1990) and Sudan (Lecoq *et al.*, 1994). The disease is transmitted by the whitefly (*Bemisia tabaci*) and by grafting, but not mechanically (Brunt *et al.*, 1990; Walkely *et al.*, 1990). It is the major disease that infects watermelon in Sudan, causing severe crop losses (Lecoq *et al.*, 1994). Efforts exerted at the National Institute for Promotion of Horticultural Exports succeeded in developing WCSV resistant lines with superior horticultural quality, some of these lines were used in this study. This project is aiming at producing WCSV resistant hybrids and to map the gene of WCSV resistance with other genes conferring vegetative and fruit characteristics in watermelon. Since WCSV resistance was found to be not simply inherited and conferred by a dominant gene with some minor genes involved (Raed *et al.*, 2008), the priority was given to elucidate crossing among the resistant lines to select for superior hybrids. Moreover, the highest the variability among these lines for a given trait the better the chance to go for mapping gene(s) conferring this trait. Therefore, the main objective of this study was to assess variability existing among the bred lines with respect to vegetative, fruit and yield

characteristics and degree of association among pairs of characteristics.

Materials and Methods

Eighteen lines resistant to WCSV were selected and used in this study. They were characterized for agronomic, fruit, and yield characteristics. They were also analyzed for degree of similarity and simple correlation between pairs of characteristics. The genetic material used in this study could be classified according to their origin into five groups:

- Charleston R: backcrossing an F1 (UG0012 x Crimson Sweet) with Charleston Gray.
- Elite Yellow, Elite Red, Elite White, Elite Ds, B4 and line 102: RILs of the cross UG0012 x Crimson Sweet (pedigree selection).
- Sugar Baby R and Sugar Baby Ice: backcrossing the F1 (UG0012 x Crimson Sweet) with Sugar Baby.
- Crimson R and Crimson Perfect: backcrossing the F1 (UG0012 x Crimson Sweet) with Crimson Sweet.
- Commercial varieties such as Charleston Gray, Crimson Sweet and Sugar Baby (From Peto Seed Company).

The accession UG0012 belonged to the species *Citrullus colocynthis* (L.) Schrader. It was collected in Medani area since 2001. It has small rounded bitter fruits, striped out skin color of dull green with light green and small brown seeds. Resistance to WCSV in this accession might be conferred by a single dominant gene with some modifiers (Unpublished data).

Description of the experimental site: Different activities exerted in this study were conducted at the University of Gezira Research Farm at Neshesiba, Medani, Sudan (14 ° 24' N and 33 ° 38' E). The climate of the area is described as arid, hot and dry. The soil is Vertisol with clay content ranging between 40 and 65%, pH value is ranging between 8 and 9.6, with less than 1% organic carbon, 300 ppm total nitrogen and 406 to 700 ppm total phosphorus (Ishag, 1994). Planting and cultural practices were done as recommended by Mohamed (1984).

Screening for WCSV resistance: Plant materials used in this study were evaluated for resistance to WCSV using a rating scale of 1-9 depending on symptoms caused by natural infection, where:

1-2= High susceptibility: The plant is completely stunted with chlorotic leaves and bears no commercial fruits.

3-4= Moderate susceptibility: Most of the plant canopy is stunted, 50-70% with clear symptoms and plants bearing few small sized fruits.

5-6=Intermediate resistance: Stunting and chlorotic symptoms are clearly observed on the top of some branches, while fruits in this area develop patches of chlorosis while older fruits remain normal.

7-8= High intermediate resistance: Plants develop slight chlorosis towards the end of the season and remain free of stunting.

9=Resistant: The plants remain healthy, vigorous and free of symptoms till the end of the season.

Parameters studied: Vegetative, inflorescence, fruit and seed descriptors were used as mentioned by watermelon descriptor list developed by the Plant Genetic Resource Unit of the Agricultural Research Corporation-Sudan (2003).

Data analysis: Collected data of characterization were subjected to statistical analysis for standard deviations and coefficients of variation. Cluster analysis of genotypes was conducted using hierarchical analysis of GENSTAT. Moreover, the standardized data matrix of characters was used to generate similarity indices based on Euclidian distances. Moreover, characterization data were subjected to statistical analysis for correlation coefficients between pairs of characters using MStat software package.

Results and Discussion

Resistance of the different lines and commercial varieties used in this study to WCSV during summer season 2010 is given in Table 1. Results showed high level of resistance of the selected lines, while the commercial varieties were severely infected by the virus causing severe crop losses. It was concluded that some minor genes were involved in WCSV resistance in these lines, since some plants showed mild disease symptoms at the end of the season. Crossing among resistant line might produce hybrids with better resistance compared by crossing with the commercial varieties. Therefore, variability existing among the resistance lines might add to the performance of the hybrids and could provide a chance of having superior quality hybrids.

Phenotypic variability and degree of similarity: High variation exists among inbred lines with respect to vegetative, inflorescence, fruit and seed characteristics, as presented in Table 2, Table 3, Table 4 and Table 5, respectively. The highest variation coefficients were obtained for 100 seeds wt (51.0), number of secondary branches (45.6), fruit wt (41.7), rind thickness (41.2) and

number of seeds/kg (40.3); whereas, no variability was observed for leaf shape.

'Perfect Crimson' and 'Crimson R', showed close phenotypic similarity to the commercial 'Crimson Sweet', with respect to agronomic and fruit characteristics; whereas, 'Sugar Baby R' showed close similarity to the commercial 'Sugar Baby'. These results were depicted in the dendrogram based on phenotypic markers (Figure 1). In this dendrogram, The 'Elite (ds)', 'Charleston Gray' and 'Charleston R2' belong to one group, while the resistant lines and commercial 'Crimson Sweet' and 'Sugar Baby' belong to a separate group. The RILs 'Elite Yellow', 'Elite Red', 'Elite White', B4 and line 102 were clustered together in a separate sub-group with 'Sugar Baby Nice'. In this figure, lines were divided into two major groups at a similarity index corresponding to 0.65. Phenotypic data collected in the second season were in the same trend as in the first season presented in Figure 1. At a similarity index corresponding to 0.85 these groups were further divided into five distinct subgroups.

Correlation among pairs of characters: Traits having significant associations, at probability level of 0.05, are presented in Table 6. Plant vine length (cm) is associated with plant canopy size (0.57) and number of secondary branches (0.6). Plant canopy is associated with male (0.51) and ovary length (0.56), whereas number of primary branches is associated with number of secondary branches (0.53) and negatively associated with fruit wt (-0.54). Number of secondary branches was negatively associated with female flower size (-0.66) while positively associated with number of seeds/kg (0.52) Earliness of flowering (50%) was negatively associated with female flower size (-0.61) and fruit wt (-0.56). Fruit weight is associated with female flower size (0.57) and male flower size (0.64).

Ovary length is associated with fruit wt (0.63), size of male flower (0.6), TSS(0.51), seed size (0.73) and wt of 100 seeds (0.63), while it is negatively associated with number of seeds/kg (-0.53). Likewise, size of male flower was positively associated with fruit wt (0.64), TSS (0.54) and seed size (0.52).

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Table 1. Evaluation of lines and commercial varieties for resistance to WCSV, summer 2010

Entry	Number of plants in each level of the rating scale*								
	1	2	3	4	5	6	7	8	9
Charleston R						1	2	8	9
Elite Yellow							5	3	12
Elite Red						2	1	3	14
Elite White						4	6	1	9
Elite Ds						1	1	6	12
B4							2	5	13
Line 102							7	2	11
Sugar Baby R							2	3	15
Sugar Baby Nice								3	17
Crimson R							1	7	12
Crimson Perfect							2	9	9
Charleston Gray (commercial variety, P.S.)	20								
Crimson Sweet (commercial variety, P.S.)	16	4							
Sugar Baby (commercial variety (P.S.)	10	4	3	3					

* Twenty plants of each entry were used to screen for WCSV resistance in this season.

Table 2. Standard deviation and coefficient of variation for vegetative characters in the first season

Vegetative characters	Minimum	Maximum	Mean	Standard deviation	Coefficient of variation
Length of main branch (cm)	131.0	361.4	227.1	52.9	23.3
Number of primary branch (No)	2.4	5.0	3.4	0.7	20.5
Number of secondary branch (No)	3.0	22.2	11.8	5.4	45.6
Leaf shape (Score (1,2,3))*	1.0	2.2	1.6	0.5	29.2
Leaf color (Score (1,3,5,7,9))*	1.0	7.0	4.9	1.6	32.4
Leaf size (Score (3,5,7))*	5.0	7.0	6.4	0.9	13.7
Leaf pubescence density(Score (1,3,5,7))*	3.0	4.8	5.0	0.5	10.9
Leaf pubescence texture(Score (3,5,7))*	4.6	7.0	5.5	0.9	16.6
Stem pubescence density (Score (1,3,5,7)) *	3.0	7.0	5.8	1.2	20.7