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Genetics and Stability of Resistance to Watermelon Chlorotic Stunt Virus in Melon (*Cucumis Melo* L).

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ABSTRACT

Experiments were carried out under field conditions at the University Gezira Research Farm, Sudan and the agroinoculation and green houses conditions in France (le Centre National de la Recherche Scientifique (CNRS*) and le Institut National de la Recherche Agronomique (INRA**) to study the inheritance and stability of resistance to watermelon chlorotic stunt virus (WCSV) in melon (*C. melo* L.). The techniques of autoradiography, using the ratioactive WCSV P^{32}, probe was used to detect WCSV in plant tissue and the phospho-imager machine was used to obtain quantified results of DNA particles within the examined plant tissue. The results indicated the presence of one dominant gene and another recessive independent gene controlling WCSV resistance in the resistant lines P1414723, P1124112 and HSD2445- 005. Multi-locational trials on resistant lines under natural field conditions revealed that the resistance to WCSV in melon is uniform and stable. Results of studying the movement of the virus within the plant tissues indicated that the blockage in the plant indigenous trafficking system was one of the mechanisms that are involved in plant resistance to WCSV in the lines P1414723, P1282448, P1124440, Pl 124112, 90625 and HSD 2445-005.

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INTRODUCTION

Watermelon chlorotic stunt virus (WCSV) is a bipartite gemini-virus transmitted by the whitefly *Bonisia tabaci* (Gennadius) in a circulative persistant manner. Its host range is restricted to the family Cucurbitaceae (Walkey *et al*، 1990). During the last decade, the disease caused significant crop losses in Yemen, Iran and Sudan (Kheyr-Pour *et al*, 2000). In the Sudan, the disease ranks the third among viral diseases that infect cucurbit crops, it came after zucchini yellow mosaic virus (ZYMV) and cucurbit aphid-borne yellows virus (CABYV). The disease is distributed over wide geographic locations in central Sudan, causing significant crop losses (Lecoq *et al.*, 1994). Leaves of infected plants are crinkled, stunted and develop striking chlorotic mottle. The infected plant looks stunted and may be devoid of marketable fruits. Fruits are usually small in size with chlorotic blotches and fail to develop (Jones *et al*، 1988).

The overall sequence identity between the Sudan and Iran isolates is 98.3% for DNA-A and 95.9% for DNA-B. A partial sequence of the virus from Yemen is slightly closer to the isolate sequence from Iran (98% identity) than that from Sudan (96%). Encapsidation and replication functions are encoded by DNA-A, while systemic spread functions are encoded by DNA-B (Kheyr-Pour *et al.*, 2000). During the course of infection, the virus particles move long distances within plants and short distances from cell to cell across both nuclear and plasmodesmal boundaries (Brough *et al*, 1988).

Natural resistance to WCSV is not common among indigenous melon germplasm, and it was only found in the accession HSD 2445 (*C. melo* var. *agrestis*), which was collected in western Sudan. Homogeneous resistance to WCSV was found in the Indian lines P1414723, P1124440، P1124112, 90625 and the. South African line P1282448 (Yousif, 2002). Inheritance of resistance to WCSV in melon will provide information needed to breed for resistance against this disease. Furthemore، identification of the mechanisms that are

involved in resistance to WCSV as well as stability of resistance are crucial for breeding strategies.

The objectives of this work were to study the inheritance and stability of resistance to WCSV, and to elucidate possible mechanisms that are involved in this resistance.

MATERIALS AND METHODS

Experiments conducted in this study were accomplished at three locations, the University of Gezira Research Farm, Sudan; the Institutes of the Centre de la Recherche Scientiflque (*CNRS*), France and the Institut National de la Recherche Agronomique (*INRA*), France.

Screening experiments were conducted under the agroinoculation conditions at CNRS, in 2000-2001. These experiments aimed at studying the inheritance of resistance to WCSV in the segregating populations of the crosses P1414723 x 'Vedrantais', P1124112 x 'Vedrantais' and HSD 2445-005 x 'Ananas'. Material studied consisted of 56 recombinant inbred lines (RILs) of the cross P1414723 x Vedrantais, 63 RIL of the cross PI 1241 12 x Vedrantais and 101 plants of the F_2 population of the cross HSD 2445-005 x Ananas. The RILs of the first two crosses were kindly supplied by the Melon Laboratory of INRA. The F_l population HSD 2445-005 x Ananas and the F_2 population of this cross were prepared at the University of Gezira Research Farm. Results of screening for WCSV resistance in the segregating populations were confirmed by hybridization of squashed dots of leaf sample of eac h plant on a nylon membrane (pore size = 0.45 nm) with the radioactive probes of WCSV prepared at CNRS (Kheyr-Pour *et al*., 2000). Quantified results of virus particles were obtained for each sample, using the phospho-imager machine at CNRS. The French variety 'Vedrantais' and the leading melon variety 'Ananas' were considered susceptible cultivars for infection by WCSV. The F_l of the three crosses and the different parents were also included in this study.

Agroinoculation assays were conducted using the infectious WCSV clones prepared at CNRS. Details on preparation of the infectious clones were described by Kheyr-Pour *et al*. (2000). Plants were inoculated at the fourth-true leaf stage three to four weeks after planting. Seedlings were injected with the combined agrobacteria into the stem and leaf petiole‹ using a I-ml disposable syringe. Data obtained were validated using the x^2--test. To determine the inheritance of resistance to WCSV in the two lines, PI 414723 and Pl 124112, the X^2 values and probabilities of the segregation of 3 resistant: I susceptible ratio and 1 resistant: 1 susceptible ratio were calculated for the data of screening of the RILs. Later‹ experiments were carried out using the first backcross generation (BC_1) of the two crosses Pl 414723 x 'Vedrantais' (62 plants) and P1124112 x 'Vedrantais' (60 plant), using the same procedure.

Experiments were conducted under field conditions to study stability of resistance to WCSV from the interaction of the virus with different cultivars. These included the WCSV resistant lines PI 414723, Pl 124112, ‹90685, HSD 2445-005, Pl 282448, Pl 124440 and the segregating lines 'Indes-5' and 'Faizabadi Phoont'. The susceptible variety 'Ananas' was used as a susceptible check. Plants of the different lines were grown in three locations in the Sudan, during summer seasons of 2000 and 2001. The three locations were Khartoum, Wad Medani and Kassala where melon is intensively cultivated. Design used was the randomized complete block design, with three replicates. Symptom screening was conducted in the field and small pieces of young leaf of each plant we squashed to a nytrocellulose membrane and hybridized with the P^{32}- radioactive labeled WCSV probe. Stability of WCSV resistance was predicted from the interaction of the virus with different melon cultivars.

The movement of WCSV virus particles in the homogeneous resistance lines was studied using grafting techniques. The melon variety 'Vedrantais' and the watermelon variety 'Sugar Baby' were used as root stocks. Three weeks prior to grafting, plants of the variety Vedrantais, which were used as rootstocks were infected using the

agroinoculation technique described by Yousif ct al. (2003). Grafting was done by cutting into the stem of the rootstocks and scions V-shaped incessions. Then, scions and rootstocks were joined together and the junction was protected from moisture loss using parafllm. Growth of the grafted plants maintained in growth chambers set at and 60% relative humidity. Scoring of symptoms was done 10,15 and 21 days after grafting. At the same time, leaf samples were squashed to nylon membranes and analysed by hybridization with the radio labeled WCSV probe. Quantification of virus DNA panicles was obtained using the phospho-imager machine at CNRS.

To test whether the blockage is in the short distance or long distance movement of the virus particles within plants, experiments conducted to study the movement of the virus panicles from the infected tissues of the rootstock to scion's tissue of the resistant lines through the vascular bundle of the main stem. Horizontal cut rings nylon the stem, at 2-5 cm above the grafting point, were squashed on a membrane. Data were taken on leaf discs from the leaf at 2-5 cm above

RESULTS AND DISCUSSION

Natural resistance is the best method of controlling pests and diseases. It is the least expensive, simple and effective means of improving stability of yield for various crops.

Results of screening of 20 plants of F_l from each of the three crosses‹ using the agroinoculation technique, indicated that resistance to WCSV is dominant in the three lines (Table l). Data on screening of ten plants of each of the RILs of the cross PI 414723 x 'Vedrantais' for WCSV resistance revealed 39 resistant lines and 17 susceptible ones, whereas‹ 43 of the RILs of the cross PI 124112 x 'Vedrantais' were found to be resistant and 13 were susceptible. The corresponding probabilities for the ratio 3:1were found to be not significant for the RILs of PI 414723 x 'Vedrantais' ($P > 0.05$) and the RILs of Pl 124112 x 'Vedrantais' ($P > 0.05$) For the 1:1 ratio, the probabilities were found

to be low for the RILs of Pl 414723 x 'Vedrantais' (P=0.3%) and highly significant for the RILs of Pl 124112 x 'Vedrantais' (P≤0.0I%). The results of screening of the RILs revealed that two genes are conferring resistance to WCSV in the two lines. Results of the F_IS and the RILs of the two lines indicated that two genes, at least one is dominant, were involved in controlling WCSV resistance in melon.

Segregation for WCSV resistance in the BC_I generation was 32 resistant: 30 susceptible and 33 resistant: 27 susceptible for the backcrosses of P1414723 x 'Vedrantais' and PI 124112 x 'Vedrantais‹' respectively. Probabilities of the X^2 values of the expected ratio 3: 1 were highly significant for BC_I of the cross P1414723 x 'Vedrantais' (P<0.001%) and BC_I of the cross P1124112 x'Vedrantais'(P ≤ 0.00I%) These results indicated that the hypothesis of two dominant genes raised out from screening of the RILs of the two crosses is not accepted. For the Ill ratio, the probability was not significant (P> 0.05) for the backcrosses of P1414723 x 'Vedrantais' and P1124112 x 'Vedrantais'. These results indicated that either one dominant or one dominant gene and another recessive gene are controlling WCSV in melon. The results of screening of the BC_I combined with those obtained from screening of the RILs indicated the presence of one dominant and another recessive independent genes controlling WCSV resistance in the two lines. This type of inheritance is simple, and the genes of WCSV resistance can easily be introgressed into the commercial cultivars to breed WCSV resistant varieties.

Table 1. Inheritance of watermelon chlorotic stunt virus (WCSV) resistance in the lines PI414723, PI124112 and HSD 2445-005, using the agroinoculation and autoradiography techniques.

Genotype	Observed values R:S	Expected ratio R:S	X^2 value	Proba-bility
PI414723	20:00			
PI124112	20:00			
HSD 2445-005	20:00			
'Vedrantais'	00:20			
'Ananas'	00:10			
F_1 (PI414723x 'Vedrantais')	20:00			
F_1 (PI124112x 'Vedrantais')	20:00			
F_1(HSD2445-005x 'Ananas')	20:00			
RILs of the cross PI414723x 'Vedrantais'	39:17	1:1	8.64	0.3 %
		3:1	0.86	35.5%
RILs of the cross PI124112x 'Vedrantais'	43:13	1:1	16.07	<0.007%
		3:1	0.95	75.76%
F_2 of the cross HSD2445-005x 'Ananas'	80:21	3:1	0.95	32.9%
		15:1	36.45	<0.001%
		13:3	0.28	59.9%
BC_1 (PI414723x 'Vedrantais')x 'Vedrantais'	32:30	1:1	0.06	79.9%
		3:1	18.09	<0.001%
BC_1 (PI124112x 'Vedrantais')x 'Vedrantais'	33: 27	1:1	0.60	60.0%
		3:1	12.80	<0.001 %

RIL = Recombinant inbred line.

The selected locations for studying the stability of WCSV resistance (Khartoum, Wad Medani and Kassala) differ in climatic conditions particularly during summer season. None of the homogeneous resistant lines was infected in the three locations during the two seasons. The variety 'Ananas' was 100% infected in the three locations in the two seasons, while the lines 'Faizabadi Phoont' and 'Indes-5' segregated in the three locations (Table 2). These results indicated that resistance, to WCSV can be described as being uniform and stable, because differences observed in the disease severity resulted mainly from differences among cultivars and not due to other factors such as the interaction between cultivars and isolates of the pathogen. These findings could encourage breeding of resistant cultivars that can be grown in the three locations. On the other hand, Kheyr-Pour *et al*. (2000) found close similarity between the virus isolates from Sudan, Iran and Yemen. The close similarly among these

isolates that originated in different climatic zones indicates the uniformity of resistance to WCSV. Therefore, bred cultivars for WCSV resistance in the Sudan are expected to be resistant in Yemen and Iran.

Table 2. Screening promising lines for resistance to watermelon chlorotic stunt virus (WCSV), under field conditions at three locations in the Sudan, during the summer seasons of 2001 and 2002.

Entry	Location (Hybridization data: sum of susceptible plants/total number of screened plants in the two seasons)		
	Khartoum	Wad Medani	Kassala
Pl 414723	0/27	0/26	0/24
90625	0/32	0/23	0/24
	5/37	6/28	4/25
	0/31	0/25	0/25
	7/38	6/29	5/23
	0/38	0/29	0/24
	0/38	0/34	0/24
	36/36	31/31	29/29

No viral DNA was found in the leaves at the stem tip and at the first node of the resistant scions, 10 and 20 days after grafting (Table 3). No accumulation of the virus particles was found in the different lines. These results suggested a blockage in the movement of the virus in these scions، since the amount of radioactivity for the squashed dots of the lines P1414723, P1124112, 90625, P1282448, HSD2445-005 was not much exceeding the background of the membrane (Table 3). Moreover، no symptoms of WCSV were found in these lines. For the scion's of 'Vedrantais' on 'Vedrantais' infected root stocks, the amount of radioactivity was high for the leaves at the first node to grafting point and at the tip of the scion implying that there was no blockage on the long and short movement of the virus within the susceptible cultivar 'Vedrantais'. Very high amount of radioactivity was scored the susceptible watermelon variety 'Sugar Baby' (Table 3).

Virus particles move both long distance along the main stem and short distances during the course of infection. Brough *et al.* (1988) stated that during infection the uncapsidated viral DNA must move from cell to cell across the nuclear and plasmodesmal boundaries and long distance within the plant. The amount of radioactivity obtained on squashed dots of agroinfected 'Sugar Baby' was very high (10805.79), while the amount of the radioactivity of the resistant lines was maintained around the radioactivity at the background of the membrane (Table 3). The results of different experiments could not be correlated, because different experiments used probes with different incorporation rates.

Table 3. Screening of WCSV resistant lines grafted in agroinfected root-stocks of 'Vedrantais'.

Grafted scion	Mean of the hybridization in				Mean of hybrid-ization of squash of grafted scions at 2-5cm above the grafting poin
	Observed symptoms	Leaf at scion's tip	Leaf at first node	Rootstocks	
PI414723	None	139.05	163.05	1642.54	3675.06
PI24112	None	142.19	176.36	1652.85	4418.96
90625	None	158.43	183.45	1142.27	3439.94
HSD 2445-005	None	186.55	172.35	1517.72	Nt
PI24440	None	Nt	Nt	Nt	5031.09
PI282448	None	Nt	Nt	Nt	3983.95
'Vedrantais' grafted scion	Severe	1470.54	1604.16	2760.23	17432.12
'Vedrantais' rootstock	Severe	-	-	3169.78	-
'Sugar baby' grafted scion	Very severe	4420.25	7754.35	-	4549.15
Background of the nylon membrance		170.32	170.32	1670.32	4549.15
Sugar Baby agronifected	Very severe	-	-	10805.79	39449.78

Means at different columns can not be compared because probes used in the different experiments were of different incorporation rates.
Nt = Not tested.

CONCLUSION

The presence of one dominant gene and another recessive independent gene conferring resistance to WCSV in melon indicated that resistance to WCSV in this species is unique and simply inherited. Moreover، resistance to WCSV in melon could be considered as uniform and stable. The blockage in the virus indigenous trafficking system was among the mechanisms that are involved in plant resistance to WCSV in the resistant lines.

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