Identification of a Natural Source of Resistance to Watermelon Chlorotic Stunt Virus in an Indigenous Accession of Cucumis Melo Var. Agrestis

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Watermelon chlorotic stunt virus (WCSV) is among the most important viral diseases of the family *Cucurbitaceae*. It is a bipartite begomovirus (DNA-A and DNA-B genome components) that belongs to the family *Geminividae* (Walkely *et al.,* 1990). It causes severe crop losses, particularly in watermelon and melon (Lecoq *et al.*,1994). In Sudan, WCSV causes high reduction in yield and quality of watermelon, melon, snake cucumber and squashes. Leaves of infected plants are crinkled, stunted and develop striking chlorotic mottle. The whole plant looked stunted and chlorotic and may be devoid of marketable fruits (Walkely *et al*., 1990). Resistance to major diseases is very common among indigenous Sudanese melons, Tibish and agrestis (C. *melo* var. *agrestis)*, compared to other melon types (Mohamed, 2000).

Experiments were conducted at the University of Gezira Research Farm in April (1996-1997) to identify a natural source of resistance to watermelon chlorotic stunt virus in *Cucumis melo* L. The screened material included: 101 accessions of C. *melo* var. *cantalupensis* and C. *melo* var *flexuousus* collected in Sudan; nine accessions belong to the indigenous Humaid type (C. *melo* var. *agrestis*) and eleven introduced lines such as P1 313970, P1 131375, Pl 255478, Vedrantais, Nantais Oblong, MR-I, Isoblon, Virgos, Margot and Zumo. The inoculation pressure of the virus in the field was increased by growing plants of the susceptible watermelon cultivar "Sugar Baby", obtained from Peto Seed Company, about one month before conducting the screening experiments.

Fifteen to forty five plants from each accession were used. Each plant was given one of the four disease scores: No observed symptoms mild symptoms including mottling and crinkling of young leaves; moderate symptoms including stunting, mottling and cri young leaves; severe symptoms which include severe mottling and crinkling of young leaves, and stunting of both leaves and internodes. The proportion of infected plants of each accession was estimated. Screened accessions were classified as resistant, slightly susceptible, moderately susceptible and highly susceptible according to their incidence rates (Hassan *et al*., 1991). The accessions with an incidence (Mean disease score) of 1 were considered resistant, the incidence rates of 2 and 3 as slightly to moderately susceptible and the in rate of 4 as highly susceptible.

The material was visually screened in the field. In the third and fourth generations of inbreeding, an additional tissue immuno blotting assay (TIBA) was performed (Marchelo, 1996). Screening in the fifth generation of inbreeding was conducted at the National Centre for Scientific Research of France (CNRS). Screening relied on agroincul ation using *Agrobacterium tumifaceins* "strain 4404" which was composed of the plasmid *Bin 19* that was harboring both genomes WCSV (Kheyr-Pour *et al*., 2000). Plants were inoculated at the fourth true leaf stage and screening was done three weeks after inoculation Young leaves of the agroinoculated plants were squashed to a nitrocellulose membrane (0.45 um pore size) and hybridized using fifty *ng* of a 2.8 kb of WCSV-SD-A and dNTP, following Sambrook *et al (* 1989) method. The membranes were then subjected to film processing and phosphoimager reading for quantification of DNAs.

Results of screening of different collections for WCSV resistance are presented in Table l. Resistance to WCSV was not commonly found in C. *melo* L. Only one indigenous accession HSD 2445 was segregating for resistance to the disease with an infection percentage of 52.6% and an incidence rate of 1.65 (Table 2). The infection rate for both Ananas and PI 313970 was 100% and the incidence rates were 3.73 and 3.83, respectively. The introduced lines MR-I, Isovac,

Isoblon and Margot were moderately susceptible, while the remaining lines were highly susceptible to the disease.

Table l. Screening different lines and accessions for WCSV-resistance at University of Gezira Research Farm, during summer 1996.

Entries	Number of accessions corresponding to Mean Disease Scores (MDS)			
	R (1.0-1.5)	S.S (1.6-2.5)	M.S (2.6-3.5)	H.S (3.6-4.0)
Sweet melon	0	2	67	25
Snake melon (*C. melo var. flexuous*)	0	7	8	2
Humaid (*C. melo var.agrestis*)	0	5	3	1
Introduced lines	0	0	4	7

R ≡ Resistant; S.S ≡Slightly susceptible; M.S. ≡ Moderately susceptible: H.S. ≡ Highly susceptible

Table 2. Screening for resistance to WCSV in the inbreeding of the accession HSD 2445.

	First screening			Visual assessment			TIBA		Agroinoculation	
	HSD 2445	Ananas	PI 313970	1_1	1_2	1_3	1_4	1_5	Sugar Body	Ananas
Infection(%)	52.6	100	100	21	15	7.1	0	0	100	100
Incidence Rate	1.65	3.73	3.83	1.54	1.21	1.17	1	1	4	3.7

1 ≡ Inbreeding generation., the corresponding number refers to the inbreeding generation.

TIBA= Tissue Immuno Blotting Assay.

Advancements in the inbreeding for WCSV resistance are given in Table 2. Similar results were obtained by TIBA and visual assessment. Former growing of highly sensitive watermelon cultivar around the experimental field was found reliable and could be applied for screening against diseases that are vectored by insects such as whiteflies. Results of screening at the fourth generation of inbreeding provided the homogeneous resistant line 005, under field conditions (Table 2) In general, results of inbreeding of HSD 2445 pointed out to be a simple mode of resistance to WCSV in this accession, since plants could

clearly be classified into resistant and susceptible. Moreover، the line 005 achieved homogeneity for resistance at an early generation of inbreeding 1_4 (Table 2).

All plants tested (20 plants) at the fifth generation were completely resistant (Table 2). They showed no disease symptoms and no considerable amount of viral DNA read by the phosphoimager machine, compared to the membrane. The incorporation rate of the probe was high (63.1×10^6). The technique of the agroinoculation was found to be very efficient for cloning the viral DNA to plants. The hybridization with radioactive probes followed by phosphor-imager reading was very much dependable in identifying resistant plants.

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