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Research Application Summary

Breeding for watermelon chlorotic stunt viral disease resistance in watermelon

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Abstract

The white fly transmitted watermelon chlorotic stunt virus (WmCSV) is the causal agent of the most devastating disease of watermelons (*Citrullus lanatus*) across many African and Middle Eastern countries including Sudan. Commercial cultivars collected from around the world proved susceptible to the disease especially during the hot dry summers and in severe situations the disease may cause complete damage of the crop. In search for sources of resistance, 27 cultivars and hundreds of landraces of *C. lanatus* were initially screened; however, none had sufficient levels of field resistance. Efforts were then expanded to include wild relatives of watermelon such as *C. colocynthis*. Large variability was noticed within the highly bitter white fleshed *C. colocynthis* in fruit size, external colour, disease and insect resistance and drought tolerance. Out of the screened collection, two accessions; C114 and Grift, were found to be highly resistant to WmCSV. The identified resistance in the wild resources was transferred by backcrossing to two of the widely cultivated watermelon cultivars; Crimson Sweet and Peacock. Both donors were readily crossable with commercial cultivars and hybrids (F1s) were fertile and no genetic barriers were noticed along the entire process of advancement of backcrossing generations.

Key words: *Citrullus colocynthis*, resistance, watermelon, watermelon chlorotic stunt virus, wild relatives

Résumé

Le virus de la cascade chlorotique transmise par la mouche blanche (VcCTMB) est l'agent causal de la maladie la plus dévastatrice des pastèques (Citrullus lanatus) dans de nombreux pays d'Afrique et du Moyen-Orient, y compris le Soudan. Les cultivars commerciaux récoltés dans le monde entier se sont révélés sensibles à la maladie, en

particulier pendant les étés chauds et secs et dans des situations graves, la maladie peut endommager complètement la culture. A la recherche de sources de résistance, 27 cultivars et des centaines de variétés locales de C. lanatus ont été initialement sélectionnés; cependant, aucun n'avait des niveaux suffisants de résistance au champ. Les efforts ont ensuite été étendus pour inclure des espèces sauvages apparentées à la pastèque comme C. colocynthis. Une grande variabilité a été observée au sein de C. colocynthis à chair blanche très amère dans la taille des fruits, la couleur externe, la résistance aux maladies et aux insectes et la tolérance à la sécheresse. Hors de la collection projetée, deux accessions; C114 et Grift se sont révélés très résistants au VcCTMB. La résistance identifiée dans les ressources sauvages a été transférée par rétrocroisement à deux des cultivars de pastèque largement cultivés; Crimson Sweet et Peacock. Les deux donneurs étaient facilement croisables avec des cultivars commerciaux et les hybrides (F1) étaient fertiles et aucune barrière génétique n'a été remarquée tout au long du processus d'avancement des générations de rétrocroisement.

Mots-clés: Citrullus colocynthis, résistance, pastèque, virus de la cascade chlorotique de la pastèque, parents sauvages

Introduction

Watermelon (*Citrullus lanatus var. lanatus* (Thunb.) Matsum. and Nakai) (2n= 2x= 22) is one of the most widely cultivated crops in the world. Global production of the crop was reported to be 117 million tons in 2017 (FAOSTAT, 2018) with China alone accounting for 68% of the total production. Watermelon is a major vegetable and cash crop in Sudan and is produced across the country and during all seasons. The hot arid climate in Sudan adds to the popularity and high consumption of the delicious fruits especially during summers.

Sudan is a country of diversified ecological and climatic conditions. There is thus a diversity of vegetation and soils that has resulted in enormous wealth of diversified indigenous genetic crop resources of which watermelon is a prominent example. The Western part of Sudan is an important region for the diversity of watermelon germplasm where an uncounted number of land races and cultivars are grown annually especially in the northern and western Kordofan regions (Goda, 2007). A very wide variability exists among these land races which may prove a valuable source of breeding material for stress environments and pest and disease resistance.

Watermelon production prone to several biotic and abiotic constraints affecting quality and leading to significant yield loss (Omara, 2017). Watermelon Chlorotic Stunt Virus disease is an endemic disease rated the most devastating disease of watermelons, inflicting heavy losses and at times total crop failure in Sudan. The disease is transmitted by the endemic whitefly (*Bemisia tabaci*) and caused by the *bipartite Geminivirus* WmCSV. Its symptoms are characterized by yellow veins, chlorotic mottling, severe stunting of young leaves, and drastically reduced fruit yield and deteriorated quality. Early infections during summers may lead to complete crop failure. Chemical control of the vector is only partially successful and few viroliferous flies are enough to transmit the population independent virus. The disease is difficult to control culturally or through insecticides. Out of the estimated cropped area of

120,000 feddan in Sudan, 70% or more are affected by the disease (Omara *et al*., 2017) leading to environmental pollution, health hazards to farm labourers, and financial losses estimated in billions of Sudanese pounds.

Watermelon cultivars produced by international seed companies and national breeding programs and grown by farmers around the world are susceptible to WmCSV (Omara, 2017).

The virus may also infect wild and commercial cucurbits including melons, squashes, cucumbers and snake cucumber (Jones, 1995). Therefore disease inoculum is always abundant in the environment and difficult to reduce. Hence, crop sanitation and vector control remains the basic options of reducing crop damage. However, these measures are difficult if not impossible to implement in the major watermelon growing regions in Sudan. Whiteflies migrate easily from bushes or forests or other host crops, while applying pesticides to completely eliminate whiteflies is expensive. Growing resistant cultivars thus remains the most reliable, cheaper, and durable measure to control the disease and sustain production of watermelon in Sudan.

Methodology

Study area. Experiments were conducted during the summer of 2016 at Shambat Research Station Farm of Agricultural Research Corporation (ARC), Khartoum North (latitude 15 36'N, longitude 32 32'E and elevation 380m). The site features a desert climate of hot dry summers where temperatures rise to 46^{o}C during April - June with relative humidity as low as 20%. Soils are mostly heavy clays with pH around 8.

Genetic material. A collection of 116 landraces of *C. lanatus* provided by the Plant Genetic Resources of ARC was used in the study. Two wild relatives (*C. colocynthis*), namely Grift and Line 114 were used as resistant checks (Elhassan *et al*., 1996) and the most widely grown cultivars Crimson Sweet and Sun Shade were used as susceptible checks. This experiment was conducted under natural infestation with infesting rows of Crimson Sweet and Sun shade.

Experimental design and cultural practices. Germplasm collected was planted following an Alpha lattice design with three replications. Land was deep ploughed, harrowed and leveled to 7 m x 2.5 m beds. Seeds were treated with the fungicide Apron star at 0.5 g/500 g to control the soil born gummy stem blight (GSB). Seeds were sown in two rows on either side of the beds with a 50 cm intra-row spacing and 2 seeds per hole.

Data collection. Disease assessment commenced 47 days after planting with the first appearance of WmCSV. Disease severity scores were made and recorded at weekly intervals until plant senescence using a scale of 0-4 employed Omara (2017) with 0 representing high resistance and 4 most susceptibility. Disease severity scores were transformed to percentage by dividing the means of scales by the maximum score used. Area under disease progress curves (AUDPC) were computed using the weekly severity ratings (Madden *et al*., 2007). Disease incidence was assessed as the proportion of plants with symptoms in the

field (Kayondo *et al*., 2014). Data were also recorded on fruit total soluble solids (TSS) using a refractometer.

Data analysis. All data were subjected to analysis of variance (ANOVA) with mean comparison performed using Fisher's protected least significant difference test (LSD) at P 0.05 (Steel and Torrie, 1997). Results were generated using ANOVA option with genotypes being considered as fixed effects and replications as random effects. Disease severity scores were used to compute area under disease progress curves (AUDPC) as described by Madden *et al*. (2007) using this formula:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} (t_{i+1} - t_i) \right)$$

Where t = time in days of each reading, y = percentage of affected foliage at each reading and n = number of readings.

Backcross breeding strategies. From screening, resistant donors (white fleshed highly bitter C. colocynthis) were identified and crossed with recurrent parents. The first generation (F1s) plants were then grown, tested for resistance to WmCSV, then selfed to produce the second generation (F2) progenies. Resistant F2 progenies with good fruit quality were then backcrossed to recurrent parents to produce BC1 plants which were in turn self-pollinated to start the second cycle of backcrossing.

Results and discussion

WmCSV disease severities and total soluble sugar. Analysis of variance of 116 genotypes, two wild relatives and two commercial cultivars tested under field conditions for WmCSV severities, AUDPC, and TSS is presented in Table 1. The results showed that blocking of the replication had significant (P 0.05) effects on initial severity and TSS. Final severity, AUDPC and disease incidence were on the other hand highly significantly (P 0.001) influenced by blocking of the replication. Genotypes did not have a significant (P>0.05) effect on initial severity but significantly affected final severity, AUDPC, disease incidence, fruit TSS.

The mean performance of materials screened under field conditions for TSS and WmCSV disease category are presented in Table 2. The ranking of the tested genotypes showed that the wild relatives, Grift and 114 exhibited the highest level of resistance to the disease with no disease symptoms (0%) until late in the season. Their AUDPC values remained 0% throughout. On the other hand, commercial cultivars, Crimson Sweet and Sun shade had high WmCSV severities (100%). Genotypes with high WmCSV severities and AUDPC also produced small fruits with white to creamy and light yellow to very light red flesh colour. Their TSS ranged between 1.5 to 4.0. On the other hand, genotypes with low WmCSV severities and AUDPC had small to medium sized fruits with white, creamy, very light red or light red flesh colour. For this category, TSS ranged between 2.0 to 4.8. Surprisingly, all the tested genotypes had lower TSS values compared to Crimson Sweet and none of them had red flesh.

Table 1. Mean squares of severities, AUDPC, disease incidence, fruit weight, TSS and evaluated in summer season of 2016

Source of Variation	Initial severity [a]	Final severity [b]	AUDPC [c]	Incidence [d]	TSS [e]
Rep	2.1	967.0	1304976.0	518.4	0.94
Rep per block	0.9*	314.2***	409583.0***	242.8***	1.48*
Genotype	0.5ns	394.2***	492192.4***	430.2***	2.36***
Residual	1.0	170.2	208135.0	219.2	1.33
LEE	0.5	91.9	112888.2	114.4	0.78
SED (p 0.05)	0.9	11.7	411.5	13.1	1.08
±SE	0.01	1.8	65.2	1.3	0.06

Significant differences at * = P 0.05 and *** = P 0.001, a= Initial severity was recorded 47 days after planting based on severities 0 – 100%. b = Final severity was taken 82 days after planting based on severities 0 – 100%, c= Area under disease progress curve computed as described by Madden *et al*. (2007), d= disease incidence, e= Total Soluble Solid using refractometer (Brix).

Table 2. Watermelon landraces, wild relatives and commercial cultivars used in the study and their classification based on disease severity

No. of genotypes	Description	TSS[a]	Category
Grift	*C. colocynthis*	1.47	Highly resistant
Line 114	*C. colocynthis*	1.64	Highly resistant
5	From ARC gene bank	2.03	Resistance
7	From ARC gene bank	2.20	Moderate resistance
36	From ARC gene bank	2.50	Moderate susceptible
57	From ARC gene bank	3.02	Susceptible
8	From ARC gene bank	3.02	Highly susceptible
Crimson Sweet	Commercial cultivar	6.47	Highly susceptible
Sun Shade	Commercial cultivar	5.80	Highly susceptible

a= Total Soluble Solid using refractometer (Brix).

Plate 1. Symptoms of watermelon chlorotic stunt virus disease on watermelon grown at Shambat Research Station Farm of Agricultural Research Corporation (ARC), Khartoum North, Sudan

Backcross breeding . Crossing of the resistant wild watermelon accessions C114 and Grift with cultivated watermelon (Crimson Sweet and Peacock) was easily done. No genetic barriers between the two populations were encountered. Back cross 1 (BC1) populations were produced, self-pollinated and will be used to start the second cycle of backcrossing. This process is continuing and will be reported in due course.

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