Inheritance of Resistance to Watermelon Chlorotic Stunt Virus Disease in Sudan

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ABSTRACT

Watermelon (Citrullus lanatus) is a major vegetable crop in Sudan produced across the country during all seasons. Its production is prone to several biotic and abiotic constraints which may lead to significant yield losses especially watermelon chlorotic stunt virus (WmCSV) disease. In order to estimate gene action for WmCSV in watermelon, six basic generations developed from crossing wild relatives Citrullus colocynthis (Grift) and a popular commercial cultivar (Crimson Sweet) were evaluated in a randomized complete block design with three replications in a field experiment. Generation mean analysis using additive, dominance and at least one of the epistatic effect (additive x additive, additive x dominance and dominance x dominance) were involved in the inheritance of the studied traits. However, simple additive-dominance model was sufficient only for initial and final severities; it was not adequate to explain the inheritance of area under disease progress curve (AUDPC) to WmCSV implying that the inheritance was more complex than this model. Both dominance and additive epistatic gene interactions were involved in the inheritance of resistance to WmCSV. The additive, dominance, additive x additive and dominance x dominance type of gene interactions were significant for final severity and AUDPC in this cross implying that selection should be postponed to later generations to attain higher homozygosity.

Keywords: Watermelon, Watermelon Chlorotic Stunt Virus, Inheritance, Epistasis, Genetic effects.

I. INTRODUCTION

Watermelon (Citrullus lanatus var. lanatus (Thunb.)Matsum.&Nakai) (2n= 2x= 22) is one of the most widely cultivated crops in the world [1]. Global production during 2002 was 89.9 million tons and this production increased to 105.4 million tons during 2012 [1]. It is considered a major horticultural crop that accounts for 2% of the world area devoted to vegetables. China is the leading world producer accounting for 67% (73 million tons) of the total world production followed by Iran, Turkey, Brazil, and Egypt [1]. Sudan is a country of diversified ecological conditions of climates, vegetation, and soils that resulted in enormous wealth of diversified indigenous genetic resources of crops 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 [2]. A broad
variability exists among these land races which may prove a valuable source of breeding material for marginal environments and pest and disease resistance. In Sudan, watermelon production spreads over all regions of the country under rainfed, irrigated and flood irrigation systems along the Nile and all year around. Farmers are more generous in applying fertilizers and chemicals to control pests and diseases. Lately, production under pivot irrigation is proving highly successful, cuts on operational costs, productivity and quality. Production is more of a family business where planting, harvesting and seed extraction is carried out by family members assisted at times by farm labours. Indigenous landraces are the main source for seeds and production spreads over hundreds of thousands of acres in western Sudan (79% of total area and 81% of total production).

The watermelon crop is prone to several constraints that may lead to significant yield losses. The lavish growth habit of watermelon attracts a wide variety of pests including whiteflies, beetles and aphids. The endemic watermelon chlorotic stunt virus (WmCSV), a bipartite geminivirus disease transmitted by the whitefly (Bemisia tabaci), is the most damaging and devastating viral disease to watermelon production in Sudan. This highly destructive endemic disease is caused by a population independent virus whereby few viruliferous flies transmit infection after few minutes of feeding on diseased plants. WmCSV inflicts heavy losses all year around and may cause total crop failure of the summer crop. Watermelon cultivars produced by international companies and national breeding programs and grown by farmers are susceptible to WmCSV [3]. The virus may also infect wild and commercial cucurbits including melons, squashes, cucumbers and snake cucumber [4] therefore; reducing the inoculum and its sources is hard to be achieved. Hence, crop sanitation and eliminating the vector are the basic measurements to avoid or reduce infection. Breeding resistant cultivars remains the most reliable and durable measure to control WmCSV and sustain watermelon production in Sudan. Therefore, this study aimed at investigating the nature of gene action governing the inheritance of resistance to WmCSV.

II. MATERIALS AND METHODS

A) Experimental design and study area
Quantitative genetics of WmCSV resistance and other agronomic characters of watermelon were studied using the F1, F2, BC1P1 and BC1P2 of a cross between wild relative Grift (P1) as male parent and Crimson Sweet (P2) as female parent in a complete randomized block design of experiment with three replications at Shambat Research Station Farm, Khartoum North (latitude 15°36'N, longitude 32°32'E and elevation 380 m) during summers and winters of 2015 and 2016. The site features a desert climate of hot dry summers where temperatures sour to 45°C during April-June and relative humidity of 20% or less. Soils are mostly heavy clays of pH equals 8 usually amended with nitrogen fertilizers.

B) Land preparation and cultural practices
Land was prepared by deep ploughing and harrowing followed by proper leveling beds were 8.0 m long and 2.5 m in width. Seeds were treated with fungicide Apron star at 0.5 g for 500 g of seeds to control the devastating soil born gummy stem blight prevailing in the study area. Seeds were sown on both sides of beds at 50 cm intra-row spacing and 2 seeds per hole. The field was irrigated weekly and hand weeded whenever necessary. These cultural practices were standard for all experiments carried out during this study.

C) Inoculation
Previous studies indicated high presence and intensity of the endemic WmCSV and its vector the whiteflies. Thus, testing and evaluation of the collected germplasm was done under natural infestation.

D) Genetic material
Quantitative genetics of WmCSV resistance and other agronomic characters of watermelon were studied using the F1, F2, BC1P1 and BC1P2 of a cross between Grift (P1) as male parent and Crimson Sweet (P2) as female parent. Grift, resistant to WmCSV, has whitish flesh, small fruit size
and highly bitter inedible fruits. Its rind is very hard and its fruits can be kept for months. The leading commercial cultivar, Crimson Sweet, susceptible to WmCSV, is adapted and highly productive with red flesh, very sweet and large fruits. A cross between the two parents was carried out to produce F1s. F1s plants were then grown and either selfed to produce F2 progenies or backcrossed to both parents to produce BC1P1 and BC1P2.

E) Data collection and analysis
Disease intensity was commenced 38 days after planting based on the proportion of diseased green leaf area. Disease scores were recorded at weekly intervals till plant senescence using a scale of 0 to 4 employed by Omara 1996, (Sadig Omara, personal communication, February 10, 2015) whereby:
0 = High resistance (HR): Plants are vigorous and productive with good fruit size and no visible symptoms on foliage till the end of the season,
1 = High intermediate resistance (HIR): Plants are vigorous but show small chlorotic spots on the top branches with no stunting,
2 = Low intermediate resistance (LIR): Stunting and chlorotic symptoms are observed on some branches while fruit size is reduced and young fruits in infected plants develop patches of chlorosis,
3 = Moderate susceptibility (MS): most of the plant canopy is stunted and plants bearing few small sized fruits,
4= High susceptibility (HS): Plants completely stunted and chlorotic and bears no commercial fruits.
Disease scales were transformed into percentage severities by dividing the means of scales by the maximum score used. Area under disease progress curves (AUDPC) were computed using the weekly percentage severity [5]. Data was also recorded for days to 50% flowering: days from sowing till 50% of plants in the entry showed at least one open male flower.
All data were subjected to analysis of variance with mean comparison performed using Fisher’s protected least significant difference (LSD) at P≤0.05 [6]. Least square means for all genotypes were generated using analysis of variance (ANOVA) option of GenStat 12th Edition (VSN International Ltd., UK) with genotypes being considered as fixed effects and replications as random effects.

Weekly assessments of disease severity were used to compute area under disease progress curves (AUDPC) as described by [5]:

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

Where,
- \( t = \) time in days of each reading
- \( y = \) percentage of affected foliage at each reading and,
- \( n = \) number of readings.
The bed length was 8 m but the number of beds per population varied from 3 for non-segregation generations i.e. P1, P2 and F1, 15 beds for the F2 while 12 beds for the BC1P1 and BC1P2. Genetic ratios were used to calculate the additive, dominance and epistatic genetics on the WmCSV inheritance among the developed populations [7].

III. RESULTS AND DISCUSSION
A) Generation mean analysis
Analysis of variance of Grift x Crimson Sweet for initial and final severities, AUDPC and 50% flowering days are presented in Table 1. The results showed significant (P≤0.05 and P≤0.001) effects among the generations for all the studied traits. While, all the replications showed non-significant effects for all the traits.
The mean performance of six generations of cross between Grift x Crimson Sweet for initial and final severities, AUDPC and flowering days are presented in Table 2. The two parents were significantly different at LSD (P≤ 0.05) = 1.7, 3.8, 153.8 and 4.9% for initial and final severities, AUDPC and flowering days, respectively. Grift (resistant parent) showed no disease symptoms (0%) until late stages of the plant age and therefore AUDPC was 0%. Crimson Sweet (susceptible parent) showed little symptom of WmCSV at initial severity (3.09%) and at final stages all plants were stunned (100%) and therefore AUDPC was high (3756.47). The mean of F1, F2 and the backcross
generations BC1F1 were lower than both mid-parent and susceptible parent suggesting that probably epistasis plays a major role in the inheritance of resistance to WmCSV [8]. Grift had more days to flower (44.7 days) than Crimson Sweet (43.7 days). F1, F2 and BC1F1 flowered earlier than the mid-parents and the resistant parent Grift.

B) Mode of inheritance explained by genetic effects
Genetic effects for resistance to WmCSV severities, AUDPC and flowering days are presented in Table 3. The simple additive dominance model was adequate to explain the inheritance of resistance to WmCSV in the cross by the non-significant lack of fit for initial and final severities and AUDPC [9]. While the simple additive dominance model did not explain the inheritance of WmCSV by the significant (p<0.05) lack of fit for AUDPC. However, significant (p<0.05) lack of fit implied that the inheritance was more complex than this model. Both dominance and additive type of epistatic gene interaction were significant at p<0.001 indicating that both were involved in the inheritance of resistance to WmCSV. However, significant lack of fit implied that the inheritance was more complex than dominance and additive x additive. Occurrence of dominance and additive epistasis interactions suggested the use of inter- and intra-mating and segregating generations to exploit both types of gene effects [10].

Generation mean analysis is commonly used to study disease resistance in which a donor parent is highly resistant and the other parent is highly susceptible [7]. It was more useful in this case because the parents were divergent and most of the favorable alleles are in Crimson Sweet and the unfavorable alleles are in Grift. The additive, dominance, additive x additive and dominance x additive type of gene interactions were significant for final severity and AUDPC in this cross implying that selection should be postponed to later generations to attain higher homozygosity[11]. However, the significant (P≤0.001) additive gene effect for AUDPC suggested that it is also possible to have early selection for the resistance. Epistasis was involved significantly in the inheritance of resistance to WmCSV in this cross as additive x additive and dominance x dominance. However, significant lack of fit of the AUDPC indicated that the trait was controlled by more than additive x additive and dominance x dominance genetic effects.

Disease gene effects were reported for resistance to WmCSV in watermelon by [12]. They suggested that the dominance seems to be incomplete since F1 mean of resistance was not equal to the donor and they contained susceptible plants with different levels on the rating scale. F2s and Bc1s were segregating between resistance and susceptibility did not fit with any known Mendelian segregation ratio [12]. These results differ from those of resistance to WmCSV identified in melon (Cucumis melo L.), which were found to be controlled by one dominant gene in addition to one recessive gene [13]. Differences in the level of resistance among progenies resulted from the crosses with the four commercial cultivars were noticed. These differences could be attributed to the differences in the genetic background of the commercial cultivars [12].

Table 01: Mean squares of initial and final severities and AUDPC for six generations from cross Grift x Crimson Sweet evaluated in 2016

| Source of variation | Initial severity | Final severity | AUDPC | Flowering days |
|---------------------|------------------|----------------|-------|----------------|
| Generations         | 4.11*            | 4309.65***     | 6212012.00*** | 41.28*         |
| Rep                 | 2.29ns           | 6.48ns         | 23210.00ns   | 36.03ns        |
| Residual            | 1.339            | 6.501          | 10418        | 10.44          |
| LSD                 | 1.744            | 3.843          | 153.8        | 4.936          |
| CV%                 | 187.30           | 11.90          | 12.60        | 7.90           |

Significant differences at * = P ≤ 0.05 and *** = P ≤ 0.001, a = Area under disease progress curve, computed as described by Madden et al. (2007). b = Initial severity. Disease severity was recorded 38 days after planting. c = Final severity was taken till senescence.
Table 02: Mean performance of six generations of Grift x Crimson Sweet evaluated in Shambat in summer season of 2016

| Populations          | Description        | No. of plants | Initial severity | Final severity | AUDPC      | 50% flowering days |
|----------------------|--------------------|---------------|------------------|----------------|------------|-------------------|
| Grift                | Resistant (P1)     | 60            | 0.00             | 0.00           | 0.000      | 44.67             |
| Crimson Sweet        | Susceptible (P2)   | 60            | 3.09             | 100.0          | 3756.0     | 43.65             |
| MP                   | -                  | 60            | 1.55             | 50.0           | 1878.0     | 44.16             |
| F1                   |                     | 60            | 0.93             | 3.76           | 142.4      | 42.00             |
| F2                   |                     | 240           | 0.00             | 8.49           | 246.2      | 37.00             |
| BC1F1 (P1)           |                     | 210           | 0.00             | 0.00           | 1.010      | 38.67             |
| BC1F1 (P2)           |                     | 210           | 0.93             | 44.7           | 1777.0     | 37.00             |
| Minimum              |                     |               | 0.00             | 0.00           | 0.000      | 37.00             |
| Maximum              |                     |               | 3.09             | 100.0          | 3756.0     | 44.16             |
| Grand mean           |                     |               | 0.62             | 21.4           | 807.0      | 40.09             |
| Mean                 |                     |               | 0.71             | 24.5           | 922.4      | 40.09             |
| LSD (p≤0.05)         |                     |               | 1.74             | 3.84           | 153.8      | 4.940             |
| CV%                  |                     |               | 187.0            | 11.9           | 12.60      | 7.900             |
| SED                  |                     |               | 0.82             | 1.80           | 72.20      | 2.290             |
| ±SE                  |                     |               | 0.57             | 1.26           | 50.30      | 1.590             |

Crimson Sweet = female parent, Grift = male parent, F1 = first generation, F2 = second generation, BC1F1 = back cross first generation, MP = mid-parents (average of Grift and Crimson Sweet).

Table 031: Mode of inheritance explained by genetic effects in Grift x Crimson Sweet evaluated in Shambat in 2016

| Source of variation | df | Initial severity | Final severity | AUDPC          |
|--------------------|----|------------------|----------------|----------------|
| A                  | 1  | 5.05***          | 5986.36***     | 8629043.00***  |
| D                  | 1  | 0.64***          | 1733.48***     | 2435349.00***  |
| aa                 | 1  | 1.33***          | 220.69***      | 353793.00***   |
| ad                 | 1  | 0.15***          | 11.36***       | 4154.00**      |
| dd                 | 1  | 0.03***          | 25.62*         | 80669.00***    |
| Lack of fit        | 1  | 0.03***          | 25.62***       | 80669.00ns     |
| Residual           | 15 | 1.34             | 6.50           | 10418.00       |
| Population type    | 5  | 1.44             | 1595.50        | 2300602.00     |

Significant differences at * = P≤ 0.05 and *** = P ≤ 0.001, a = additive, d = dominance, aa = additive x additive ad = additive x dominance and dd = dominance x dominance.

IV. CONCLUSION

The study revealed that the inheritance of WmCSV disease is more complex than the simple additive dominance model and epistasis was involved significantly in the inheritance of resistance to WmCSV in this cross. Due to the presence of greater non-additive gene effects combined with significant epistasis gene action, selection for almost all of the studied traits in this cross, especially in early generations, remains complex using only conventional methods.

BIBLIOGRAPHY

[1] FAOSTAT. (2016). Food and agriculture organization of the United Nations, statistics division online available at http://faostat3.fao.org
[2] Goda, M. (2007). Diversity of local genetic resources of watermelon Citrullus lanatus (Thunb.)
[3] Annual report. http://www.arcsudan.sd/English.html.
[4] Jones, David L. (1995). Palms throughout the World. Washington, D.C: Smithsonian Institution Press. p. 86. ISBN 1-56098-616-6.
[5] Madden, L.V. Hughes, G. and van den Bosch, F. (2007). The Study of Plant Disease Epidemics. American Phytopathological Society. St. Paul Minnesota, USA.
[6] Steel, R.G.D. and Torrie, J.H. (1997). Principles and procedures of statistics: a biometrical approach. McGraw-Hill, New York.
[7] Bernardo, R. (2002). Breeding for quantitative traits in plants. Library of congress control Number 2002091971. Stemma press Woodbury, Minnesota USA
[8] Kearsey, J. M. and Pooni, S. 1996. The genetical analysis of quantitative traits, Chapman and Hall, London, UK.
[9] Abebe, H., Setegn, G. and Habtamu, Z. 2013. Generation mean analysis and heritability of drought resistance in common bean (Phaseolus vulgaris L.). African Journal of Agricultural Research 8 (15):1319–1329.
[10] Gaikwad, K., Lal, J. and Kumar, H. (2009). Genetic Architecture of Yield Attributing Characters in Sesame (SesamumindicumL.). Crop Improvement 36 (1):1-5.
[11] Jawahar, L. J., Kuldeep, S. D., Sudheer, K. S., Bhandari, H. R. and Tripathi, M. K. 2013. Estimation of gene effects based on joint scaling test and sequential model fit scheme for quantitative traits in Sesame (SesamumindicumL.). Journal of Agricultural Science 5 (3):224–235.
[12] Elhassan, R. M., Omara, S.K., Dafalla, G. A., Yousif M.T, El-jack A.E., (1996). Evaluation of Watermelon (Citrullus spp.) germplasm for resistance to Watermelon Chlorotic Stunt Virus. Sudan Journal of Agricultural Research Vol. 12 (2008) PP. 79-84.
[13] Mohamed T. Y., Mohamed T. Y., Gasim A. D., Kheyir-Pour, A., Gronenborn, B., Pitrat, M. and Dogimont, C. (2005). Genetic Stability of Resistance to Watermelon Chlorotic Stunt Virus in melon (Cucumis melo L.). Gezira Journal of Agricultural Science 3 (1): 54-64.