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EFFECT OF COTTON CHROMOSOME SUBSTITUTION AND RENIFORM NEMATODE RESISTANT LINES IN RESISTANCE TO NON-DEFOLIATING PATHOTYPE OF *VERTICILLIUM DAHLIAE*

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Abstract. Verticillium wilt (VW) is an important and widespread disease of cotton and once established is long-lived in soil and difficult to manage. Non-defoliating pathotype of Verticillium dahliae fungi is extremely virulent. Identification and development of new cotton lines with increased VW resistance is the only effective method of disease management. Chromosome substitution (CS) lines are unique germplasm with identical genetic background from TM-1 double haploid line and one introgressed chromosome from *G. barbadense* specie which considered having natural resistance to VW. This paper discusses results of first evaluation of VW resistance of 17 CS lines, their parental lines and five reniform nematode resistant lines using a controlled environment bioassay. Chromosome substitution lines displayed contrasting levels of resistance to VD11 pathotype of VW. Five CS lines and five nematode resistant lines demonstrated high level (0.4-0.6DSI) of resistance after inoculation by spore suspension of pathogen. Based on results of membrane damage assay M713 Ren5 line showed high resistance to pathogen inoculum. The comparative analysis of such unique genetic materials as chromosome substitution lines greatly empowers the detection of chromosomal effects on disease resistance. Crosses between identified VW resistant CS lines can provide unique opportunity to combine alleles associated with disease resistance from two substituted chromosome in one genome.

Keywords: *Cotton, Verticillium wilt, chromosome substitution, membrane damage, spore suspension, inoculation.*

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1. Introduction

Cotton is an important fiber crop grown in more than 30 countries around the world (Ullah *et al*., 2017). Cotton is commonly regarded as being a partially cross-pollinated crop. Insect pollination, in particular honey bee pollination, can aid in this process and result in higher yields and better quality lint. Among the cotton species, *Gossypium hirsutum* accounts for 95% of world cotton production due to its high productivity and fiber quality (Zhang *et al*., 2014). Different biotic and abiotic stress factors are important factors limiting cotton production (Gul *et al*., 2025). Serious losses occur every year due to various diseases caused by microbes, viruses, fungi and nematodes affecting cotton yield and fiber quality. According to recent reports in the USA, 480 million bales of cotton

were lost between 1990 and 2014 due to the wilting diseases (Disease database; Lawrence *et al*., 2016). In China, more than 40% of cotton-growing areas are currently infected with *V. dahliae*, with a direct economic loss of US$250-310 million (Palanga *et al*., 2021).

Verticillium wilt was first found in Virginia and later spread to various cotton-growing countries around the world. Fungi in particular account for 2 out of 3 infectious plant diseases (Carris *et al*., 2012; Salimov *et al*., 2025). Recent climate changes have driven many widespread epidemics of Verticillium wilt. The monoculture system for a long time, the frequent introduction of new germplasm from different countries are the main reasons of such widespread infections (Jing *et al*., 2018; Khalid *et al*., 2023; Salimov *et al*., 2025).

Cotton has a number of fungal diseases that affect the growth and development of the plant; these include Anthracnose, Powdery mildew, Ascochytosis, black rot, leaf spot, Verticillium wilt, Fusarium wilt, etc. (Nyvall, 2013). In recent years, the introduction of germplasm resistant to Fusarium wilt has brought the disease under control, but control of Verticillium wilt still requires further research. The two main pathogens of Verticillium wilt in cotton are *V. dahliae* and *V. albo-atrum.* Of these, *V. dahliae* is more widespread (Shen, 1985). When fungal spores come into contact with the plant surface, the microclimate, air temperature and humidity affect their ability to break cell membranes of host plant. *V. dahliae* enters through the roots and invades the vascular system of plants (Chen, 1994). Mycelia grow faster at 22-25 ^{o}C. *G. hirsutum* (Upland cotton) cultivars are more susceptible compared to the *G. barbadense* (Egyptian cotton). Based on research reports *G. barbadense* are considered to have genes of resistance against the *V. dahliae* fungus (Jenkins *et al*., 2018). Currently, an important target of scientists working in the field of plant genetics and breeding is the detection of wilt resistance genes in *G. barbadense* L. and their use in the creation of transgenic plants (Wang *et al*., 2004).

The initial symptoms of infection with wilt disease are the formation of necrotic zones on the leaves, chlorosis, wilting, browning of the vascular system. Vascular browning is a characteristic symptom before wilting, if the external environmental factors are favorable for the development of fungal mycelia, the plant is quickly destroyed (Fradin & Thomma, 2006; Fakhari *et al*., 2025). The disease also affects bolls, penetrates into the seed and causes post-infection symptoms. This significantly affects the quality of the fiber, reducing the micronaire and fiber length (Mammadova *et al*., 2024b; Karademir *et al*., 2012).

The virulence mechanisms of fungal pathogens are quite complex and controlled by multiple signaling pathways (Amrahov *et al*., 2023a). The study of how these signaling pathways interact with each other will lead to the development of effective disease control strategies. Due to the lack of effective fungicides and with only a few exceptions, sufficient genetic resistance, the study of these mechanisms becomes more important (Amrahov *et al*., 2024). Since plants and pathogens interact interactively with each other, there are several signaling pathways through which this interaction is carried out. There are two types of stress resistance mechanisms in plants, intrinsic resistance and induced resistance (Mammadova *et al*., 2024a). Induced resistance is activated by pathogen infection and involves a number of molecular regulatory mechanisms at the cellular level; involves structural changes in plants to resist pathogen invasion and proliferation and ultimately allow plants to gain resistance. Several reports have shown structural variation among cotton cultivars with different disease resistance to *V. dahliae* (Song *et al*., 2020)

Unlike other fungal pathogen, *V.dahliae* produces hiphopodium, narrowed infective structures to the penetration pole to enter cotton roots (Zhao *et al*., 2016). To depolarize

the plant cell wall, *V. dahliae* secretes cell wall degrading enzymes (Amrahov *et al*., 2023b). After successfully penetrating the host, fungal pathogens adapt to the host intercellular environment and induce some defense responses to survive against plant immunity and nutrient deficiency conditions. The rapid release and secretion of toxins results in the death of host plants and eventually provides the environment for the transition to the necrotrophic phase (Gao *et al*., 2010).

Much research has been done to understand the genetics and inheritance of Verticillium wilt in cotton, but the results have been inconsistent. Many experts use the Sea-island cotton resistance model to understand the resistance of Upland cotton to Verticillium wilt. However, resistance in Sea-island cotton is largely controlled by one or more genes and has relatively stable resistance to different strains of *V. dahliae*. It is generally accepted that resistance to Verticillium wilt in Upland cotton is a quantitative trait controlled by multiple genes (Jiang *et al*., 2009). In parallel with the development of molecular biology, genetic engineering technologies, breeding programs for disease resistance have been developed, but still a deeper investigation of the key factors of the defense mechanism in the pathogen-plant interaction is required. Identification of chromosomal location of the genes of resistance to biotic and abiotic stress in plants has big importance in transferring of certain chromosomes or chromosome arms using different techniques (Jenkins *et al*., 2018; Nazik *et al*., 2025).

Gossypium barbadense exhibits a higher resistance to Verticillium wilt compared to G. hirsutum (upland cotton). This difference in resistance is linked to faster detection of the pathogen by G. *barbadense*, stronger defense gene expression and the involvement of specific genes. Understanding the molecular mechanisms underlying Verticillium wilt resistance in G. *barbadense* provides valuable insights for breeding programs aimed at enhancing resistance in upland cotton, a more widely cultivated specie. Chromosome substitution lines with introgressed chromosome from resistant genotypes or exotic germplasm are unique material to determine chromosomal location of genes associated with resistance.

This paper discusses results of first artificial inoculation of cotton chromosome substitution lines and reniform nematode resistant mutant lines by *V.daliae* spore suspension to identify chromosomal location of Vertcillium wilt resistance genes.

2. Material and Methods

Plant material

Plant material for this study consisted of seventeen chromosome substitution lines with introgressions of chromosome or chromosome arm from *G. barbadense* cv. Pima 3-79 into genetic background of *G. hirsutum* TM-1 via hybridization of their parental ines – TM-1 and Pima 3-79 and five reniform resistant (Ren) lines. Ren lines originated from the photoperiodic *G. barbadense* accession GB713 and were selected using the tightly linked simple sequence repeat (SSR) markers. Reniform nematode resistant mutant lines (M713 Ren1, M713 Ren2, M713 Ren3, M713 Ren4 and M713 Ren5) resistant to the reniform nematode, *Rotylenchulus reniformis* Linford and Oliveria were developed and released by the USDA-ARS and the Mississippi Agricultural and Forestry Experiment Station in 2012. List of plant material, their genetic background represented on Table 1.

Seeds of chromosome substitution lines were obtained from the USDA-ARS, Mississippi State, MS, United States (Jenkins, 2018).

Table 1. Plant material used for *V.dahliae* (ND VD11) resistance evaluation

N	Genotye	Specie	Genetic background
1	Pima 3-79	G. *barbadense*	double haploid line
2	TM-1	G. *hirsutum*	highly inbred line
3	CS-B01	CS line	substitution of 1st chromosome from Pima 3-79 in TM-1genome
4	CS-B02	CS line	substitution of 2nd chromosome from Pima 3-79 in TM-1genome
5	CS-B04	CS line	substitution of 4th chromosome from Pima 3-79 in TM-1genome
6	CS-B05	CS line	substitution of 5th chromosome from Pima 3-79 in TM-1genome
7	CS-B06	CS line	substitution of 6th chromosome from Pima 3-79 in TM-1genome
8	CS-B07	CS line	substitution of 7th chromosome from Pima 3-79 in TM-1genome
9	CS-B11sh	CS line	substitution of short arm of 11th chromosome from Pima 3-79 in TM-1 genome
10	CS-B12sh	CS line	substitution of short arm of 12th chromosome from Pima 3-79 in TM-1 genome
11	CS-B14sh	CS line	substitution of short arm of 14th chromosome from Pima 3-79 in TM-1genome
12	CS-B15sh	CS line	substitution of short arm of 15th chromosome from Pima 3-79 in TM-1genome
13	CS-B16	CS line	substitution of 16th chromosome from Pima 3-79 in TM-1genome
14	CS-B17	CS line	substitution of 17th chromosome from Pima 3-79 in TM-1genome
15	CS-B18	CS line	substitution of 18th chromosome from Pima 3-79 in TM-1genome
16	CS-B22sh	CS line	substitution of short arm of 22th chromosome from Pima 3-79 in TM-1genome
17	CS-B22Lo	CS line	substitution of long arm of 22th chromosome from Pima 3-79 in TM-1genome
18	CS-B25	CS line	substitution of 25th chromosome from Pima 3-79 in TM-1genome
19	CS-B26Lo	CS line	substitution of long arm of 26th chromosome from Pima 3-79 in TM-1genome
20	M713 Ren1	*G.barbadense*	
21	M713 Ren2	*G.barbadense*	
22	M713 Ren3	*G.barbadense*	
23	M713 Ren4	*G.barbadense*	
24	M713 Ren5	*G.barbadense*	

Soil preparation and V.dahliae inoculation with pathogen conidia suspension

Soil was sterilized at 120ºC for 1 hour, mixed in equal parts with perlite and turf. Pots were filled with mixture and three seeds were planted in each plastic pot. After germination seedlings were thinned and one seedling per pot was left. Plants were maintained in growth chamber and watered with distilled water until treatment. At 2-3 true leaf stage pants were treated by pathogen spore suspension. Five biological and 3 technical replicates were used for disease severity ranking. Ten plants per replicate were used.

Preparation of V.dahliae spore suspension

The VD 11 non-defoliating pathotype isolate of *V.dahliae* was isolated from infected cotton was used in this study. The resistance level of cotton genotypes to *V.dahliae* was determined using spore suspension inoculation method.

V.dahliae isolate (VD 11) was grown in Potato Dextrose Agar (PDA). Two week old culture of *V.dahliae* was used for inoculation. To collect pathogen conidia petri dishes with *V.daliae* culture were flooded with water. Fungal conidia was harvested from PDA surface using a sterile spatula. To remove mycelium particles spore suspension was filtered with double layers of sterilized cheesecloth. The spore suspension was adjusted to 4 x 10^6 spores per ml^{-1} with the use of haemocytometer.

Inoculation of seedlings

Inoculation was performed at 3-4 true leave stage of seedlings. Five ml of spore suspension was injected to the bottom of each plastic pot. Mock inoculated plants were used as control. The experiment was carried out in a randomized plot design with four replications per treatment for each line and two treatments (VD 11 -treated and non-treated control) in the growth chamber (25±1ºC, 12h light/12h dark cycle). The mock inoculated (non-treated) controls were planted and grown at the same conditions as the treated plants.

Randomly selected seedlings (5%), were assayed to determine if they were infected by *V. dahliae*. For this analysis, collected seedling stems were washed in running tap water, surface disinfested in 0·5% sodium hypochlorite for 1 min. Stem pieces of seedlings were placed on potato dextrose agar (PDA) plates and incubated at 25°C in the dark for 7 days. The identity of *V.dahliae* was confirmed by microscopic observations of conidiophores and by the formation of microsclerotia.

Disease Severity Index

Raiting of Verticillium wilt symptoms was conducted starting from 21 DPI (days post inoculation). Experimental plants (control and inoculated) were evaluated based on progression of the disease using a scale 0 to 5 (Tsror *et al*., 2001).

Disease Severity Index (DSI) was calculated using Karman method (1971).

$$DSI=[(a\times0)+(b\times1)+(c\times2)+(d\times3)+(e\times4)+(f\times5)]/M \quad (1)$$

DSI: Disease severity index;
a, b, c, d, e, f: Number of plants with 0, 1, 2, 3, 4, 5 rating respectively,
M: Total number of plants.

0=a healthy plant with no visible leaf symptoms; 1=mild to moderate leaf symptoms or <25% chlorotic/necrotic leaves; 2=severe leaf symptoms or 25-50% chlorotic/necrotic leaves; 3= 60-70%; 4= >75% chlorotic/necrotic leaves or some terminal dieback, plant often stunted and 5= defoliation, with stems dying back or dead to ground level. An average severity rating is then calculated for each genotype on a replication basis from the sum of rating x number of plants divided by the total number of plants evaluated.

Statistical analysis

All data obtained in the experiments were analyzed statistically by using JMP statistical software program (5.0.1, SAS Institute, Cary, NC) and SPSS v25 for analysis of variance.

Quantification of membrane damage/cell death using Evan's Blue Staining technique

Membrane damage (cell death) was quantified using Evan's Blue Staining method.

One of the most resistant CS line (CS-B01) and its resistant parental line (Pima 3-79) was subjected to Evan's blue assay. Three biological replicates were used to quantify the uptake of Evan's blue dye and the data was analyzed using the GenStat program. Root tissues collected from 30 days old inoculated and non inoculated seedlings, were transfered to 2ml Eppendorf tubes. 2ml Evan's blue solution was added to each tube. Volumes were adjusted to ensure that tissues are immersed in the solution. Tubes were shaked in an orbital shaker at 50 oscillations/min for 20 min. To extract the Evan's blue dye from the roots 1ml of 1% SDS cell lysis buffer was used. 100 mg of root tissue was grinded using tissue lyser. After grinding the extract was transfered to 2ml Eppendorf tubes and centrifuged at 7.167 xg for 5 min at room temperature. An aliquot of supernatant was transferred to new tubes. The optical density was measured at 600nm spectrophotometrically using a cuvette by taking 1% SDS as blank. Each measurement was performed on 3 technical replicates. 1 µg of Evan's blue was dissolved in 1 ml of distilled water to get 1 µg ml-1 stock solution. From stock solution five concentrations were prepared by making up the volume to 1 ml. The absorbance was measured at 600 nm in spectrophotometer (Figure 2). A minimum of three biological replicates were used to quantify the uptake of Evan's blue dye and the data were analyzed using the GenStat program. Concentration of Evan's blue dye was estimated by reffering to a standard curve. Significance level was tested using analysis of variance (ANOVA) to determine statistically significant differences between the means of independent (unrelated) groups.

3. Results and Discussion

Experiments were conducted under controlled conditions in growth chamber to minimize environmental variables that affect assessment of VW resistance of cotton lines. The two parental lines TM-1 and Pima 3-79 displayed contrasting levels of resistance to the ND VD11 pathogen (Table 2).

Table 2. Tukey's honestly significant difference test of mean DSI values of genotypes (DSI values) is key but poorly formatted and should be redone with clear labeling (columns: Genotype, Species, Mean DSI, Statistical Group).

Genotype	Statistical group								Mean DSI values of genotypes
CS-B14sh	A								1,8000000
CS-B17	A								1,8000000
CS-B11sh		B							1,6000000
CS-B16		B							1,6000000
CS-B18		B							1,6000000
CS-B25		B							1,6000000
CS-B07			C						1,4000000
CS-B12sh			C						1,4000000
CS-B15sh			C						1,4000000
CS-B22sh			C						1,4000000
CS-B26Lo			C						1,4000000
CS-B06				D					1,2000000
TM-1					E				0,8000000
M713 Ren1					E				0,8000000
M713 Ren2					E				0,8000000
M713 Ren4					E				0,8000000
CS-B02					E				0,8000000
CS-B04					E				0,8000000

Genotype	Statistical group								Mean DSI values of genotypes
CS-B05					E				0,8000000
M713 Ren3						F			0,6000000
CS-B01							G		0,4000000
M713 Ren5							G		0,4000000
CS-B22Lo							G		0,4000000
Pima 3-79								H	0,2000000

CV (%) = 1,49
LSD = 2,99

Summary of Fit

RSquare	1
RSquare Adj	1
Root Mean Square Error	1,822e-9
Mean of Response	1,075
Observations (or Sum Wgts)	72

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	25	16,875000	0,675000	2,03e+17
Error	46	1,5266e-16	3,32e-18	**Prob > F**
C. Total	71	16,875000		<,0001*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Genotypes	23	23	16,755000	2,2e+17	<,0001*
Replication	2	2	0,120000	1,81e+16	<,0001*

The high level of (Verticillium wilt) VW resistance (0.2 mean DSI- Disease Severity Index) was observed in Pima 3-79 line. Second group was consisted of tree genotypes - two cromosome substitution lines CS-B22Lo, CS-B01 and reniform nematode resistant line M713 Ren5 with 0.4 mean DSI. Three chromosome substitution lines – CS0B02, CS-B04, CS-B05, their parental line TM-1 and three nematode resistant lines – M713 Ren1, M713 Ren2, M713 Ren4 showed moderate resistance to VW with 0.8DSI.

M713 Ren3 line with 0.6 DSI was separated from both E and F groups showing high resistance. Genotypes demonstrating VW disease severity index between 0 to 1 are considered as disease resistant. Twelve genotypes (Pima 3-79, CS-B22Lo, M713 Ren5, CS-B01, M713 Ren3, CS-B05, CS-B04, CS-B02, M713 Ren4, M713 Ren2, M713 Ren1 and TM-1) demontrated second high level (0.8 DSI) of resistance to ND VD11 pathotype of VW. Chromosome substitution lines CS-B14sh and CS-B17 were characterised as most susceptibe lines with high (1.8) disease severity index.

G. hirsutum and *G. barbadense* species have a common allotetraploid ancestor and originated from a natural hybridization between diploid *G. arboreum* and *G. raimondii* (Paterson *et al*., 2012; Alizade & Mammadova, 2023). The different evolution and domestication process resulted in significant differences including resistance to biotic and abiotic stress. Hu et al. (2021) reported that *G. hirsutum* possesses high resistance to

abiotic stress while *G. barbadense* cultivars show superior performance in terms of disease resistance, such as Verticillium wilt and Fusarium wilt.

The adavantage of chromosome substitution lines is that each line is genetically fixed and have identical genetic background from highly inbred line - TM-1 (*G.hirsutum*) with only one introgressed chromosome or chromosome arm from Pima 3-79 double haploid line (*G.barbadense*). Substitution of one chromosome or chromosome arm from *G.barbadense* into *G.hirsutum* background is a new approach to transfer the superior traits including disease resistance from *G. barbadense* into *G. hirsutum* cultivars (Ma *et al*., 2021).

Results showed that all nematode resistant Ren lines demonstrated resistance to VW pathogen. Nematode resistance of germplasm lines -M713 Ren1 (PI 665928), M713 Ren2 (PI 665929), M713 Ren5 (PI 665930) were derived from photoperiodic *G.barbadense* accession GB713 (McCarty *et al*., 2013). McCarty et al. (2013) reported that the lines demonstrated high level of nematode resistance in greenhouse experiments. Evaluation of Ren and BARBREN-713 lines in reniform nematode-infested fields in Alabama, Texas and Louisiana resulted in suppression of reniform nematode populations by 55% (Bell *et al*., 2015). In our experiments M713 Ren1, M713 Ren2 and M713 Ren5 lines exhibited high level of resistance to VW pathogen. Based on current and previous experiment results M713 Ren1, M713 Ren2, M713 Ren3, M713 Ren4 and M713 Ren5 lines can be characterized as double stress (VW and nematode) resistant lines.

In this study soil treatment with *V.dahliae* spore suspension was used for rapid assesement of resistance of cotton lines against pathogen. Different inoculation methods such as root-dipping, root dipping with cutting (wound inoculation), soil tratment with spore suspension or conidia sispension are used in experiments for evaluation of resistance of young cotton seedlings to Vertcillium wilt (Platt & Sanderson, 1987).

We used soil treatment with spore suspension which is more similar with natural inocualtion in the field. This method is eliminating stress induced by changing evironment which is characteristic for root-dipping method. Wound inoculation (root cutting or inoculation of stem using syringe) is mechanically breaking first membrane defence of plant and causing double stress. *V.daliae* is soil borne fungi and soil treatment with spore suspension is more accurate method for evaluation of natural resistance of cotton lines.

Quantification of membrane damage

The plant cell walls, consisting of lignin, pectin, hemicellulose, cellulose, lipids and structural proteins, that play an important role on protecting plant from the invasion of pathogens (Ishida & Noutoshi, 2022). Toxins and enzymes including xylanase, protease, pectinase and cellulase produced by *V. dahlia* acting on intracellular components including plasma membrane and cell walls causing cytotoxicity, degrading the host plant cell membrane (Zhang *et al*., 2022). Degradation of plant cell membrane has correlation with penetration, germination and growth of VW microsclerotea. Evans blue dye method allows quantifying the level of damaged cells after pathogen inoculation. Standard graph for Evans blue was prepared using 1 µg ml-1 of Evan's blue stock solution. Different concentrations of Evan's blue were diluted and absorbance was recorded at 600 nm.

For evaluation of membrane damage three genotypes (Pima 3-79, CS-B01 and M 713 Ren5) with high level of resistance were subjected to Evans blue assay.

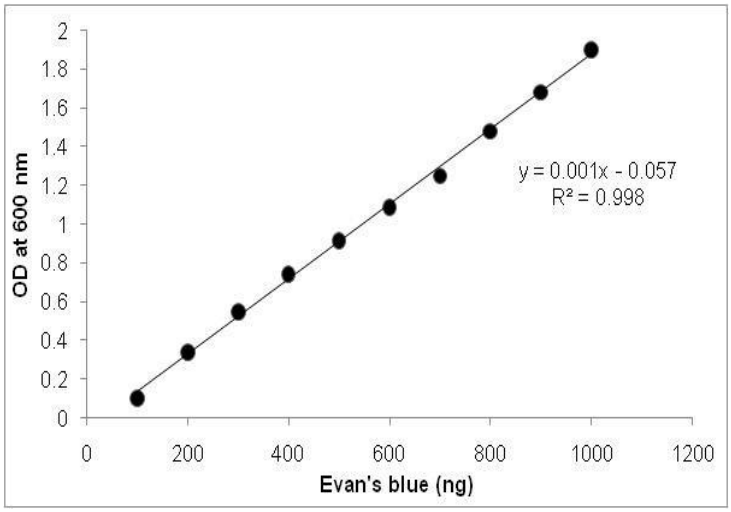

Figure 1. Standart graph for Evan's blue staining

Root samples cotton seedlings inoculated by pathogen conidia suspension and mock inoculated control plants were stained in Evans blue dye solution. After staining absorbed dye was extracted and quantified spectrophotometrically (Figure 2).

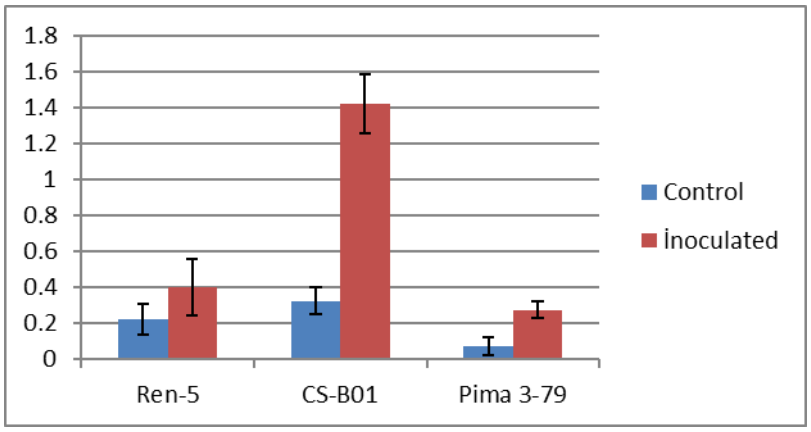

Figure 2. Evans blue quantification assay of VW resistant genotypes

Genotypes demonstrated different results of membrane damage assay. Comparatively less membrane damage was observed both in control and inoculated seedlings of Pima 3-79. Ren5 line demonstrated less membrane damage compared to CS-B01 chromosome substitution line.

Based on assessment of chromosome substitution lines CS-B0, CS-B02, CS-B04, CS0B05 and CS-B22Sh demonstrated high resistance to VW non-defoliant pathotype. M713 Ren1, M 713 Ren2, M713 Ren4 and M 713 Ren5 lines possessing reniform nematode resistance also demonstrated high resistance to VW VD11 non defoliating pathotype. These lines can be characterized as double stress resistant genotypes.

4. Conclusion

Cotton genotypes have evolved morphological, biochemical and physiological responses by accumulating antifungal macromolecules, modifying the structure of tissue

and other important immune responses. Number of genes is involved in plant protection mechanisms of plants against pathogens. Introgression of target alien genes from unadapted species into Upland cotton using conventional breeding is extremely difficult. CS lines are unique germplasm and innovative approach to introgress resistance to disease into commercial varieties. CS lines are isogenic to parental line TM-1 and each other for 24 chromosome pairs except for one alien introgressed chromosome or chromosome arm. Use of *V.dahliae* resistant CS lines identified in this study will eliminate challenges characteristic for interspecific hybridization. Results of this study will provide important gene resources for the breeding of new cotton germplasm resistant to Verticillium wilt. Findings of this study will allow to narrow search of Verticillum wilt resistant genes from whole genome of resistant tetraploid *G. barbadense* genome to certain chromosomes of resistant CS line. First screening results of CS lines will contribute to the development effective strategy of plant protection and disease management which is complicated due to the lack of available resistant germplasm in Upland cotton.

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