SEGREGATION ANALYSIS OF VERTICILLIUM WILT RESISTANCE IN FOURTEEN *GOSSYPIUM HIRSUTUM* × *GOSSYPIUM BARBADENSE* HYBRIDS

Huiping Zhou
Hui Fang
Department of Plant and Environmental Sciences, New Mexico State University
Las Cruces, NM
Soum Sanogo
Department of Entomology, Plant Pathology and Weed Science, New Mexico State University
Las Cruces, NM
Jinfa Zhang
Department of Plant and Environmental Sciences, New Mexico State University
Las Cruces, NM

Abstract

Verticillium wilt (VW) caused by *Verticillium dahliae* is one of the limiting factors in Upland cotton (*Gossypium hirsutum*) production. VW resistance in Pima cotton (*Gossypium barbadense*) has not been successfully transferred into commercial Upland cotton cultivars. The objective of this current study was to investigate the genetic basis of VW resistance in multiple Upland x Pima hybrid populations. F_2 progenies from 14 crosses between four Upland and four Pima cotton cultivars or breeding lines were evaluated for VW resistance in the greenhouse using artificial inoculation method. The results showed that VW resistance in seven of the 14 F_2 cross combinations fitted the segregation ratio of 3:1, indicating that the VW resistance in Pima cotton is controlled by a major gene.

Introduction

In cotton production, Verticillium wilt (VW) caused by *Verticillium dahliae* is one of the major limiting factors affecting cotton yield and fiber quality. VW caused 0.5 ~ 3.5% of annual yield loss in the U.S., and 3.5 ~ 5.0% in New Mexico (Blasingame and Patel, 2005). Breeding and planting VW resistance cultivars is the most economic method to control this disease (Lüders et al., 2008). However, there is no consensus on whether VW resistance is controlled by one major gene or multiple minor genes (Bolek et al., 2005; Fradin et al., 2006; Mert et al., 2008). Understanding the genetic basis of the resistance to this disease will be beneficial to breed for Verticillium wilt resistant cultivars. Pima cotton is known as a good source VW resistance; however, its resistance has not been successfully transferred into commercial Upland cotton cultivars due to hybrid breakdown and low selection efficiency for VW resistance. The objective of this current study was to investigate the genetic basis of VW resistance in multiple Upland x Pima hybrid populations.

Materials and Methods

Materials

Four elite Upland cotton genotypes, Acala 1517-08, LA 35RS, UA 48 and MD 25-27Y were used as males to cross with four Pima cotton cultivars, Pima S-7, DP 340, Cobalt and Phy 830. The resulting F_1 hybrids, except for LA 35RS x Phy 830 and UA 48 x DP 340, were selfed to produce F_2 populations. The 14 F_2 populations and their parental lines were used for evaluation of VW resistance.

VW evaluation

Seeds from the 14 F_2 populations and their parents were planted in 4-inch plastic pots with 2 seed/pot (2 hills/pot, 1 seed/hill) in the greenhouse on May 18th, 2012. The pots were filled with potting soil (Scott 450, Marysville, OH, USA) mixed with slow release Osmocote® fertilizer (Marysville, OH, USA). The pathogen, *V. dahliae* was isolated from infected cotton plants and was cultured in Czapek-Dox broth on a rotary shaker at 140 rpm at 22-25℃ for 15 days. The suspension was filtered through a double layer of cheesecloth to separate spores (conidia) from mycelia and the spore concentration was adjusted using a hemacytometer. When seedlings were at the 2nd/3rd true leaf stage, root inoculation was made by pouring 100 mL of conidia suspension per pot on June 6th (4.07×10^6 conidia/mL). The second inoculation was made on June 17th (3.37×10^6 conidia/mL) to ensure a consistent infection by *V. dahliae*.

The assessment of VW severity in each plant was made based on plant symptoms on August 10th (65 days after inoculation) using a 0-5 scoring scale (Figure 1) following Zhang et al (2011):

0	No symptom
1	<25% chlorotic/necrotic leaves
2	25-50% chlorotic/necrotic leaves
3	50-75% chlorotic/necrotic leaves
4	>75% chlorotic /necrotic leaves
5	Complete defoliation or plant death

Figure 1. Scoring scale of Verticillium wilt.

The number of infected leaves and defoliated leaves were also recorded for calculating the percentage of infected plants, average severity (rating) and percentage of defoliated leaves.

Data Analysis

The data were subjected to analysis of variance (ANOVA) and Chi-square analysis using SAS 9.3 (SAS Institute Inc., 2012). The Chi-square at $\alpha = 0.05$ was used to verify if the segregation ratio of an F_2 population fits 3:1. The least significant difference (LSD) at the 5% significant level was used to compare differences between genotypes.

Results and Analysis

Differences among parents

The results of ANOVA for rating, percentage of infected plants and percentage of defoliated leaves indicated that significant differences existed among the eight parents (Table 1). Two Upland cotton cultivars, UA 48 and LA 35RS were the most resistant genotypes with the lowest values of these three traits. However, the other two Upland cotton Acala 1517-08 and MD 25-27Y, and two Pima cotton Pima S-7 and Pima DP 340 had higher mean values of the three traits, indicating that they were more susceptible to VW. In fact, Pima DP 340 had the highest rating (3.5), percentage of infected plant (67%), and percentage of defoliated leaves (53.3%), and was therefore the most susceptible genotype tested in the current study.

Table 1. Response of eight parental genotypes of Pima and Upland cotton to *Verticillium dahliae*.

Parents	Rating	Infected plants %	Defoliated leaves %	Species
DP 340	3.46a	66.99a	53.30a	*G. barbadense*
Acala 1517-08	3.17ab	66.08ab	43.36abc	*G. hirsutum*
Pima S-7	3.15ab	57.77bc	49.59ab	*G. barbadense*
MD 25-27Y	3.08ab	58.72bac	36.39c	*G. hirsutum*
Cobalt	2.82bc	55.47dc	41.64bc	*G. barbadense*
Phy 830	2.69bc	54.85dc	36.36c	*G. barbadense*
LA 35RS	2.32c	48.20de	21.84d	*G. hirsutum*
UA 48	2.24c	46.08e	20.58d	*G. hirsutum*
F Value	4.61	6.49	11.29	
Pr> F	0.0042	0.0006	<0.0001	
LSD (0.05)	0.59	8.72	10.18	

Mean values followed by the same letter are not significantly different at α= 0.05.

Segregation analysis of VW resistance in F_2 populations

Out of 14 F_2 populations, segregation of VW resistance in seven combinations, i.e., LA 35RS × DP 340, MD 25-27Y × Pima S-7, MD 25-27Y × DP 340, MD 25-27Y × Cobalt, MD 25-27Y × Phy 830, Acala 1517-08 × Cobalt, and Acala 1517-08 × Phy 830, fitted a 3:1 (Resistance : Susceptible) ratio (Table 2) according to the Chi-square analysis. The results indicated that one major gene is involved in controlling resistance to VW in these seven F_2 populations.

Table 2. Segregation of VW resistance in 14 F_2 populations after inoculation with *Verticillium dahliae*.

F2 population	No. R	No. S	Total	Expected ratio	χ^2 value	p-value
LA 35RS × Pima S-7	75	9	84	3:1	9.143	0.0025
LA 35RS × DP 340	66	21	87	3:1	0.035	0.8527
LA 35RS × Cobalt	59	26	85	3:1	31.395	<.0001
MD 25-27Y × Pima S-7	59	23	82	3:1	0.407	0.5237
MD 25-27Y × DP 340	62	26	88	3:1	0.970	0.3248
MD 25-27Y × Cobalt	76	21	97	3:1	0.581	0.446
MD 25-27Y × Phy 830	64	24	88	3:1	0.242	0.6225
Acala 1517-08 × Pima S-7	47	29	76	3:1	7.018	0.0081
Acala 1517-08 × DP 340	66	35	101	3:1	5.020	0.0251
Acala 1517-08 × Cobalt	75	32	107	3:1	1.374	0.2412
Acala 1517-08 × Phy 830	62	18	80	3:1	0.267	0.6056
UA 48 × Pima S-7	60	34	94	3:1	6.255	0.0124
UA 48 × Cobalt	70	18	88	3:1	26.727	<.0001
UA 48 × Phy 830	66	21	87	3:1	39.084	<.0001

$\chi^2_{0.05,\ df=1} = 3.814$

Discussion

Fourteen F_2 populations from crosses between Upland and Pima cotton were used to evaluate the resistance to VW in order to verify whether the resistance is controlled by a single gene in cotton. The results showed that a major resistance gene is involved in segregation of VW resistance in 7 cross combinations. These results are similar to those obtained by Mert et al. (2008). However, the findings from this study are different from the research of Lüders et al. (2008) and Bolek et al. (2005), where the resistance to VW was controlled by polygenes.

Eight parents showed different levels of resistance to VW. Two Upland cotton genotypes, UA 48 and LA 35RS, were the most resistant to VW, while two Pima cultivars Cobalt and Phy 830 also conferred similar VW resistance. Transgressive segregation in VW resistance was also observed in this study, indicating that both Pima and Upland cotton parents carried genes conferring resistance to VW.

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