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Inheritance of resistance to *Verticillium wilt* in cotton

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ABSTRACT – *The control of genetic resistance to* Verticillium wilt *in cotton was studied by specific designs for mean and variance analysis. It was found that the trait is highly heritable and mainly controlled by additive gene effects. According to the method of disease evaluation and the procedure for calculation, two or three genes would be involved in the determination of resistance. Although the results by the three methods used for disease evaluation were rather similar, a probable tolerance mechanism, observed mainly in the resistant parent, indicated the possibility of inconsistencies in studies of resistance to this* Verticillium wilt *in cotton, in the case of evaluation based on external leaf symptoms.*

Key words: Cotton diseases, *Verticillium wilt*, study of genetic inheritance.

INTRODUCTION

Wilt caused by *Verticillium dahliae* Kleb is highly damaging and ranks among the major cotton diseases (Cia and Salgado 2005). It was one of the first diseases of the crop reported in the United States, where it spread rapidly throughout several cotton-producing states. After first occurrences stated in Virginia, in 1914 (Carpenter 1914), the disease has currently reached almost all cotton-producing regions in the world. It is known in Brazil since 1926 (Silveira 1965), where it is found mostly in purple Latosols, rich in organic matter, especially in the states of Parana and Sao Paulo (Cia and Salgado 2005). The fungus infects around 40 different families (Schnathorst 1981) in 400, almost exclusively dicotyledonous (Parry 1982) plant species (Bell 1992).

The symptoms caused by the pathogen are typically irregular leaf chlorosis, possibly necrosis, epinasty and discoloration of the stem vessels. The intensity of the symptoms depends on several factors, among them, pathogen variants (Schnathorst and Mathre 1966); inoculum density (Pullman and Devay 1981; Schnathorst and Mathre 1966); temperature (Garber and Presley 1971); infected cotton genotypes (Balmer et al. 1969, Cia et al. 1975, Katsantonis et al. 2003) as well as the interactions between them. Vascular symptoms are safer indicators for an identification of pathogen-infected plants (Paplomatas et al. 1992) than leaf symptoms. The latter are more influenced by environmental factors (Devay et al. 1974) and can be confounded with other diseases, pests or nutritional deficiency. According to Cia et al. (1975), the best

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method for assessing a cultivar's resistance is to rate the intensity of darkening of the plant vessels in field studies using a rating scale. In studies by Pullman and Devay (1981), on the other hand, the internal symptoms were not correlated with the yield, in contrast to the external, where this correlation was observed.

Although cultural practices such as crop rotation may be helpful, it is virtually impossible to control the disease without the use of resistant cultivars on plantations. Genotypes with this feature and studies of breeding for resistance to this disease are fairly well-known, both abroad (Brown 1927, Christidis and Harrison 1955, Bassett 1974, Bell 1982, 1999) and in Brazil (Balmer et al. 1969, Cia et al. 1970, 1975, 2003, Gridi-Papp et al. 1994, Galbieri et al. 2006a, b). However, studies on the mechanism of genetic resistance to Verticillium wilt in cotton are scarce and not seldom contradictory (Mert et al. 2005, Wilhelm et al. 1974). With regard to the possibility that the disease might become a problem in Brazilian cotton cultivation as well, this study aimed to clarify issues related to inheritance of Verticillium resistance in cotton, to establish a sound basis for breeding programs.

MATERIAL AND METHODS

Several experiments were conducted at the Instituto Agronômico (IAC) in 2005 and 2006 focusing on Verticillium wilt in cotton, including an assessment of the performance of cotton genotypes challenged by the disease (Galbieri et al. 2006a, b). Cultivar DELTAOPAL and line IAC 02-2190 were the most resistant and most susceptible of the genotypes studied, respectively. These genotypes were therefore used in a scheme of hybridization, for the genetic study of means and variance. The six resulting populations (parents, F_1, F_2 and backcrossings) were studied in a greenhouse experiment in 2006, in random blocks, with three replications. Four plants of each population were planted in 5 L pots. Plants were inoculated 30 days after emergence when the seedlings had 3-4 true leaves, by root immersion for five minutes, in a solution at a concentration of 10^6 spores mL^{-1}.

The evaluation was conducted at the plant level, 65 days after inoculation, by three different methods: a) based on external symptoms, i.e., the proportion of chlorotic area on leaves of the infected plants, b) based on internal symptoms, i.e., the presence and intensity of darkened xylem vessels - due to the presence of the fungus - estimated by cutting at the stem basis, and c) also based on internal symptoms, considering the height reached by the darkened vessels within the stem. The three methods were used to evaluate each plant using 1 – 5 rating scales, increasing with the intensity of the described symptoms. For statistical analyses the scores were converted into $\sqrt{x}$. In the study of nature and magnitude of the genetic effects the method of Mather and Jinks (1977) was employed.

RESULTS AND DISCUSSION

The population means and variances and a summary of the conventional analysis of variance are shown in Table 1. The results of the latter indicate good experimental accuracy and consistent data for the three methods of evaluation, as shown by the coefficient of variation and intra-class correlation. Moreover, the differences between the population values by the "F" test were substantial, and Tukey test mean comparison confirmed differences in resistance between the parents and also between the susceptible parent and backcross to the resistant parent. Also, in the three methods, the contrasts $Ya = 2RC_1 – P_1 – F_1$; $Yb = 2RC_2 – P_2 – F_1$; and $Yc = 4F_2 – 2F_1 – P_1 – P_2$ were not significant, indicating absence of epistatic effects, as required by the method used for genetic analysis.

In the genetic study of means, the simplified additive/dominant model was adopted, since the parameters explained more than 90% of the results and the interactions of the complete model were irrelevant. The data analysis suggested that disease resistance is mainly due to additive gene effects while the dominance effects are practically zero (Table 2).

In the main aspects the genetic analysis of variance (Table 3) confirmed the results based on means. However, the data are consistent only for the external evaluation of disease symptoms. In the methods based on internal symptoms, the high values of variance of resistant parental causes surprise (Table 2), which differ considerably from the susceptible parent and the F_1 generation, as this parameter should represent nothing but the environmental variance in the three populations. One possible explanation for this is that the parent in

question was not highly resistant, especially in view of the inoculum potential, which shows, in these conditions, genetic variability in the disease response. It is also possible that the fact was not observed in case of external symptoms due to the existence of a mechanism of disease tolerance in this genotype. Whatever the reason, the problem is that this abnormality results in inconsistencies in the estimation of genetic and additive variances and an underestimated heritability index. In such cases it might be advisable to use only the variances of the susceptible parent and F_1 to estimate the environmental variance. In this case the value is comparable to the mean square of the residue in conventional analysis of variance, which in some way also represents the environmental variance. Curiously, the estimated degree of dominance in the study of variances, now in the three methods, is completely at odds with the means of P_1, P_2 and F_1 and with the values of the same parameter when estimated based on the means. It is worth emphasizing that similar problems in genetic analysis of variances were pointed out by Warner (1952) and by Fuzatto et al. (2007), who suggested, as in this case, caution in the interpretation of results. In any case, despite the cited limitations, the study of variances confirmed the predominance of additive effects in trait control and suggested that the heritability rates are very favorable for an enhanced disease resistance.

Table 1. Mean scores, variances and results of conventional statistical analysis, obtained in studies of inheritance of resistance to *Verticillium wilt* in cotton

Generation	N[1]	Means[2]			Variances[3]		
		Methods[4]			Methods[4]		
		a	b	c	a	b	c
P_1	35	1.28	1.97	2.03	0.041	0.114	0.125
P_2	40	2.88	3.40	3.60	0.043	0.046	0.018
F_1	26	2.14	2.52	2.73	0.026	0.026	0.021
F_2	142	1.77	2.53	2.70	0.108	0.127	0.146
RC_1	37	1.38	2.42	2.56	0.061	0.078	0.072
RC_2	77	1.82	3.34	3.46	0.099	0.070	0.100
"F"		47.1**	16.1**	19.1**			
C.V.		4.40	4.50	3.90			
r_I		0.94	0.85	0.94			
Tukey 5 %		0.53	0.64	0.58			

[1] Number of plants evaluated in each generation
[2] Scores from 1 to 5, non-transformed
[3] Variances with scores transformed by $\sqrt{x}$
[4] a = external symptoms on the leaves; b = intensity of darkened xylem vessels; c = height reached by darkened xylem vessels in the stem

Table 2. Parameter obtained in genetic analysis of means made in experiment to study the inheritance of resistance to *Verticillium wilt* in cotton

Parameters[1]	Method a[2]		Method b[2]		Method c[2]	
	Estimate	"t"	Estimate	"t"	Estimate	"t"
m	1.3607	53.17**	1.6106	53.25**	1.6572	57.78**
a	0.2880	11.36**	0.2169	7.42**	0.1883	6.76**
d	-0.0020	0.04	-0.0306	0.49	0.0037	0.06

[1] m = mean of homozygous of all genes; a = additive effects; d = dominance effects
Methods: a = external symptoms on the leaves; b = intensity of darkened xylem vessels; c = height reached by the darkened xylem vessels in the stem

Table 3. Genetic parameters of the F_2 population, obtained in experiment to study the inheritance of resistance to *Verticillium wilt* in cotton

Parameters	Estimates		
	Method a[(1)]	Method b[(1)]	Method c[(1)]
Phenotypic variance	0.1084	0.1270	0.1464
Environmental variance	0.0340	0.0530	0.0463
Genotypic variance	0.0744	0.0740	0.1001
Additive variance	0.0565	0.1057	0.1205
Dominance variance	0.0179	-0.0149	0.0050
Heritability (broad sense)	0.69	0.58	0.68
Heritability (restricted sense)	0.52	0.83	0.82
M.D.D. (based on variances)	0.796	0.531	0.288
M.D.D. (based on means)	0.075	-0.147	-0.108
Number of genes[(2)]	2.1	1.3	1.1
Number of genes[(3)]	3.2	2.9	2.6

[(1)] Methods: a = external symptoms on leaves; b = intensity of darkened xylem vessels; c = height reached by the darkened vessels in the stem
[(2)] Calculated based on the frequency of the extreme class in the F_2 population
[(3)] Calculated by Wright's formula

Based on the frequency of the extreme class in the F_2 population and, alternatively, on Wright's formula (Vencovsky and Barriga 1992), one or two genes in the first case and two or three in the second were involved in the trait control. It is useful to point out that the estimated number of genes differed too, according to the method of disease evaluation. It appears that the mean grades in all populations were lower in the evaluation of external symptoms and in the case of the resistant parent. Furthermore, the correlation between the grades of the two methods that include internal symptoms was r = 0.78 and between those involving internal - any of them - and external symptoms was only r = 0.50. These facts may indicate, as mentioned above, that a tolerance mechanism is involved in the disease expression, in the case of the parents used here.

CONCLUSIONS

The resistance to *Verticillium wilt* in cotton is a highly heritable trait and due, predominantly, to additive gene effects. According to the method of disease evaluation and the procedure adopted for calculation two to three genes may be involved in resistance control. A probable tolerance mechanism, observed mainly in the resistant parent, indicated inconsistencies may occur in the study of resistance to Verticillium in cotton, in the case of evaluation based on external leaf symptoms.

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Herança da resistência do algodoeiro à murcha de *Verticillium wilt*

RESUMO – *O controle genético da resistência do algodoeiro à murcha de* Verticillium *foi estudado por meio de delineamento específico para a análise de médias e variâncias. O caráter mostrou-se de alta herdabilidade e devido, predominantemente, a efeitos aditivos dos genes. Conforme o método de avaliação da doença e o procedimento adotado para o cálculo, de dois a três genes, estariam envolvidos na determinação da resistência. Embora os resultados tenham sido bastante parecidos, nos*

três métodos utilizados para a avaliação da doença, um provável mecanismo de tolerância, observado principalmente no progenitor resistente, aponta para a possibilidade de que inconsistências possam ocorrer em estudos da resistência a essa doença, no caso de avaliação com base nos sintomas externos nas folhas.

Palavras-chave: doenças do algodoeiro, murcha de *Verticillium*, estudo de herança genética.

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