

## Notas Científicas

### Inheritance of resistance to cotton blue disease

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**Abstract** – The objective of this work was to determine the inheritance of cotton blue disease resistance by cotton plants. Populations derived from the CD 401 and Delta Opal resistant varieties were evaluated, through a greenhouse test with artificial inoculation by viruliferous aphids. Cotton blue disease resistance is conditioned by one dominant gene, both in CD 401 and Delta Opal varieties.

**Index terms:** *Gossypium hirsutum*, *Aphis gossypii*, genetic resistance, luteoviruses.

### Herança da resistência do algodoeiro à doença-azul

**Resumo** – O objetivo deste trabalho foi determinar a herança da resistência do algodoeiro à doença-azul. Populações derivadas das variedades resistentes CD 401 e Delta Opal foram avaliadas em casa de vegetação, por meio da inoculação de pulgões virulíferos. A resistência à doença-azul do algodoeiro é condicionada por um gene dominante, tanto em 'DC 401' quanto em 'Delta Opal'.

**Termos para indexação:** *Gossypium hirsutum*, *Aphis gossypii*, resistência genética, luteovírus.

Diseases constitute one of the main causes of losses in cotton crop, mostly in high productivity areas. One of the diseases of great economic importance in cotton crop, in Brazil, is commonly called cotton blue disease (CBD). It was first described in 1962, in the town of Ribeirão Bonito (Embrapa, 2001), SP, Brazil, as a particularly virulent type of Veinal Mosaic, capable of inflicting very relevant damage.

The disease is transmitted by the cotton aphid *Aphis gossypii* Glover, a highly polyphagous and cosmopolitan species, which has over 80 host plant species (Ebert & Cartwright, 1997). The virosis transmission by the *Aphis gossypii* is of persistent type (Costa et al., 1997; Santos, 2001).

It was recently proved that the pathogen is a virus of the Luteoviridae family, and it was confirmed, for the first time, that it is in fact a new virus of the *Polerovirus* genus, named "Cotton leaf roll dwarf virus" (CLRDV) (Corrêa et al., 2005). When inoculated into a plant, the symptoms develop in 9 to 28 days (Cauquil & Vaissayre, 1971; Cauquil & Follin, 1983).

Knowledge about genetic inheritance pattern of plant resistance to diseases must allow better planning for breeding populations and must offer guidance in choosing the best strategy for mapping the genes linked to this resistance. Genetic inheritance studies are relatively simple, when the characteristic being studied is qualitative and, therefore, follows a discreet distribution. In these cases, adherence tests are performed in order to verify whether the observed segregation matches the expected one, considering different hypotheses.

The objective of this work was to study the genetic inheritance of CBD resistance, aiming to facilitate the planning of breeding populations, in order to incorporate this resistance characteristic into new cultivars. The knowledge of how the cotton plant inherits the resistance to CBD might also allow the genetic mapping of this characteristic, making the molecular marker assisted selection possible in breeding programs.

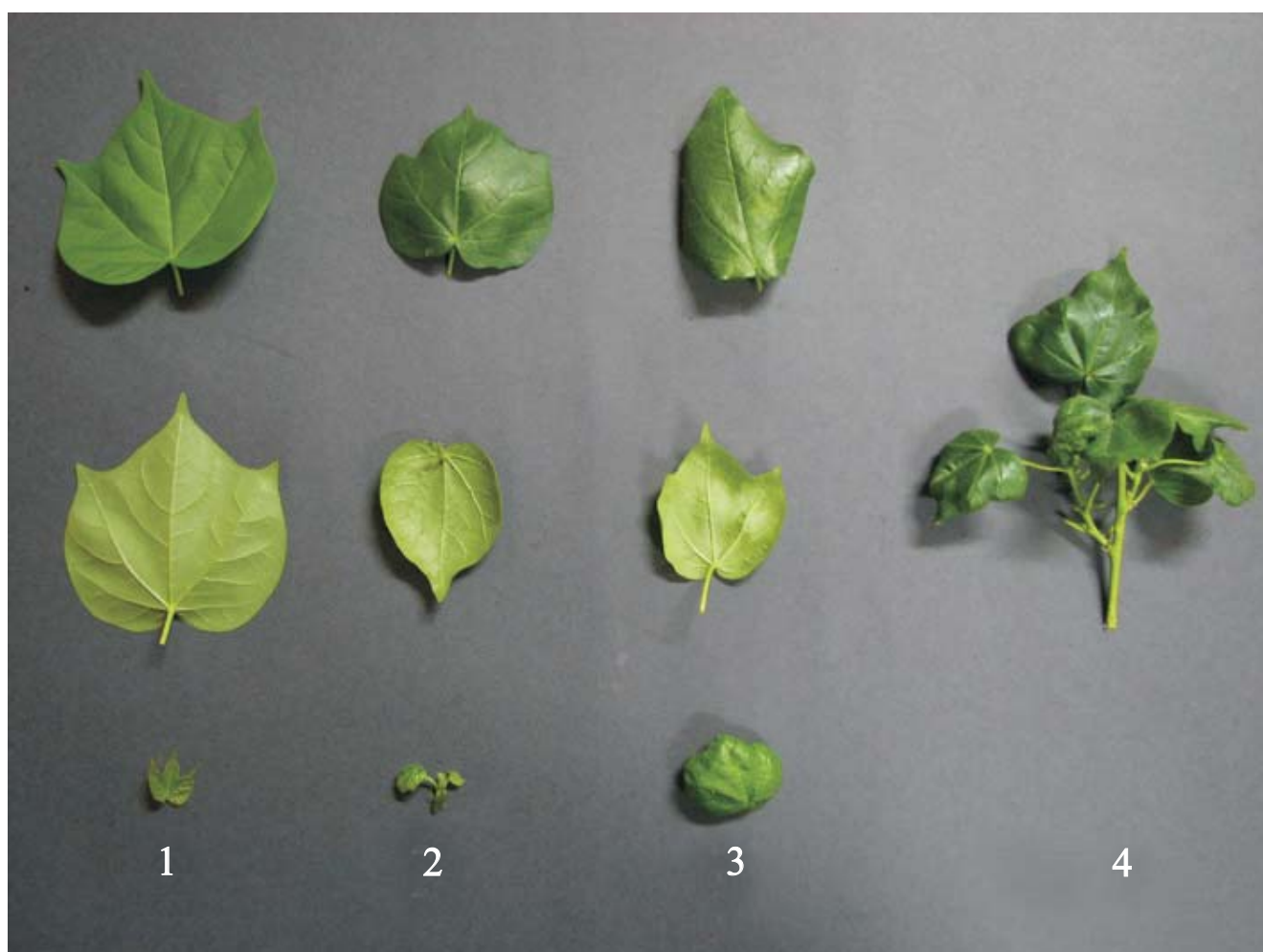
The work took place in Brazil, at Cooperativa Central de Pesquisa Agrícola Experimental Station, in Cascavel, PR, and at Embrapa Algodão, in Campina Grande, PB.

The populations for the inheritance study were obtained from the following crosses: 'CD 401' (resistant) x 'FM 966' (susceptible); 'Delta Opal' (resistant) x 'CNPA ITA 90' (susceptible) and 'Delta Opal' (resistant) x 'Mákina' (susceptible). From 'CD 401' x 'FM 966' cross, the following progenies were evaluated: F<sub>1</sub>, F<sub>2</sub>, F<sub>2:3</sub>, BC<sub>1</sub>F<sub>1s</sub> (obtained by crossing F<sub>1</sub> plants and the susceptible parent) and BC<sub>1</sub>F<sub>1:2</sub> (obtained by selfing BC<sub>1</sub>F<sub>1s</sub> plants). From 'Delta Opal' x 'CNPA ITA 90' and 'Delta Opal' x 'Mákina' crosses, the following progenies were evaluated: F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub>F<sub>1r</sub> (obtained by crossing F<sub>1</sub> plants and the resistant parent) and BC<sub>1</sub>F<sub>1s</sub>.

Populations derived from 'CD 401' x 'FM 966' were evaluated in the plantlet stage. These populations were sown in trays with 72 cells each. For the artificial inoculation of CBD in the plantlets, viruliferous aphids were used. To obtain infected aphids, they were placed for 15 days on

plants infected with the disease. After aphids acquired the virus, they were collected through an adapted vacuum cleaner, and transferred onto healthy plantlets. The inoculation took place in an interval of 10 to 12 days after sowing, i.e., 5 to 6 days after the emergence. After this period, plantlets already displayed a well-developed first true leaf. The number of viruliferous aphids inoculated was not exactly the same on each plantlet, but the distribution was homogeneous, so that every plantlet could have approximately the same quantity of aphids. The viruliferous aphids remained on plantlets for 8 days. Subsequently, they were eliminated from the plantlets through endosulfan CE insecticide pulverization (2.8 g L<sup>-1</sup> active ingredient).

The evaluation of the blue disease symptoms took place 30 and 45 days after infestation, and symptoms were graded according to the following ranks (Figure 1): 1, no symptom; 2, normally colored and slightly deformed



**Figure 1.** Scale for evaluation of blue disease resistance. Grade 1 is resistant and grades 2, 3 and 4 are susceptible.

leaves; 3, dark-colored and visibly deformed leaves; 4, thin bluish-green leaves, highly deformed with yellow veins. The plants graded 1 were considered resistant, and the others, susceptible. The experiment took place inside cages lined with a thin mesh, in order to avoid the entrance of the aphid parasites (*Lysiphlebus* sp.), in a greenhouse.

Plant populations derived from 'Delta Opal' x 'CNPA ITA 90' and 'Delta Opal' x 'Mákina' crosses were evaluated in their adult stage. The plants were cultivated in a greenhouse, in 10 L buckets containing fertilized soil as substrate. Inoculations were made using aphids collected from plants displaying typical symptoms of CBD, found in the field and in the greenhouse. Inoculations started around 30 days after germination and were repeated twice, in the 45 and 60 days after germination. Five or more viruliferous aphids were placed on each plant at each inoculation. Vectors remained on the plants for at least 48 hours. Plants were evaluated at the end of the cycle for verification of the presence of blue disease symptoms.

The results were submitted to chi-square test, for comparisons of the observed and expected ratios between resistant and susceptible plants, in each population. By the hypothesis of one dominant gene, the expected ratio was 1:1 in BC<sub>1</sub>F<sub>1s</sub>, 3:1 in BC<sub>1</sub>F<sub>1:2</sub>, and 1:2:1 in F<sub>2:3</sub> population. In BC<sub>1</sub>F<sub>1r</sub>, all plants were expected to be resistant. The hypothesis was considered true, if the associated probability was higher than 5%. Consistency of chi-square test among populations was

evaluated through the heterogeneity test applied for all populations with the same hypothesis for ratio test.

Results obtained for populations derived from 'CD 401' x 'FM 966' (Table 1) matched the segregation of one dominant gene for resistance to CBD, in CD 401 cultivar. On BC<sub>1</sub>F<sub>1:2</sub> generation, the expected ratio was 3:1 for the hypothesis of segregation of one dominant gene, because only resistant RC<sub>1</sub>F<sub>1</sub> plants were self-pollinated in order to obtain these populations. Seven BC<sub>1</sub>F<sub>1:2</sub> populations were evaluated and, in all of them, the 3:1 segregation hypothesis was accepted. The heterogeneity test demonstrated that data from these seven populations were very consistent (P = 26.14%). In F<sub>2:3</sub> generation, 74 families were evaluated, and 14 were homozygous resistant, 42 were segregant and 18 were homozygous susceptible, as it was expected for 1:2:1 segregation hypothesis (P = 40.99%).

Populations obtained from the cross of Delta Opal resistant cultivar with two susceptible varieties (CNPA ITA 90 and Mákina) also indicated that resistance to CBD in Delta Opal cultivar is granted by one dominant gene (Table 2). Both F<sub>2</sub> populations segregated at 3:1 ratio, and these two populations were highly comparable (P = 50.7% on the heterogeneity test). Also, both BC<sub>1</sub>F<sub>1s</sub> populations, obtained by crossing F<sub>1</sub> plants with the susceptible cultivars, segregated at 1:1 ratio, and the results were consistent across these two populations, with P = 27.41% on the heterogeneity test.

The results obtained so far don't make possible to conclude whether the same gene is present in both cultivars or each cultivar has a different gene.

**Table 1.** Reaction of CD 401 and FM 966 cultivars, and of the generations F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub>F<sub>1</sub> and BC<sub>1</sub>F<sub>1:2</sub> to the inoculation of cotton blue disease (CBD) agent, and adherence test ( $\chi^2$ ) of the inheritance of cotton plant resistance to CBD<sup>(1)</sup>.

Population	Number of individuals	Observed		Expected		Theoretical ratio (R:S)	$\chi^2$	P (%)
		R	S	R	S			
CD 401	116	116	0	116.0	0.0	1:0		
FM 966	116	0	116	0.0	116.0	0:1		
F <sub>1</sub>	25	25	0	25.0	0.0	1:0		
F <sub>2</sub>	74	56	18	55.5	18.5	3:1	0.018	89.32
BC <sub>1</sub> F <sub>1s</sub>	109	53	56	54.5	54.5	1:1	0.082	77.38
BC <sub>1</sub> F <sub>1:2</sub> - Fam2	104	84	18	76.5	25.5	3:1	2.941	8.63
BC <sub>1</sub> F <sub>1:2</sub> - Fam15	113	92	21	84.7	28.2	3:1	2.481	11.52
BC <sub>1</sub> F <sub>1:2</sub> - Fam23	110	90	20	82.5	27.5	3:1	2.727	9.86
BC <sub>1</sub> F <sub>1:2</sub> - Fam25	89	72	17	66.7	22.2	3:1	1.652	19.87
BC <sub>1</sub> F <sub>1:2</sub> - Fam26	104	85	19	78.0	26.0	3:1	2.513	11.29
BC <sub>1</sub> F <sub>1:2</sub> - Fam30	122	94	28	91.5	30.5	3:1	0.273	60.12
BC <sub>1</sub> F <sub>1:2</sub> - Fam33	119	83	36	89.2	29.7	3:1	1.751	18.58
Heterogeneity test for the seven BC <sub>1</sub> F <sub>1:2</sub> families (6 df)						3:1	7.693	26.14

<sup>(1)</sup>R: resistant; S: susceptible; P: probability; df: degrees of freedom.

**Table 2.** Reaction of cultivars and generations F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub>F<sub>1</sub>r and BC<sub>1</sub>F<sub>1</sub>s, in the crosses 'Delta Opal' x 'Mákina' and 'Delta Opal' x 'CNPA ITA90' to the inoculation of the cotton blue disease (CBD) agent, and segregation test ( $\chi^2$ ) for the inheritance of cotton plant resistance to CBD<sup>(1)</sup>.

Cross	Population	Number of plants	Observed		Expected		Theoretical ratio (R:S)	$\chi^2$	P (%)
			R	S	R	S			
Delta Opal x Mákina	Delta Opal	18	18	0	18.0	0.0	1:0	1.110	29.18
	Mákina	16	3	13	0.0	16.0	0:1		
	F <sub>1</sub>	14	14	0	14.0	0.0	1:0		
	F <sub>2</sub>	30	20	10	22.5	7.5	3:1		
	BC <sub>1</sub> F <sub>1</sub> r	16	16	0	16.0	0.0	1:0		
	BC <sub>1</sub> F <sub>1</sub> s	18	9	9	9.0	9.0	1:1	0	100.00
Delta Opal x CNPA ITA 90	Delta Opal	16	16	0	16.0	0.0	1:0	0.035	85.14
	CNPA ITA 90	19	3	16	0.0	19.0	0:1		
	F <sub>1</sub>	12	12	0	12.0	0.0	1:0		
	F <sub>2</sub>	38	28	10	28.5	9.5	3:1		
	BC <sub>1</sub> F <sub>1</sub> r	17	17	0	17.0	0.0	1:0		
	BC <sub>1</sub> F <sub>1</sub> s	16	11	5	8.0	8.0	1:1	2.250	13.37
Heterogeneity test for the F <sub>2</sub> generations (1 df)							3:1	0.440	50.70
Heterogeneity test for the BC <sub>1</sub> F <sub>1</sub> s generations (1 df)							1:1	11.19	27.50

<sup>(1)</sup>R: resistant; S: susceptible; P: probability; df: degrees of freedom.

An allelism test, from the segregation analysis of populations derived from the cross of the two resistant varieties (CD 401 x Delta Opal), will make it possible to identify whether the resistance in these two cultivars is bestowed by the same or by different genes. Furthermore, the mapping of this resistance gene will turn possible to evaluate other sources of resistance, through the use of the molecular markers linked to the resistance gene, and to identify those that have the same gene.

This simple inheritance pattern is also known for other cotton diseases. Zandoná et al. (2006) have studied the cotton plants inheritance mechanism of resistance to ramulosis, caused by *Colletotrichum gossypii* var. *cephalosporioides*, in the resistant cultivars BRS ANTARES and IAC 23, when crossed with the susceptible cultivar STO 474, and have identified that resistance was conditioned by one dominant gene. In the same way, the cotton plants resistance to angular leaf spot, caused by *Xanthomonas axonopodis* pv. *malvacearum* bacterium, is controlled by one dominant gene in Delta Opal and Epamig Liça cultivars, and by two dominant genes in Fibermax 986 cultivar (Zandoná et al., 2005). Metha & Arias (2001) have studied F<sub>2</sub> e F<sub>2.3</sub> populations descending from the crosses: 'PR 94-82' (R) x 'IAPAR 71' (S), 'PR 94-215' (R) x 'IAPAR 71' (S) and 'CNPA PRECOCE 2' (R) x 'IAPAR 71' (S). The authors have identified that the resistance to *Stemphylium solani*, present in PR 94-82

and CNPA-PRECOCE 2 cotton cultivars, is individually determined by one dominant gene; and in 'PR 94-215', resistance is determined by the presence of two dominant genes of complementary epistatic effect.

This is the first report of cotton plants inheritance of resistance to blue disease. Resistance to CBD, in CD 401 and Delta Opal cultivars, is conditioned by one dominant gene. For this reason, it is suggested that this gene be called *Rghv1* (*Resistance to Gossypium hirsutum Virus 1*).

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