



Phenotypic variability among isolates of *Ramularia areola* from Brazilian cotton

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ABSTRACT

There is a lack of information about the level of resistance of cotton genotypes to a wider range of *Ramularia areola* isolates occurring across the cotton growing areas of Brazil. For this purpose, firstly it is necessary to know the existence or not of genotypic and phenotypic variability among the *R. areola* isolates from different geographical origins. The objective of the present investigation was to verify the existence of phenotypic variability among 23 *R. areola* isolates collected from six cotton growing states of Brazil. Two resistant genotypes, FMT 02102996 and CNPA BA 2003-2059, and the susceptible genotype FMT 701 were individually inoculated with 23 *R. areola* isolates under glasshouse conditions and the severity of infection was evaluated 30 days after inoculation. Genotypes CNPA BA 2003-2059 and FMT 02102996 were susceptible to three isolates and resistant to the rest of the isolates. Genotype FMT 701 was susceptible to all the isolates except the isolates 22.3 and 42.7. Results indicate the existence of variability among *R. areola* isolates and that the three genotypes are useful in distinguishing phenotypic variability within isolates of this pathogen.

Key words: *Gossypium hirsutum*, *Ramularia* leaf spot, varietal resistance.

Ramularia leaf spot of cotton caused by *Ramularia areola* can be controlled through the use of resistant cultivars provided sources of resistance to this pathogen are made available. However, there is a lack of information about the level of resistance of cotton genotypes to a wider range of Brazilian *R. areola* isolates. For this purpose, firstly it is necessary to understand about the existence or not of genotypic and phenotypic variability between the isolates occurring across the cotton growing areas of Brazil.

Although occurrence of the teleomorph of *R. areola* in nature is not known, there is an indication of existence of “field strains” of this pathogen in Brazil. Frequently inconsistent reaction under field conditions of some resistant cultivars is observed, possibly because of the existence of such field strains, resulting in frustration for breeders as well as for cotton farmers (Cia et al., 2010, 2011). Nonetheless, this situation can be altered provided knowledge about some of the fundamental aspects, like phenotyping and genotyping of *R. areola* isolates, is made available. Phenotyping and genotyping are two important steps for genetic breeding that help in developing a differential set of cultivars capable of distinguishing “field strains” of *R. areola* originated from different geographical regions and/or from different *Gossypium* species. This consequently leads to the identification of sources having a wide spectrum of resistance. Once such sources of resistance are identified they can be used

in national breeding programs aimed at pyramiding resistance genes in commercially desirable cultivars.

In earlier studies, Novaes et al. (2011) and Zandoná et al. (2012) identified genotypes CNPA BA 2003-2059 and FMT 02102996 as resistant to the isolate 44 of *R. areola*, collected from Ipameri, State of Goiás, Brazil, from the culture collection of Instituto Agronômico do Paraná (IAPAR). Zandoná et al. (2012) also reported that each one of the two resistant genotypes has a different dominant gene. Some other resistant genotypes to isolate 44 were also identified by Barbosa et al. (2011). However, their resistance is not found to be stable under variable field conditions (Wilson P. Almeida, personal communication). The objective of the present investigation was to verify the phenotypic variability among 23 *R. areola* isolates of different geographical origins to aid in the identification of sources with a wider spectrum of resistance.

Seeds of three genotypes, two resistant (CNPA BA 2003-2059 and FMT 02102996) and one susceptible (FMT 701) to the isolate 44 of *R. areola* were provided by Camilo de Lelis Morello (Embrapa Algodão, Campina Grande, PB, Brazil) and Paulo H. Aguiar (Fundação MT, Rondonópolis, MT, Brazil). Plants of both resistant genotypes were segregating for resistance to *R. areola* under glasshouse inoculations. For this reason, plants of each genotype were inoculated during two successive generations to purify and to confirm their resistant and susceptible reactions. Seeds derived from second

generation by selfing a single plant of each one of the genotypes were further multiplied and used to make sure the homozygosity of the genotypes (Zandoná et al., 2012).

Leaf samples showing typical disease symptoms were collected from different cotton growing areas of Brazil by FRAC (Fungicide Resistance Assessment Committee) technicians between 2009 and 2010, and were received in the laboratory for monospore isolations. Twenty-three *R. areola* isolates representing different geographic areas of Brazil were stored in V8-agar for further studies.

Fungal isolates were multiplied for 7-10 days on V8-juice agar, the conidia were scraped from the sporulating cultures, conidial suspension of the inoculum was adjusted to 10^4 /mL, and a few drops of Tween 20 were added to the inoculum. Twenty-five days old plants were inoculated using a hand sprayer and an approximately equal amount of inoculum per plant was sprayed. Twelve plants of each genotype were inoculated separately by each one of the 23 isolates of *R. areola*. Soon after inoculation the plants were incubated in a growth chamber at 21°C in the dark and to near saturated humidity for 48 h. After this period the plants were transferred to the glasshouse bench and randomized. The glasshouse temperature varied between 20 and 30°C. Disease severity on the leaves was assessed 30 days after inoculation using a visual scale between 0 and 100% of the leaf area infected (LAI).

The genotypes CNPA BA-2003-2059 and FMT 02102996 identified in earlier studies as resistant to *R. areola* isolate 44 (Novaes et al., 2011) showed variation to some isolates originated from different geographical origins. These genotypes showed a susceptible reaction to three isolates (13.2, 17.5 and 58.4) originated from the states of Paraná (PR), São Paulo (SP) and Mato Grosso do Sul (MS), respectively (Figure 1, Table 1), but a resistant reaction to the rest of the isolates. With the exception of isolates 22.3 and 42.7, genotype FMT 701 was susceptible to all isolates of *R. areola*.

As stated earlier, isolates of *R. areola* used in the present investigation were collected from different geographical origins in six Brazilian states which are representatives of the major cotton growing regions of Brazil. The results indicate the existence of variability among *R. areola* isolates and show that the three cotton genotypes used in the present investigation are useful in distinguishing phenotypic variability in the pathogen. While these genotypes are capable of distinguishing a few isolates, further research on the identification of other genotypes suitable to distinguish differences among the isolates will be necessary in order to develop a set of differential cultivars.

Phenotyping and genotyping form basic pillars for breeding resistant cultivars and for this reason due emphasis needs to be given in the future to this kind of investigation. Such studies would assist in establishing a set of differential cultivars for the “field strains” of *R. areola* occurring in

different geographical regions, and consequently would help towards a more efficient breeding.

The results also indicate that the resistance of genotypes CNPA BA 2003-2059 and FMT 02102996, although governed by a single dominant gene, may not

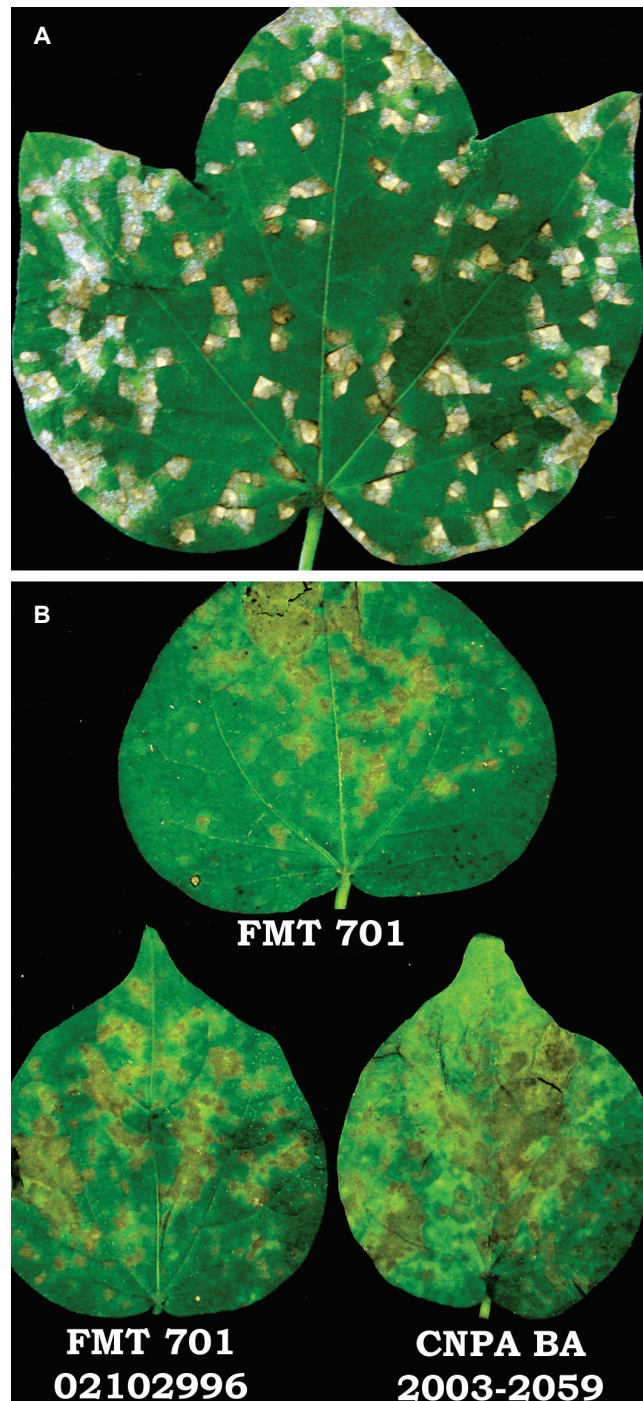


FIGURE 1 - A. Phenotypic reaction of isolate no. 44 of *R. areola* on leaf of genotype FMT 701, 30 days after inoculation under glasshouse conditions; **B.** Phenotypic reaction of isolate no. 58.4 of *R. areola* on three cotton genotypes, 30 days after inoculation under glasshouse conditions.

TABLE 1 - Phenotypic variability of 23 isolates of *R. areola* in three cotton genotypes assessed under glasshouse conditions

Isolate	Origin	% Leaf area infected in three cotton genotypes*		
		FMT 701	FMT 02102996	CNPABA 2003 - 2059
11.5	Primavera do Leste, MT	50.0	0.0	0.0
12.8	Moreira Sales, PR	36.4	0.0**	0.0**
13.2	Sto. Ant. da Platina, PR	42.5	78.6	16.7
17.5	Riolândia, SP	27.6	23.5	13.3
18.4	Riolândia, SP	43.3	0.0	0.0
19.4	Riolândia, SP	25.0	0.0	0.0
20.8	Itumbiara, GO	50.5	0.0	0.0
22.3	Location unknown, BA	0.0**	0.0	0.0
25.1	Location unknown, BA	50.0	0.0	0.0
26.1	Location unknown, BA	43.5	0.0	0.0
29.4	Lucas do Rio Verde, MT	51.0	0.0	0.0
40.6	Novo São Joaquim, MT	51.8	0.0	0.0
41.1	Primavera do Leste, MT	22.5	0.0	0.0
42.7	Campo Verde, MT	0.0**	0.0	0.0
44	Ipameri, GO	54.1	0.0	0.0
46.4	Ipameri, GO	60.0	0.0	0.0
50	Rondonópolis, MT	22.7	35.4	---
51.3	Rondonópolis, MT	44.5	0.0**	0.0**
54.1	Primavera do Leste, MT	16.6	0.0	0.0
58.4	Chapada do Sul, MS	45.0	23.0	48.3
60.1	Ipameri, GO	41.8	0.0	0.0
63.3	Mineiros, GO	54.2	0.0	0.0**
64.1	Mineiros, GO	50.4	0.0	0.0

*Average of 12 plants; **Only two plants showed small necrotic lesions which failed to sporulate after 24 hours of incubation in humid chamber under laboratory conditions.

be stable and may be isolate dependent. These results would create stimulus for further work on phenotypic and genotypic variability among the Brazilian *R. areola* isolates so as to improve our knowledge of the cotton - *R. areola* interaction and to develop a broader set of differential cotton cultivars.

Considering the fact that the inheritance of resistance is simple, pyramidation of resistance genes seems feasible. The use of resistant cultivars would avoid the constant use of fungicides and would lower the cost of cultivation.

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