

A SSR marker linked to the B_{12} gene that confers resistance to race 18 of *Xanthomonas axonopodis* pv. *malvacearum* in cotton is also associated with other bacterial blight resistance gene complexes

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Abstract The SSR marker CIR246, which is linked to the B_{12} gene that confers resistance to race 18 of *Xanthomonas axonopodis* pv. *malvacearum* (*Xam*), was used to evaluate a series of cotton germplasms that were also tested for their response to inoculation with race 18 of *Xam* (S-295, Delta Opal, 101-102B, Guazuncho-2, Acala 44, Mebane B1, and Stoneville 2B-S9). The allele associated with resistance was amplified in genotypes that carry the B_{12} gene (S-295 and Delta Opal) as well as in those that carry B_2B_3 (101-102B) and $B_{9L}B_{10L}$ (Guazuncho-2), indicating that the molecular marker is able to identify genotypes resistant to the *Xam* races up to race 18, with resistance genes other than B_{12} .

Keywords Molecular marker · *Gossypium hirsutum* · Bacterial blight · Resistance gene

Introduction

Cotton bacterial blight is caused by the bacterium *Xanthomonas axonopodis* pv. *malvacearum* (*Xam*) (Smith) Vaut. About 19 *Xam* races have been described worldwide, and additional hypervirulent races have appeared in Africa (Follin et al. 1988). Among the *Xam* races, race 18 occurs most frequently in almost all cotton-producing areas in the world (Thaxton and El-Zik 2001).

There are at least 22 reported resistance genes or gene complexes in cotton that confer differing degrees of resistance to various *Xam* races carrying different avirulence genes in a typical gene-for-gene manner (Delannoy et al. 2005). These genes and gene complexes have quantitative effect and may confer complete or partial resistance to specific races of the pathogen. Gene complexes B_2B_3 and $B_{9L}B_{10L}$ have been shown to confer resistance up to race 18 (Innes 1965; Innes et al. 1974), while B_{12} confers resistance to all races, including the hypervirulent isolates from Africa, that overcome the former (Girardot et al. 1986; Wallace and El-Zik 1989).

Breeding for cotton resistance to *Xam* is traditionally been carried out under natural infection due to the practical impossibility to make artificial inoculations on the thousands of genotypes produced every year by the cotton breeding programs. Natural infection can occur at a high level, but escapes are common among plants in an infected field. Artificial inoculation methods used for screening cotton plants for resistance to *Xam* can result in more reliable information, improving the selection program and the effectiveness of breeding programs; however, these methods demand time and require controlled environments (Hillocks 1992).

Xiao et al. (2010) identified SSR and SNP markers in chromosome 14 that are tightly associated with the B_{12} gene that confers resistance to race 18 of *Xam*. These markers could be used for marker-assisted selection and germplasm screening for bacterial blight resistance. In this study, we show that the CIR246 SSR is also associated with other genes complexes that confers resistance to race 18 of *Xam* such as B_2B_3 and $B_{9L}B_{10L}$. Thus, the CIR246 marker does not allow the discrimination of the gene or gene complex involved in resistance, being useful only to identification of alleles for resistance up to race 18 of *Xam*.

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Materials and methods

Cotton genotypes (Table 1) were inoculated with an isolate of *Xam* race 18, kindly provided by Dr. Michel Nicole at IRD, UMR RPB “Résistance des Plantes aux Bioagresseurs”, France, which was grown at 30 °C on 523 medium (1.0 % w/v sucrose, 0.4 % w/v yeast extract, 0.8 % w/v hydrolyzed acid casein, 0.2 % w/v K₂HPO₄, 0.03 % MgSO₄) solidified with 2.0 % w/v agar. After approximately 48 h of growth, the cultures were washed with a sterile saline solution (0.85 % NaCl), and the bacterial suspension was adjusted to 10⁸ cfu/ml. Approximately 0.5 ml of the freshly prepared bacterial suspension was gently injected at three sites of the abaxial side of two leaves of plants with 6 to 8 fully expanded leaves. Inoculated plants were maintained in a greenhouse at a 25/30 °C light/dark cycle with relative humidity averaging 80 %, and the symptoms were scored 5 days after inoculation.

The same genotypes were evaluated for the alleles amplified using marker CIR246. The DNA was purified according to McDonald et al. (1994), amplified using the procedures described by Nguyen et al. (2004), electrophoresed in 6 % denaturing polyacrylamide gels and silver stained (Creste et al. 2001).

The experimental design was completely randomized and all experiments were performed in triplicate. This assay was repeated twice.

Results

Accessions S295, Delta Opal, 101-102B and Guazuncho-2 were resistant to race 18 of *Xam* (Table 1), displaying a hypersensitive reaction (HR) to bacterial inoculation, while Acala 44, Mebane B1, and Stoneville 2B-S9 showed a water-soaked lesion.

When CIR246 was used to genotype the accessions, Delta Opal presented an amplification product of 146 bp previously associated with resistance in this accession (Xiao et al. 2010). The same allele was present in all other accessions that were resistant to race 18 of *Xam*, such as S295, 101-102B, and Guazuncho-2 (Table 1). In contrast, Acala 44 presented an

allele of 166 bp, while Mebane B1 and Stoneville 2B-S9 each presented an allele of 156 bp. Both alleles were previously associated with susceptibility to race 18 of *Xam* (Xiao et al. 2010).

Discussion

The 146 bp allele amplified by marker CIR246 is thus present in all accessions resistant to race 18 of *Xam*, regardless of the resistance gene or gene complex present (*B*₁₂, *B*₂*B*₃, or *B*_{9L}*B*_{10L}). The resistance of 101-102B to race 18 is attributed to a gene complex formed by the major genes *B*₂ and *B*₃ and the minor gene *B*_{sm} (Innes et al. 1974). Despite the fact that *B*₂ and *B*₃ were mapped independently on chromosome 20 at a distance of about 50 cM from each other (Wright et al. 1998), they segregate as a single locus in 101-102B and derived genotypes. Innes et al. (1974) suggested that a chromosomal inversion may have brought *B*₂ and *B*₃ together in 101-102B, creating a “supergene” that segregates as a single locus. The resistance of S295 to all known races of *Xam* (Girardot et al. 1986) is attributed to the *B*₁₂ gene (Wallace and El-Zik 1989), which mapped to chromosome 14 (Wright et al. 1998). The resistance in S295 is genetically linked to the *B*₂*B*₃ locus found in 101-102B and may involve a single dominant gene closely associated with this locus or, more probably, a complex inherited as a single locus and constituted by the race 18 resistance locus and a minor gene that modifies and amplifies its effect (Follin et al. 1988). The resistance in Guazuncho-2 is attributed to the *B*_{9L} and *B*_{10L} genes (Lagière R (1959), cited in Follin et al. (1988)) that also segregate as a single locus homologous to (or that co-segregates with) the *B*₂*B*₃ locus (Follin et al. 1988).

In their study using Delta Opal as a donor for resistance, Xiao et al. (2010) identified molecular markers associated with resistance to *Xam* race 18 on chromosome 14. Based on genetic analysis of the resistance to race 18 and the chromosomal location, they inferred the resistance gene to be *B*₁₂. However, gene complexes that behave as single loci and impart resistance to race 18 other than *B*₁₂ have also been localized on the same chromosome. In this study, we show

Table 1 Genotypes evaluated, allele size (in bp) for marker CIR246, response to inoculation with race 18 of *Xam*, resistance gene present and original reference for the resistance gene

Genotype	Allelic size (bp)	Response	<i>R</i> Gene(s)	Reference
S-295	146	Resistant	<i>B</i> ₁₂	Wallace and El-Zik, 1989
Delta Opal	146	Resistant	<i>B</i> ₁₂ ^a	Xiao et al., 2010
101-102B	146	Resistant	<i>B</i> ₂ <i>B</i> ₃ <i>B</i> _{sm}	Innes et al., 1974
Guazuncho-2	146	Resistant	<i>B</i> _{9L} <i>B</i> _{10L}	Innes, 1965
Acala 44	166	Susceptible	–	Brinkerhoff, 1970
Mebane B1	156	Susceptible	<i>B</i> ₂ <i>B</i> _{sm}	Brinkerhoff, 1970
Stoneville 2B-S9	156	Susceptible	<i>B</i> _{sm}	Brinkerhoff, 1970

^a The identity of the *R* gene present in cultivar Delta Opal is based on circumstantial evidence

that all genotypes carrying these gene complexes exhibited the same 146 bp allele for the CIR246 marker. Thus, the CIR246 marker does not allow the discrimination of the gene or gene complex involved in resistance. This drawback is not important in the majority of cases because the CIR246 marker allows for the accurate identification of alleles for resistance up to race 18, the most aggressive and widespread race in the majority of the cotton-producing areas. Nevertheless, only the *B₁₂* gene confers resistance to the hypervirulent African strains that are capable of overcoming the resistance conferred by other genes or gene combinations (Delannoy et al. 2005). Thus, breeding for *Xam* resistance in those regions where these hypervirulent strains occur should be based on both marker and pedigree.

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