



An AFLP marker linked to the *Pm-1* gene that confers resistance to *Podosphaera xanthii* race 1 in *Cucumis melo*

Ana Paula Matoso Teixeira, Fatima Aparecida da Silva Barreto and Luis Eduardo Aranha Camargo

Departamento de Entomologia, Fitopatologia e Zoologia Agrícola, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, São Paulo, Brazil.

Abstract

Brazil produced 330,000 metric tons of melons in 2005, principally in the Northeast region where one of the most important melon pathogens is the powdery mildew fungus *Podosphaera xanthii*. The disease is controlled mainly by incorporating single dominant resistance genes into commercial hybrids. We report on linkage analysis of the *Pm-1* resistance gene, introgressed from the AF125^{*Pm-1*} Cantalupensis Charentais-type breeding line into the yellow-fleshed melon (Group Inodorus) breeding line AF426-S by backcrossing to produce the resistant line AF426-R, and the amplified fragment length polymorphism (AFLP) marker M75/H35_155 reported to be polymorphic between AF426-S and AF426-R. Segregation analysis of M75/H35_155 using a backcross population of 143 plants derived from [AF426-R x AF426-S] x AF426-S and screened for resistance to *P. xanthii* race 1 produced a recombination frequency of 4.9%, indicating close linkage between M75/H35_155 and *Pm-1*. Using the same segregating population, the M75/H35_155 marker had previously been reported to be distantly linked to *Prv*¹, a gene conferring resistance to papaya ringspot virus-type W. Since M75/H35_155 is linked to *Prv*¹ at a distance of 40.9 cM it is possible that *Pm-1* and *Prv*¹ are also linked.

Key words: AFLP marker, *Cucumis melo*, disease resistance, linkage analysis, powdery mildew.

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Melon (*Cucumis melo* L., Cucurbitales, Cucurbitaceae) is of great economic importance in Brazil where about 330 thousand metric tons are produced per year (FNP, 2005), principally in the Northeast region due to the adoption of advanced technology and the favorable climate of this region which allows melons to be grown practically throughout the year. This region benefits from the fact that the harvest occurs during September through March during the off-season in Spain, the most important European producer. More than half the melon yield from the Brazilian Northeast is destined for export, with about US\$ 108 million US dollars worth of melons, equivalent to over 204 thousand tons of fruit, having been exported in 2007 (Portal do Agronegócio, 2007).

The fungus *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff (2000) (Erysiphales, Erysiphaceae) is the causal agent of powdery mildew in Brazil. The first report of the destructive effects of this pathogen in melon crops was in 1925 in California (Jahn *et al.*, 2002). This pathogen can infect plants in protected crops as well as in

the field, with high temperature and low humidity increasing the intensity of infection. In Northeast Brazil this disease is more frequent at the end of the vegetative cycle, causing additional losses by shortening the harvesting time and affecting the quality of late fruits due to intensive exposure to sunlight as a consequence of defoliation (Rego, 1995, Viana *et al.*, 2001).

All resistance genes to *P. xanthii* present in American, European and Japanese melon varieties derive from germplasm collected in India, which is one of the centers of origin of melons (Pitrat *et al.*, 1998, cited by Jahn *et al.*, 2002). According to Pitrat (1998), there are 18 sources of resistance genes against *P. xanthii*, and 10 resistance genes described in the literature that differ from each other not only according to their origin but also in their specificity relative to the pathogen race and mode of action (Pitrat, 2002). The *Pm-1* gene from PI78374 confers resistance to *P. xanthii* race 1 and was introduced into the 'Cinco' cultivar (Thomas and Webb, 1982). It is a dominant-resistance gene commonly used in melon breeding programs aimed at obtaining varieties adapted to the Brazilian Northeast, in view of the occurrence of *P. xanthii* race 1 in that region (Reis and Buso, 2004).

Molecular markers linked to resistance genes are commonly used in marker-assisted breeding, especially

Send correspondence to Luis Eduardo Aranha Camargo. Departamento de Entomologia, Fitopatologia e Zoologia Agrícola, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Av. Pádua Dias 11, 13418-900 Piracicaba, SP, Brazil. E-mail: leacamar@esalq.usp.br.

when the trait under consideration is controlled by a single gene. In melon, for example, there have been reports of linkage between molecular markers and resistance genes to *Fusarium oxysporum* f. sp. *melonis* (Wechter and Dean, 1998; Karsies *et al.*, 2000; Brotman *et al.*, 2005), zucchini yellow mosaic virus (ZYMV: Danin-Poleg *et al.*, 2002), melon necrotic spot virus (MNSV: Baudracco-Arnas and Pitrat, 1996), cucumber mosaic virus (CMV; Dogimont *et al.*, 2000), and papaya ringspot virus-W (PRSV-W: Brotman *et al.*, 2005; Teixeira and Camargo, 2006). In addition, two candidate resistance genes have been identified by positional cloning, one conferring resistance to race 2 of *F. oxysporum* f. sp. *melonis* (Joobeur *et al.*, 2004) and another conferring resistance to MNSV (Nieto *et al.*, 2006).

Although several studies exist on resistance genes against melon powdery mildew, there are few published studies dealing with linkage analyses. Working with the melon line WMR29, Pitrat (1991) reported linkages among a resistance gene to races 1 and 2 of *P. xanthii*, the *Fn* (*flaccida necrosis*) gene, that confers resistance to ZYMV strain F, and the *Vat* gene, that confers resistance to virus transmission by the aphid *Aphis gossypii*. Pitrat (1991) also described linkage between a resistance gene to race 2 of *P. xanthii* in the *C. melo* line PI 414723 and the morphological marker “halo cotyledons”. Finally, Perchepped *et al.* (2005) mapped one major quantitative trait loci (QTL) conferring resistance to *P. xanthii* race 3 and another to race 5. Since the allelic relationships of powdery mildew resistance genes are not clear, it is not known if one of these QTL corresponds to the *Pm-1* gene.

We conducted a linkage analysis between the *C. melo* amplified fragment length polymorphism (AFLP) marker M75/H35_155 and the *Pm-1* resistance gene using a segregating population from a cross between two near-isogenic *C. melo* lines differing from each other not only with regard to the presence of *Pm-1* but also with respect to the presence of *Prv¹*, a resistance gene to PRSV-W (Teixeira and Camargo, 2006). The M75/H35_155 marker was previously identified in this *C. melo* population by Teixeira and Camargo (2006) as being distantly linked (40.9 cM) to the *Prv¹* gene. The present work tested the linkage hypothesis between M75/H35_155 and *Pm-1* due to the highly-isogenic nature of the parental lines.

The resistant *C. melo* line AF125^{*Pm-1*} was used as the donor of *Pm-1*. This line is a Cantalupensis Charentais type, derived from the self-pollination of *C. melo* line ‘Cinco’ which has resistance genes to several pathogens, including the *P. xanthii* resistance gene *Pm-1* and the PRSV-W resistance gene *Prv¹*. We introduced the *Pm-1* gene into the susceptible (S) recurrent *C. melo* line AF426-S during six backcrosses to produce the resistant (R) line AF426-R, thus lines AF426-S and AF426-R are near-isogenic lines (NILs). A backcross population of 143 plants (BC₁F₁) resulting from the [AF426-S x AF426-R] x

AF426-S cross was used for segregation analysis between the M75/H35_155 AFLP marker and the *Pm-1* gene.

To analyze the reaction of BC₁F₁ plants to *P. xanthii* race 1, plants were grown in a growth chamber at 25 °C to 30 °C with a relative humidity varying from 80 to 90% and a 12 h photoperiod provided by 20W daylight type fluorescent lamps (Sylvania, Brazil) providing 18.000 to 20.000 lux. To assess susceptibility to *P. xanthii* race 1 we collected leaf samples from each plant 26 days after emergence. The samples were disinfested with 70% (v/v) alcohol for a few seconds, rinsed with distilled water and then four 1.5 cm diameter leaf-disks were cut from each leaf and placed in petri dishes. With the aid of an eyelash glued to a pasteur pipette, the leaf disks were inoculated by transferring a small number of conidia and conidiophores of isolate R1 of *P. xanthii* (provided by Sakata Seeds Sudamerica Ltda., Brazil) maintained on cotyledons of the cucumber (*Cucumis sativus* L) Safira. After inoculation the plates were maintained in a growth chamber at 20 ± 2 °C, 70% relative humidity under a 12-hour photoperiod provided by 4 20W daylight type fluorescent lamps (Sylvania, Brazil). Leaf-disks from the parent lines and from the AF125^{*Pm-1*} donor line were inoculated as controls in addition to leaf-disks from *C. melo* plants from the differential varieties Hale’s Best Jumbo, PMR 45, PMR 5, PMR 6 and Edisto, which were used to confirm the race identity of isolate R1 according to a standard classification system (Thomas, 1978). Symptoms were evaluated 12 days after inoculation, using a stereoscopic microscope. Plants whose leaf-disks showed signs of the pathogen (mycelium, conidiophores, and conidia) on their surface were considered susceptible.

We extracted DNA from leaves of the plants when they were about 20 days old according to the method of Hoisington *et al.* (1994). Amplification of the M75/H35_155 marker (155 bp) was made as described by Teixeira and Camargo (2006) after DNA digestion with the *MseI* and *HindIII* (Invitrogen, USA) enzymes followed by amplification with the selective primers *HindIII*-ACA and *MseI*-GTA (Invitrogen, USA).

Proportions of genotype classes for the marker and for the *Pm-1* gene were tested using the chi-squared test. The recombination frequency between the marker and the resistance gene was used to estimate the genetic distance between both.

As expected, parental line AF426-S showed symptoms of susceptibility while no development of the pathogen was observed on lines AF426-R and AF125^{*Pm-1*}. The frequency of resistant and susceptible plants in the backcross population did not differ from the expected frequency for segregation of a dominant resistance gene in a backcross population (Table 1), confirming the type of inheritance expected in this cross.

Segregation of marker M75/H35_155 was also dominant and monogenic, as expected (Table 1). Linkage analy-

Table 1 - Number of resistant (Rr) and susceptible (rr) backcross (BC₁F₁) *Cucumis melo* plants showing the presence (+) or absence (-) of the amplified fragment length polymorphism (AFLP) marker M75/H35_155. Chi-squared (χ^2) and recombination frequency (rf) values are also shown.

Segregation in the BC ₁ population				Total number of plants	χ^2 M75/H35_155 (1:1)	χ^2 <i>Pm-1</i> (1:1)	rf
Rr/+	Rr/-	rr/+	rr/-				
62	0	7	74	143	0.062	2.52	4.9

sis between M75/H35_155 and *Pm-1* revealed linkage between both at a distance of 4.9 cM. Only 7 recombinant plants were found and all were susceptible with the presence of the M75/H35_155 fragment, which excludes the possibility that these plants were not inoculated with *P. xanthii* (i.e. disease escapes). Since M75/H35_155 is distantly linked to the *Prv1* gene as previously described by Teixeira and Camargo (2006) which, in turn, is located in linkage group IX of the melon reference map (Périn *et al.*, 2002; Brotman *et al.*, 2002), it is possible that *Pm-1* is also located in this group. This could be tested by using more markers from group IX, but the probability of finding a polymorphic marker in the presumed interval between *Prv1* and *Pm-1* would be low due to the highly isogenic nature of the parental lines. An alternative would be to map marker M75/H35_155 in the reference map, if possible.

Because *P. xanthii* is a pathogen present in various producing regions worldwide, use of resistant plant cultivars is the most suitable control strategy (Perchepped *et al.*, 2005). The importance of the *Pm-1* gene stems from the fact that *P. xanthii* race 1 frequently occurs in production fields in the Brazilian Northeast (Reis and Buso, 2004). Therefore, molecular markers linked to the *Pm-1* gene are important tools for the development of resistant varieties. Further efforts should be made to convert the AFLP marker M75/H35_155 into a simpler and specific type of marker such as a sequence characterized amplified region (SCAR) based marker or a cleaved amplified polymorphic sequences (CAPS) marker. This is the first report of a marker linked to the *Pm-1* gene.

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Internet Resources

Portal do Agronegócio, <http://www.portaldoagronegocio.com.br/> (January 31, 2007).

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