Breeding melon for resistance to *Fusarium* wilt: recent developments

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Received: 3 December 2012 / Accepted: 28 February 2013 / Published online: 15 March 2013
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Abstract Melon *Fusarium* wilt (MFW), caused by *Fusarium oxysporum* f. sp. *melonis* (*Fom*), is one of the most destructive diseases of melon (*Cucumis melo* L.). The development and deployment of resistant cultivars is generally considered to be the best approach to control MFW. Based on the host resistance genes associated with variants of this pathogen, *Fom* isolates were classified into four physiological races designated 0, 1, 2, and 1,2. Two dominant resistance genes, *Fom-1* and *Fom-2*, control resistance to races 0 and 2, and 0 and 1, respectively. *Fom* isolates classified as race 1,2 are able to induce disease in melon lines carrying the above resistance genes. Many sources of resistance to *Fom* races 0, 1, and 2 have been reported. Partial resistance to race 1,2 controlled by polygenic recessive genes was only detected in a few Far Eastern melon accessions, except for the breeding line BIZ where complete resistance was described. Identification of

DNA markers tightly linked to genes conferring resistance to *Fom* has immediate application in MFW resistance breeding programs. The *Fom-2* gene has been cloned, and it encodes a protein with a nucleotide binding site (NBS) and leucine-rich repeats domain (LRR). Based on the sequence of this domain, some molecular markers linked to this gene were developed. Several DNA markers linked to *Fom-1* have also been described. However, the usefulness of these markers was variety-dependent. Therefore, their combined use would be very useful in marker assisted selection for introducing resistance to *Fom* races 0 and 2 in melon. Recently, these markers were used for the positional cloning of this gene, which encoded a protein with a NBS–LRR domains that shows similarity to the toll and interleukin-1 receptors (TIR). Regarding *Fom* race 1,2, nine QTL were detected on five linkage groups by composite interval mapping. In this paper we review the current knowledge of MFW disease, and focus on genetic resistance to *Fom* and marker-assisted selection for resistance.

Keywords *Fusarium oxysporum* f. sp. *melonis* · *Cucumis melo* · Race · Gene · QTL · Molecular markers

Introduction

Melon (*Cucumis melo* L.) is an important horticultural fruit crop in tropical and subtropical regions, but it is

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also grown extensively in temperate zone countries. World production of cantaloupes and other melons in 2009 was about 26 million tons (www.fao.org). As many other crops, melon is susceptible to numerous foliar and root fungal pathogens that induce disease and reduce yield and fruit quality. Among these, Fusarium wilt is caused by a soil-borne pathogen, *Fusarium oxysporum* Schlechtend: Fr. f. sp. *melonis* (H.N. Hansen) W.C. Snyder & H.N. Hans (*Fom*). This fungus survives in the soil as chlamydospores, and is capable of colonizing crop residues and roots of most crops grown in rotation with melon (Gordon et al. 1989). Therefore, crop rotation has only provided limited protection against melon Fusarium wilt (MFW) disease (Crino et al. 2007).

Soil disinfection using various chemical products mainly methyl bromide (Cebolla et al. 2000) was a traditional practice to control *Fom* in greenhouses. Because of environmental and human health concerns (Brimner and Boland 2003), this fumigant was banned in industrialized countries. Developing countries have a different methyl bromide control schedule mandating a 20 % reduction in 2005 and total phase-out by 2015 (Gullino et al. 2003).

Soil solarization is another effective strategy to reduce soil inoculum and control wilt disease (Tamietti and Valentino 2006), but is not readily applicable for intensive vegetable farming systems, where time required to solarize the soil is very limited. Furthermore, soil solarization is often limited by local climate constraints such as temperature and relative humidity (Shlevin et al. 2004). Grafting of melons onto resistant rootstocks is also considered a promising practice to control soil-borne diseases in vegetables, particularly for MFW (Cohen et al. 2002; King et al. 2008). However, the added cost still limits its feasibility only to melon varieties with great economic value.

The use of resistant cultivars, therefore, is probably the most effective and practical means of controlling MFW. The success of breeding programs for MFW resistance is influenced by many factors, including: the nature of the pathogen and diversity of virulence in the population; availability, diversity and type of genetic resistance; or the effectiveness of methods and tools, such as molecular markers, used for assessing plant resistance.

The objective of this paper is to review the present state of knowledge on the MFW disease, the existing sources of genetic resistance, and the availability and

usefulness of molecular markers and quantitative trait loci (QTL) linked to resistant to *Fom*. Such knowledge of is needed to develop resistant cultivars to this disease.

Pathogen and pathogenesis of MFW

Species, races and vegetative compatibility groups

Fusarium oxysporum Schlechtendahl emend. Snyder and Hansen is a cosmopolitan species (Booth 1971) comprising both pathogenic and nonpathogenic isolates (Gordon and Martyn 1997). The pathogenic isolates of *F. oxysporum* cause Fusarium wilt on several agricultural crops, and are accordingly subdivided into *formae speciales* (Snyder and Hansen 1940; Baayen 2000).

One of the economically more important and destructive *f. speciales* is the causal agent of MFW, *F. oxysporum* f. sp. *melonis* Snyder and Hansen (Leach and Currence 1938). The first report of MFW in New York was in 1930 (Chupp 1930), and it later has been found in many melon-growing areas worldwide, including America (Leach and Currence 1938), Europe, Asia (Quiot et al. 1979; Sherf and Macnab 1986), and Africa (Schreuder et al. 2000). The nature of the diversity comprised within *Fom* has direct bearing on the prospects for MFW control through genetic resistance; therefore a range of approaches are typically employed for the characterization of *Fom* isolates.

In general, to define genetic relationships within *f. speciales*, *F. oxysporum* strains have been grouped into vegetative compatibility groups (VCGs) based on their ability to form heterokaryons (Puhalla 1985). Strains that belong to the same VCG normally have identical alleles at their compatibility loci, enabling the exchange of nuclear material (Glass et al. 2000). In *Fom*, Jacobson and Gordon (1990a) identified eight VCGs: 0130, 0131, 0132, 0133, 0134, 0135, 0136 and 0137, in a worldwide collection of *Fom* isolates. Also, various DNA-based tools have been used to separate *Fom* into a number of clonal lineages that more or less correspond to their VCG grouping (Jacobson and Gordon 1990b; Namiki et al. 1998).

Currently, variation in virulence within a *f. speciales* has been categorized by assigning pathotypes to

pathogenic races. Races are defined by their differential interaction with host genotypes (Armstrong and Armstrong 1978), which, in some cases, are cultivars known to carry one or more major genes for resistance. *Fom* isolates have been divided into four common races designated as 0, 1, 2 and 1,2 (Table 1), based on pathogenicity on three melon differential cultivars, ‘Charentais-T’, ‘Doublon’ and ‘CM-17187’ (Risser et al. 1976). The resistance genes effective against the respective races have been characterized in these differential cultivars: ‘Doublon’ and ‘CM-17187’ possess single dominant resistance genes, *Fom-1* and *Fom-2*, respectively; ‘Charentais-T’ has no known resistance gene. The race nomenclature corresponds to the resistance genes that are overcome (Risser et al. 1976). Race 1,2 overcomes these two resistance genes and is further divided into pathotype 1,2y, which induces leaf yellowing symptoms before the death of the plants, and 1,2w, which causes wilting and death without prior yellowing symptoms (Bouhot 1981). More recently, race 1,2 has spread through the world (Velooso et al. 2000; Perchevied and Pitrat 2004; Herman and Perl-Treves 2007). The spread of this race has become a problem for melon cultivation, and the development of genotypes with resistance to multiple races of the pathogen represents a major objective in melon breeding programs. Until recently, only a few cultivars (hybrids) tolerant to race 1,2 have been available commercially, and they are mainly used as rootstocks.

Origin of pathogenic races

In general terms, the appearance of a new *Fom* race, as might be evidenced by a previously resistant cultivar succumbing to the disease, could be explained in one of two ways: either the new race was introduced from another geographic area, or it originated locally. The latter scenario may be

explained either by derivation from a preexisting race, through selection pressure for virulence by extensive spread of resistant cultivars, or by selecting from the local population of non-pathogenic strains of *F. oxysporum*. A good example of introduction from distant areas is *Fom* race1/VCG 0134, that is known to occur in Europe (Jacobson and Gordon 1990a), Central Asia (Mohammadi et al. 2004), North America (Jacobson and Gordon 1990a), and South Africa (Schreuder et al. 2000). Molecular markers, including mitochondrial (Jacobson and Gordon 1990b) and nuclear DNA haplotypes (Appel and Gordon 1995), confirm that VCG 0134 corresponds to a clonal lineage. Thus, identified infestations of race 1/VCG 0134 in California (Gwynne et al. 1997), Maryland (Jacobson and Gordon 1990a), New York (Zuniga and Zitter 1993), and South Africa are very likely the result of introduction from areas where the race was already established.

Although movement of strains as a result of human activities is clearly dominant in the establishment of new infestations of MFW, de novo origin of pathogenic forms is also possible. Several lines of evidence support this view. First, in these *f. speciales*, there is a complex relationship between pathogenic races and VCGs (or clonal lineages). That is, a given race may be associated with more than one VCG and some VCGs are associated with multiple races. For example, VCG 0134 of *Fom* is associated with all four known races. Furthermore, races 0, 1, and 1,2, are all associated with identical mtDNA (Jacobson and Gordon 1990b) and nuclear DNA haplotypes (Schroeder and Gordon 1993). The close relationship between these three different races may indicate that relatively simple genetic changes can lead to a change in cultivar specificity, i.e. one pathogenic race can give rise to another.

Factors such as co-evolution with the plant host and the spread of virulence determinants via processes

Table 1 Classification of *Fusarium oxysporum* f. sp. *melonis* races based on Risser et al. (1976)

<i>Fusarium oxysporum</i> f. sp. <i>melonis</i> races	Differential cultivars and their gene for resistance ^a		
	Charentais-T	Doublon (<i>Fom-1</i>)	CM-17187 (<i>Fom-2</i>)
Race 0	[S]	[R]	[R]
Race 1	[S]	[S]	[R]
Race 2	[S]	[R]	[S]
Race 1,2	[S]	[S]	[S]

^a [R] and [S] indicate resistance and susceptible phenotype respectively

such as parasexuality, heterokaryosis, and sexual recombination have also been implicated in the evolution of this pathogen (Gordon and Okamoto 1992; Guadet et al. 1989). Although parasexuality and heterokaryosis are known to occur in *F. oxysporum* (Beckman and Roberts 1995), sexual fruiting structures have never been observed in the species and only indirect evidence for sexual recombination has been detected (Snyder and Hansen 1940).

Molecular methods for *Fom* detection and quantification

Current methods used for *Fom* detection include visual observation of disease symptoms, fungal mycelia and spores (Baayen 2000), as well as isolation of the fungus using a selective culture medium (Komada 1975). Because the selective medium is not species- or strain-specific, proper identification can be time-consuming and non-definitive, and results are not immediately available due to the time required for fungal growth. Molecular methods based on polymerase chain reaction (PCR), such as real-time PCR, offer advantages over classical detection methods because they are sensitive, reliable, and quick (Scheda et al. 2004).

More recently, López-Mondéjar et al. (2012) developed and validated a real-time PCR method that allowed detection and quantification of *Fom* in asymptomatic melon seedlings and in their organic substrates. This technique allowed a sensitive and rapid detection of *Fom* in melon plant material and substrate as soon as 48 h after inoculation, compared with 5–6 days required by the culture-dependent techniques.

At present, however, no molecular tools are available to discriminate among *Fom* races and therefore further work will be necessary to develop new DNA-based technology for this purpose. In tomato, assays for xylem-secreted effector-transcripts of *F. oxysporum* f. sp. *lycopersici* were shown to correctly diagnose the fungal race (Lievens et al. 2009); such effectors, however, are yet to be discovered in f. sp. *melonis*. More recently, 75 cDNA fragments with differential expression between the races 1 and 1,2 were identified (Sestili et al. 2011). Such transcripts could provide markers for race identification, but this would require further studies.

Epidemiology and defense responses

Fom infects the root system, wherein it progresses through the epidermis, cortex and endodermal tissues and penetrates the xylem vessels through the pits. From this stage on, the fungus uses the xylem for upward movement and establishment throughout the plant (Bishop and Cooper 1983). Whilst in the xylem, the mycelium sporulates, and microconidia are carried upwards by the xylem stream. At vessel ends, conidia germinate and the secondary mycelium penetrates the next vessel.

Plant infection by *F. oxysporum* is therefore a complex process that comprises several stages of host–pathogen interaction: recognition of the host roots and adsorption; penetration of hyphae through the different root tissues; penetration and progression in the xylem; and adaptation to the internal plant environment. To be successful, the fungus must overcome different plant defense responses at each stage (Beckman and Roberts 1995; Di Pietro et al. 2003; Michielse and Rep 2009). During the final stage of infection, the fungus secretes lytic enzymes and toxins that lead to disease symptoms, including necrotic lesions, chlorosis and wilting. Melon resistance sources have been identified and genetically characterized, but the defense mechanisms that confer resistance remain elusive.

The interaction and dynamics of *Fom* race 1,2 colonization in a susceptible melon cultivar ‘Ein Dor’ versus resistant melon line BIZ has been documented using green fluorescent protein (GFP) as a marker (Zvirin et al. 2010). At 1–2 days post-inoculation, the fungus grew on the root epidermis and adhered to epidermal cell borders. By day 4, the mycelium crossed the cortex and endodermis through narrow pores in cell walls and reached xylem vessels, where it sporulated and produced secondary hyphae that grew upwards. Race 1,2 colonized the resistant plant’s vascular system, but the incidence of seedling infection was lower than in susceptible ‘Ein Dor’, suggesting stronger defense responses in BIZ expressed at the pre-xylem stage of infection. Infection of the vascular system of BIZ was slower; at 11 days post-inoculation, race 1,2 only colonized the lower hypocotyl of BIZ, whilst the upper hypocotyls of ‘Ein Dor’ were already infected. Thus, Zvirin et al. (2010) established that resistant plants were not immune to the pathogen, but were able to quantitatively inhibit its progression

by expressing an efficient defense response. They also demonstrated that transcript levels of phenylalanine ammonia lyase, chitinase and hydroperoxide lyase were induced to a greater extent in the resistant line.

Pretreatment of melon plants with dinitroaniline herbicides markedly increased their resistance to Fusarium wilt (Grinstein et al. 1976; Lotan-Pompan et al. 2007). Cohen et al. (1986) reported that the dinitroaniline herbicides trifluralin and dinitramine were the most effective for inducing resistance to *Fom* and suggested that reduction of wilt symptoms is associated with a reduction in ethylene production. In addition, higher glutathione levels following dinitroaniline treatment have been suggested to confer protection against *Fom* (Bolter et al. 1993).

Lotan-Pompan et al. (2007) identified, using suppression subtractive hybridization and cDNA-AFLP, seven genes whose expression is associated with resistance to *Fom* race 2 following trifluralin treatment. Furthermore, the authors demonstrated that expression of four stress-related and up-regulated genes was enhanced when the plants were subjected to salinity stress, suggesting that trifluralin induces a general stress response which protects the plant against Fusarium wilt.

Resistance to *Fom* races 0, 1 and 2

Sources of resistance to *Fom* races 0, 1 and 2

The *Fom-1* gene, originally identified in melon cultivar ‘Doublon’, confers high-level of resistance to races 0 and 2, whereas the *Fom-2* gene, originally identified in melon cultivar ‘CM17187’, confers high resistance to races 0 and 1 (Risser et al. 1976). To date, these genes have been extensively used in melon breeding and were already introduced to the majority of modern melon cultivars. However, it is desirable to have additional resistance sources available, because future adaptation of the pathogen could render specific resistance genes ineffective. Therefore, many studies searched for novel melon resistance sources to *Fom* (Zink 1983; Champaco et al. 1992; Pitrat et al. 1996; Álvarez et al. 2005). These studies revealed that, in the melon germplasm, resistance to *Fom* races 0, 1 and 2 is more frequent than that to *Fom* race 1,2. Resistance traits were found in melon accessions belonging to different botanical varieties of *C. melo* L. from

different geographical origins, mostly areas of greater melon genetic diversity: the Iberian Peninsula, Far East and Middle East. Pitrat et al. (1996) screened an extensive collection of *C. melo* for resistance to *Fom* and found that 14.7 % of the 353 accession tested were resistant to both races 0 and 2, and 13.8 % were resistant to races 0 and 1. In another study, Álvarez et al. (2005) found new resistance sources to *Fom* races 0, 1 and 2 in a collection of 139 accessions from different geographical origins. Resistance to race 1 was less common than resistance to the other two races, since only four accessions showed race 1 resistance, whereas twelve were resistant to races 0 and 2.

Genetics of resistance to *Fom* races 0, 1 and 2

The first genetic studies on *Fom* resistance were carried out by Messiaen et al. (1962). They described *Fom* resistance among French ‘Cantaloupe Charentais’ genotypes and selected open pollinated cultivars homogeneous for resistance, such as ‘Doublon’ and ‘Védrañtais’. This resistance was conditioned by a dominant gene called *Fom-1* (Messiaen et al. 1962). Screening of the genetic resources led to the discovery of an independent dominant gene (*Fom-2*) in some accessions from the Far-East (CM 17187). This protection, however, was short-term, since it was overcome by novel *Fom* strains of race 1,2 (Risser et al. 1969).

Later, Zink and Gubler (1985) also described a dominant gene, *Fom-3*, as responsible for resistance to races 0 and 2 in melon line Perlita-FR. *Fom-3* is tightly linked to *Fom-1* and the two are suspected to be allelic. Risser (1987) affirmed that resistance in Perlita-FR is controlled by *Fom-1* and the susceptible plants detected in the F₂ generation of the cross Perlita-FR × ‘Doublon’, used to test for allelism, could result from residual segregation occurring in the Perlita-FR parent used by Zink and Gubler (1985).

Recently, Oumouloud et al. (2010) reported a new recessive gene (*fom-4*) that confers, together with *Fom-1*, resistance to races 0 and 2 in the Tortuga melon line. Similar to the other *f. oxysporum* *F. speciales*, that have exclusively asexual reproduction and little potential for gene flow, *Fom* presents only a low risk to overcome major resistance genes (McDonald and Linde 2002). However, this situation may change because of the extensive commercial

cultivation of melons carrying the resistance controlled by *Fom-1* and *Fom-2*.

The use of different resistance genes such as *fom-4* could provide higher levels of resistance to *Fom* races 0 and 2; Tortuga may therefore constitute a new alternative source of resistance. In addition, resistance controlled by more than one gene might increase its durability (Khetarpal et al. 1998). Thus, better protection against race 2 could be achieved by combining two resistant genes, *Fom-1* and *fom-4*. The combined use of the identified molecular markers linked to *Fom-1* gene (Oumouloud et al. 2009) and the ones linked to *fom-4* we are developing at this moment (Oumouloud et al. 2012) would be very useful in marker assisted selection for introducing the two resistance genes into the melon varieties.

Molecular markers linked to *Fom-1* and *Fom-2* genes

The expression of MFW symptoms following artificial inoculation is affected by the virulence of the pathogen isolates (Namiki et al. 1998), the genetic background of the plant (Mas et al. 1981), and environmental factors such as temperature and light intensity (Cohen et al. 1996; Burger et al. 2003). Furthermore, some genetically susceptible plants may escape wilting following standard inoculation. Escapes as well as ambiguous symptom expression in controlled inoculation tests result in selection of false negatives that reduce selection progress for resistance to MFW. Molecular markers linked to genes conferring resistance to *Fom* have the potential to reduce or eliminate this problem, by ensuring that the chromosome segment that carries the resistance gene is selected and maintained even when the inoculation test is not very reliable, or strictly on the basis of the presence of the marker without disease testing.

Inoculation with non-pathogenic *Fom* races may confer cross-protection (Mas et al. 1981; Alabouvette and Couteaudier 1992; Freeman et al. 2001). Thus, inoculation with one pathogenic race could impede subsequent selection of the same plant for resistance to an additional race in a sequential testing series. It was found, in fact, that melon plants that survived inoculation with race 1 did not show any disease symptoms following subsequent inoculation with race 2 (Chikh-Rouhou et al. 2006). Hence, molecular markers enable

simultaneous selection of genes for resistance to two or more *Fom* races.

Development of molecular markers to the *Fom-2* gene

The bulk segregant analysis (BSA) strategy, described by Michelmore et al. (1991), was used to detect random amplified polymorphic DNA (RAPD) markers linked to *Fom-2* (Wechter et al. 1995). These fingerprint markers have been transformed to more stable and convenient single-locus ones, such as cleaved amplified polymorphic sequences (CAPS; Zheng et al. 1999), or sequence characterized amplified region (SCAR; Zheng and Wolf 2000).

Combining the BSA strategy with amplified fragment length polymorphism (AFLP) methodology, Wang et al. (2000) found some AFLP markers linked to the *Fom-2* locus, and converted them into co-dominant SCAR markers, designated ‘AM’ and ‘FM’ (Table 2). The usefulness of these SCAR markers for indirect selection of *Fom-2* was confirmed across 24 melon accessions from diverse race 1 resistant and susceptible origins. These and other markers are regularly used by melon breeders and served as starting points for map-based cloning of the *Fom-2* gene (see below).

Cloning and characterization of *Fom-2* gene

The *Fom-2* resistance gene was isolated by a map-based cloning strategy using a population derived from the cross ‘Vedrantais’ × PI 161375 (Joobeur et al. 2004). Two additional populations have been derived from two F₇ recombinant inbred lines (RILs) segregating for *Fom-2*. A BAC contig was built from the MR-1 library (Luo et al. 2001), and the sequencing of two overlapping partial BAC clones identified three candidate genes. Research efforts concentrated on one of these genes because of its high similarity to resistance genes of the NBS–LRR class (Joobeur et al. 2004).

The putative *Fom-2* gene is 3 kb long and contains an uninterrupted open reading frame predicted to encode a 1,073 amino acid polypeptide that includes the different features of non-TIR NBS–LRR proteins. In contrast to most members of this class, no evidence of CC structure was found in the N terminus of the *Fom-2* protein.

Table 2 Single-locus markers linked to *Fom-2* gene

Marker name	Marker type	Primers	Restriction enzyme	Reference
AM	SCAR	5'-CTTCATCACTATTCGAGGATGAC-3' 5'-CTTTCTGCACACCAACCAAAAAGG-3'	–	Wang et al. (2000)
FM	SCAR	5'-GAAGATGCAAAGAAAAAGAGAAGG-3' 5'-TCAATTATTAAACATTCTGATGCC-3'	–	Wang et al. (2000)
CAPS2	CAPS	5'-GGAAAGTGAGGTGTTGAATT-3' 5'-TACACATTGGTCCGTTAGAC-3'	<i>EcoRI</i>	Wang et al. (2011)
CAPS3	CAPS	5'-AGACGTAGCATTGCTTCTCTAG-3' 5'-TGGCATCCTTCAGCACCTTC-3'	<i>XbaI</i>	Wang et al. (2011)
Fom2-R ₄₀₈	SCAR	5'-GAGAAATTTGCAATGGGTGG-3' 5'-TTACTACTATTATGCTCAACTTGC-3'	–	Oumouloud et al. (2012)
Fom2-S ₃₄₂	SCAR	5'-ATGAAAAGAAAAGATAACGACGA-3' 5'-ATTGCTCTAAGTTGATCATATTCTG-3'	–	Oumouloud et al. (2012)

SCAR sequence characterized amplified region, CAPS cleaved amplified polymorphic sequences

Additionally, Wang et al. (2011) described, using the Pfam software (<http://pfam.sanger.ac.uk/>), the detailed characteristics of this NBS–LRR protein. The protein harbours two significant Pfam-A match structures, an NB-ARC domain, and an LRR-1 domain. Another seven possible LRR-1s were observed at the C terminus of the *Fom-2* protein, that conformed with the consensus motif LxxLxxLxxLxx (N/C/T)x(x)L observed in cytoplasmic *R*-gene products (Jones and Jones 1997). The *Fom-2* protein has also one Sfi1 C (spindle body associated protein C-terminus) domain and an EAF (ELL-associated factor) domain.

Joobeur et al. (2004) revealed that the amino acid sequences from three susceptible cultivars ('Védrantais', 'Ananas Yokneum' and 'Durango') were identical to each other; however, when compared with the amino acid sequences deduced from resistant genotypes (MR-1 and PI 161375), 25 amino acids out of 541 were different. The sequences of the LRR fragment from the two resistant lines were identical, except of three nucleotides. These differences resulted in the substitution of the amino acid residues V and K in MR-1 by M and E in PI 161375, respectively. So far, however, functional validation of the *Fom-2* gene by transgenic complementation/silencing of resistance, or by TILLING, has not been reported. A preliminary study using transgenic roots of composite melon plants reported the expression of the *Fom-2* promoter fragment along the vascular tissues (Normantovich et al. 2012). Using the same system to express the *Fom-2* coding sequence in a *Fom*-susceptible

background resulted in partial resistance in most, but not all experiments (Normantovich et al. 2012).

Recently, Oumouloud et al. (2012) reported the cloning and sequencing of a partial LRR fragment of *Fom-2* from 11 melon resistant accessions from various geographic regions. They identified three alleles of *Fom-2* and their results revealed that the structure of the *Fom-2* LRR domain is highly conserved, since 8 of the 11 resistant genotypes showed similar alleles to the resistant one characterized in the PI 161375 line. Conversely, PI 124111 was the only line that presented the same resistant allele previously described in MR-1. This could be explained by the ancestral relations between the two lines (Monforte et al. 2003), as the MR-1 breeding line was derived from PI 124111 (Thomas 1986). Finally, accession Cum-355 carried a novel resistance allele that differs from both PI 161375 and MR-1.

The information generated from the *Fom-2* LRR region sequences allowed systematic development of "Functional Markers" that were developed based on the nucleotide polymorphisms detected between the susceptible and resistant *Fom-2* alleles (Wang et al. 2011; Oumouloud et al. 2012). Such markers were first documented in plants by Andersen and Lubberstedt (2003) and have been recently developed for several cloned *R*-genes. They are highly predictive of phenotype as they target the functional ("causal") polymorphism within a desired gene and overcome the problem of recombination between marker and trait.

In this context, Wang et al. (2011) reported two CAPS markers, representing allele-specific markers based on SNP in the LRR region of *Fom-2* (Table 2). In a parallel study, Oumouloud et al. (2012) developed two simple and efficient SCARs, *Fom2-R*₄₀₈ and *Fom2-S*₃₄₂, that represent a pair of allele-specific markers (Table 2). The *Fom2-R*₄₀₈ primers amplify a single band of 408 bp only in the resistant genotypes, whereas the *Fom2-S*₃₄₂ primers amplify a 342 bp band only in the susceptible ones. The two primer pairs can be combined in a multiplex PCR reaction, providing together a co-dominant marker. Such SCARs resulted in good identification of 27 resistant genotypes representing several melon horticultural types, enhancing the reliability and cost effectiveness of marker assisted selection for the *Fom-2* gene.

Molecular markers linked to *Fom-1* gene

The first molecular markers linked to this gene, were reported by Brotman et al. (2002, 2005) using two recombinant inbred line populations developed at “Institut National de la Recherche Agronomique-France” (INRA). Based on BSA, Brotman et al. (2005) identified a RAPD marker that generated a 1,235-bp fragment linked to the *Fom-1* gene in repulsion phase. This RAPD was converted to a CAPS marker designated as 62-CAPS (Table 3). This marker mapped at a distance of 0.7 and 6.3 cM from *Fom-1* in the two populations, respectively. An additional marker, based on cloned resistance gene homologues (RGH), was designated NBS-1 (Brotman et al. 2005), transformed into a CAPS marker (Table 3), and mapped at 2.8 cM from *Fom-1*.

Testing 62-CAPS and NBS1–CAPS in a set of 14 genotypes (seven resistant to *Fom* race 2 and seven susceptible) revealed a complex situation. The markers did not separate all the genotypes according to their resistance phenotype; instead, they seemed to group the accessions according to their horticultural or botanical groups, with group-specific marker haplotypes.

In a study that combined BSA and RAPD markers, Oumouloud et al. (2008) identified three molecular markers (*B17*₆₄₉, *V01*₅₇₈ and *V06*₁₀₉₂) linked to the *Fom-1* locus, using the F₂ generation from the cross Charentais-*Fom1* (*Fom* race 2 resistant) × TRG-1551 (susceptible). The polymorphic fragments were cloned, sequenced and converted to SCAR markers *SB17*₆₄₅, *SV01*₅₇₄ and *SV06*₁₀₉₂ respectively (Table 3).

The markers *SB17*₆₄₅ and *SV01*₅₇₄ amplified DNA fragments of 645 and 574 bp, respectively, in the resistant parent, while *SV06*₁₀₉₂ amplified a 1,092 bp fragment only in the susceptible parent. Markers *SB17*₆₄₅ and *SV01*₅₇₄ were linked in coupling phase to *Fom1*, at 3.5 and 4 cM, respectively, whereas *SV06*₁₀₉₂ was more distant (15.1 cM). The application of these three markers across 24 melon *Fom* race 2 resistant and susceptible accessions from diverse origins revealed different marker haplotypes and supported multiple, independent origins of this resistance trait. Nevertheless, since the markers are not very tightly linked to the gene, recombination could also explain cases of incorrect genotyping.

In a different study, four AFLP markers linked to *Fom-1* (GTC/ATG-260, TCG/GGT-400, TAG/GCC-470 and GTG/ACC) have been identified using the BSA approach (Tezuka et al. 2009). These markers were mapped in 125 F₂ individuals derived from the cross between line MR-1 (*Fom* race 2 resistant), and the susceptible Japanese line P11. Markers GTC/ATG-260 and TCG/GGT-400 were linked in coupling phase to *Fom-1* at 0.5 and, 4.9 cM respectively, whereas TAG/GCC-470 was linked in repulsion to the resistant allele at 0 cM. The fourth marker, GTG/ACC, was considered co-dominant and mapped at 10.3 cM from *Fom-1*. TAG/GCC-470 was converted to a SCAR designated S-TAG/GCC-470 that amplified a single fragment (347 bp) only from P11 and the susceptible bulks (Table 3). Sequencing of regions flanking TCG/GGT-400 allowed the development of two CAPS markers, C-TCG/GGT-400 and CAPS2 (Table 3).

The usefulness of these DNA markers in determining the *Fom-1* genotype was tested with several fixed lines and commercial F₁ melon hybrids. The results, like the ones discussed above, differed among horticultural types. S-TAG/GCC-470, GTC/ATG-260 and C-TCG/GGT-400 corresponded well to the *Fom-1* genotype in var. *reticulatus* and *cantalupensis* but not in vars. *chinensis*, *conomon* and *makuwa*.

Tezuka et al. (2011) developed a SCAR marker (S-MRGH9) and three CAPS markers (CAPS3, C-MRGH12 and C-MRGH13) based on the published BAC 31O16 sequence (Van Leeuwen et al. 2005), which contains a cluster of melon RGH and maps around the *Fom-1* locus (Table 3).

These markers were mapped using the same F₂ population (Tezuka et al. 2009), as well as a collection

Table 3 DNA markers linked to *Fom-1* gene

Marker name	Marker type	Primers	Restriction enzyme	Reference
NBS1–CAPS	CAPS	5'-TATTGCTAAAGCTGTTTTCAAAAGCG-3' 5'-AACAAAACTTTTCGATTTCCTAAGTT-3'	<i>Alw26I</i>	Brotman et al. (2005)
62-CAPS	CAPS	5'-GGAGAAGATGCTAGAGCCATTC-3' 5'-AATCGGGCATCCTGTTTTGG-3'	<i>NcoI</i>	Brotman et al. (2005)
SB17 ₆₄₅	SCAR	5'-AGGGAACGAGTTGAGAGAGCTAGA-3' 5'-CGAGGATTCTTAAGTAGCATGGA-3'	–	Oumouloud et al. (2008)
SV01 ₅₇₄	SCAR	5'-TGACGCATGGAATGAAATAAA-3' 5'-GCATGGCCAAGGTGCAATA-3'	–	Oumouloud et al. (2008)
SV06 ₁₀₉₂	SCAR	5'-ACGCCCAGGTATCATATACACC-3' 5'-ACGCCCAGGTTACGAAGTCA-3'	–	Oumouloud et al. (2008)
CAPS2	CAPS	5'-CAATTTTGGTTTCTTTGGATGG-3' 5'-TTTCGAGGTTAGAGGTTTGTC-3'	<i>TaqI</i>	Tezuka et al. (2009)
S-TAG/GCC-470	SCAR	5'-GAATTCTAGACTGAGCTTATAAACCC-3' 5'-TTAAGCCTAAAAGGGAATGGCCCCC-3'	–	Tezuka et al. (2009)
C-TCG/GGT-400	CAPS	5'-TTCAAAATCAAAGGAAATGCAA-3' 5'-GGACCCAAACTTACCCTACACTT-3'	<i>EcoRI</i>	Tezuka et al. (2009)
618-CAPS	CAPS	5'-CTGGAGCCCAAATGAACAAAC-3' 5'-GCTGGAGCATTCTAGTAATGAAA-3'	<i>TfiI</i>	Oumouloud et al. (2010)
S-MRGH9	STS	5'-GGTTGGCGATCTCACTGGAG-3' 5'-TTTACCAATTCGCCCCATCC-3'	–	Tezuka et al. (2011)
CAPS3	CAPS	5'-GTTGGAGATGTTCCCTTGA-3' 5'-ACCTGGCAACTTTGGTTTG-3'	<i>HaeIII</i>	Tezuka et al. (2011)
C-MRGH12	CAPS	5'-CGTCGGGTATGTCTCCATCT-3' 5'-TGATGCTGCTGATGGACTTC-3'	<i>XmnI</i>	Tezuka et al. (2011)
C-MRGH13	CAPS	5'-CCACCCATCCCCATATTCC-3' 5'-TGAGGAAGCAGGAGGGGAAC-3'	<i>TaqI</i>	Tezuka et al. (2011)
160P4-T7	CAPS	5'-GGTTAGATGTTGAATTAATGG-3' 5'-TCTCGAGTGTTAGTGAGTTGG-3'	<i>TaiI</i>	Brotman et al. (2012)
RG10-1	CAPS	5'-CCTGTACTCTTGAAATCGAACAA-3' 5'-TTGTGGAAGACTAAAAGAGGTTCA-3'	<i>DdeI</i>	Brotman et al. (2012)
RG-A	CAPS	5'-GCCATGCTTCTTCTCTGAGC-3' 5'-CCAATTCTCTCAAACACCACTTT-3'	<i>SwaI</i>	Brotman et al. (2012)
RG9-2	CAPS	5'-TCTGTTGGAAGCGTTTGATG-3' 5'-TTGGCTCCAAATCATTTAGCTT-3'	<i>DdeI</i>	Brotman et al. (2012)
RG-G	CAPS	5'-AGCGTGTGGTATGCAGACAG-3' 5'-CAAGAATCCAACACGGGAGT-3'	<i>BsuRI</i>	Brotman et al. (2012)
160P4-SP6	CAPS	5'-GCATGTTAGGGATTGTACATTC-3' 5'-TTGTTGGCTTTTAGTTTTTCTGTCCATTTCTGTTTC-3'	<i>TaqI</i>	Brotman et al. (2012)

SCAR sequence characterized amplified region, CAPS cleaved amplified polymorphic sequences

of 104 recombinant inbred lines derived from the cross MR-1 × P11. A set of 70 melon genotypes, that included 43 fixed lines and 27 commercial F₁ hybrids

were used to test the usefulness of the above markers. According to the authors, C-TCG/GGT-400 seems suitable for *Fom-1* genotyping in var. *cantalupensis*,

while CAPS2, NBS1-CAPS, C-MRGH12 and 62-CAPS are suitable, with few exceptions, for genotyping accessions from vars. *chinensis*, *conomon* and *makuwa*.

The variety-dependent usefulness of DNA markers might indicate that there is no linkage disequilibrium between *Fom-1* and the markers, and crossing over has occurred in some cultivars and lines. Another explanation is that the *R* gene cluster around the *Fom-1* locus is very polymorphic; the four alleles detected by C-MRGH13 (Tezuka et al. 2011) could also reflect such polymorphisms.

Characterization of *Fom-1* gene

The *Fom-1* gene was isolated by a map-based cloning strategy (Brotman et al. 2012). A backcross ('Védrantais' × PI 414723) × PI 414723 population of 1190 individuals was screened for recombination events within the interval delimited by markers NBS1 and 62-CAPS flanking the *Fom-1* locus. In parallel, all BC₁S₁ families were inoculated with *Fom* race 2. The region was sequenced using libraries, and progenies displaying recombination events were genotyped by additional BAC-based markers. The results indicated that *Fom-1* was separated by a single recombinant from marker RG9-2 (Table 3), residing in exon 2 of the NB-LRR homologue, RGH9. On its other side, *Fom-1* was flanked by marker RG-G (Table 3) that maps between RGH8 and RGH9. This identified RGH9 as the *Fom-1* gene. The sequence analysis revealed that this gene belongs to the TIR–NB–LRR type. Further studies will be required to elucidate the molecular polymorphism between resistant and susceptible alleles and provide functional validation of the *Fom-1* gene action.

Resistance to *Fom* race 1,2

Sources of resistance to *Fom* race 1,2

As previously stated, many resources of high resistance to race 0, 1, and 2 have been reported, and incorporated into commercial melon cultivars; nevertheless, no gene that confers complete resistance to either race 1,2w or 1,2y has been reported, although partial resistance has been found.

The first report of melon resistance to *Fom* race 1,2 was recorded by Risser and Rode (1973) from accessions Ogon-9 and Piboule. Later, Pitrat et al. (1996) established that sources of resistance to race 1,2 are restricted to a few Far-Eastern accessions belonging to *C. melo* subsp. *agrestis*. When screening melon accessions from different geographical origins for resistance to race 1,2, they found that only 3 % of the 271 accessions tested showed some resistance, all of them from the Far East. The resistance to race 1,2 described in accessions Ogon-9 and Piboule allowed breeding of partially resistant lines, such as Isabelle (Risser and Rode 1973), two doubled-haploid lines, Nad-1 and Nad-2 (Ficcadenti et al. 2002), and some commercial hybrids.

Additionally, Hirai et al. (2002) selected two melon rootstocks cultivars 'Dodai No. 2' and 'Dodai No.1' that showed partial resistant to the race 1,2y. Also, Herman and Perl-Treves (2007) reported a near complete resistance to race 1,2 of *Fom* in the breeding line BIZ, and showed that BIZ definitely had a higher level of resistance than Isabelle. An F₁ hybrid, 'Adir', derived by crossing BIZ with a non-resistant counterpart, displayed good field resistance to race 1,2. 'Adir' also was used as a rootstock for the susceptible melon cultivar 'Ophir', and provided good protection against MFW (Horev 2002).

High resistance levels to race 1,2 have been described in three Japanese melon accessions (Kogane Nashi Makuwa, C-211, and C-40), and useful levels in one Russian (C-160) and two Spanish (C-300 and Mollerusa-7) accessions (Oumouloud et al. 2009). Chikh-Rouhou et al. (2010) screened 110 melon accessions from diverse origins for resistance to race 1,2 and reported partial resistance to this race within a Portuguese accession BG-5384 belonging to var. *cantalupensis*. Matsumoto et al. (2011) found high-level of resistance to race 1,2y in 34 of 76 accessions from 7 of 11 wild *Cucumis* spp. Nevertheless, this high-level resistance to race 1,2y could not be introduced into melon cultivars, because of strong reproductive barriers between melon and the wild *Cucumis* spp.

Genetics of resistance to *Fom* races 1,2

Resistance to *Fom* race 1,2 is complex and appears to be under polygenic control. Perchepped and Pitrat (2004) reported the mode of inheritance of resistance to *Fom* race 1,2 in the partially resistant line Isabelle

using a RIL population derived from an F_1 hybrid between Isabelle, and the susceptible cultivar, ‘Védrantais’. The authors confirmed that resistance to race 1,2 in Isabelle is under polygenic control, and established that the heritability of resistance was high (0.72–0.96), and the minimum number of effective factors controlling the resistance ranged between 4 and 14. They also showed that some RILs were significantly more susceptible than ‘Védrantais’; this result may indicate transgressive segregation, which implies that the susceptible parent ‘Védrantais’, may also have alleles for resistance.

Herman and Perl-Treves (2007) studied the nature of *Fom* race 1,2 resistance in the F_2 and backcross generations from the cross between BIZ and PI 414723. Segregation of the resistance response in these populations supported a model in which two complementary, recessive genes, designated *fom-1,2a* and *fom-1,2b*, are required to obtain full resistance. Recently, Chikh-Rouhou et al. (2011) determined the mode of inheritance of resistance to *Fom* race 1,2 in lines BG-5384, Shiro Uri Okayama, Kogane Nashi Makuwa and C-211. The F_1 , F_2 , and reciprocal backcross generations from the crosses between the above four resistant accessions and ‘Piel de Sapo’, a *Fom* race 1,2 susceptible melon, were analyzed by inoculation with isolates belonging to the 1,2y and 1,2w pathotypes. The distribution of the area under the disease progress curve (AUDPC) values in the F_2 and BC generations after inoculation with both pathotypes of race 1,2 of *Fom* was continuous, suggesting quantitative inheritance of resistance. Broad-sense heritabilities ranged from 0.48 to 0.59. Such relatively moderate values could be explained by the existence of several factors involved in the resistance and the epistatic effects detected in this study. Additive, dominance, and epistatic effects were significant in all crosses, which indicates that the resistance is under complex genetic control in the four accessions. Additive effects were always positive and significant, regardless of the pathotype, indicating that it should be possible to increase resistance levels by accumulating resistance genes.

QTL mapping

Following the above reported inheritance study, Perchepped et al. (2005) performed QTL-analysis of the resistance to *Fom* 1,2 in breeding line Isabelle using the same 120 RIL population described above.

They identified a total of nine QTL located on five linkage groups, most of them at linkage group ends, together explaining between 41.9 and 66.4 % of the total variation. Most of these QTL appear to be recessive. The resistant alleles of seven QTL originated from the partially resistant parent Isabelle, whereas resistance alleles of two QTL came from the susceptible parent, ‘Védrantais’. This agreed with the observed significant transgression towards susceptibility (Perchepped and Pitrat 2004).

Among the QTL, *fomIII.1* and *fomVI.1* were specific for the Y pathotype, while *fomV.2* and *fomXII.1* were only identified following inoculation with pathotype W strains. On the other hand, five QTL, *fomIII.2*, *fomIII.3*, *fomV.1*, *fomXI.1*, and *fomXII.1*, were effective against both pathotypes Y and W. These results suggested that partial resistance to *Fom* race 1,2 is governed by pathotype-shared loci, as well as by pathotype-specific loci.

QTL *fomV.2* co-localized with the resistance genes *Vat*, which confers resistance to aphid colonization and virus transmission, and *Pm-w* for powdery mildew resistance, within a cluster of resistance gene homologs (RGH; Brotman et al. 2002; Garcia-Mas et al. 2001). QTL *fomXI.1* co-localized with the resistance gene *Fom-2* (marker AM), which confers resistance to *Fom* races 0 and 1, and with the RGH sequence NBS3 (Brotman et al. 2002). The *Fom-2* gene itself has not been reported to contribute to race 1,2 resistance, therefore the loci implicated in resistance to *Fom* race 1,2 and races 0 and 1 may be different, but tightly linked. The presence of both quantitative and qualitative resistance genes in the same genomic regions suggests that QTL may correspond to allelic variation of qualitative resistance genes with intermediate phenotypes (Robertson 1989).

More recently, analysis of the melon genome sequence revealed that the resistance genes were organized in clusters. In particular, 79 R-genes were located within 19 genomic clusters, 16 with genes belonging to the same family. In the genomic interval of less than 135 kb, 7 TIR–NBS–LRR genes were clustered and located at the region harbouring the *Fom-1* resistance gene (Garcia-Mas et al. 2012).

Herman et al. (2008) reported a preliminary QTL analysis for resistance to *Fom* race 1,2 in the breeding line BIZ using a set of 154 F_3 families derived from the cross between this line and PI 414723. A major recessive QTL for resistance to *Fom* race 1,2

originated from Biz was detected in linkage group 2 (Gonzalo et al. 2005) that doesn't harbour the QTL described by Perchepped et al. (2005). The authors hypothesized that BIZ and Isabelle might carry different loci for resistance and not just different alleles in similar loci.

Conclusion

Two dominant resistance genes, *Fom-1* and *Fom-2*, control resistance to races 0 and 2, and 0 and 1, respectively. Many sources of resistance to these races have been found in several accessions belonging to different melon botanical varieties and the resistance was introduced to many melon commercial cultivars. Many molecular markers, linked to *Fom-1* and *Fom-2*, potentially useful for marker-assisted selection have been identified. The *Fom-2* gene was cloned and characterized, and SNP-based functional markers associated with resistance to race 1 were developed. These markers are expected to enhance the reliability and cost effectiveness of marker-assisted selection for the *Fom-2* gene in melon.

Regarding the *Fom-1* gene, several molecular markers linked to this gene have been reported; nevertheless, their usefulness was variety-dependent. The recent cloning of this gene, which encodes a TIR–NB–LRR protein, now paves the way to its full molecular characterization.

The new recessive gene *fom-4*, reported in Tortuga, should be studied in depth since it could prevent future breaking of resistance. Development of markers associated with *fom-4* is under way, and will be used for pyramiding genes to further increase the durability of resistance to race 2.

Sources of high resistance to *Fom* race 1,2 predominantly come from Far Eastern accessions belonging to *C. melo* subsp. *agrestis* such as Ogon-9 or Kogani Nashi Makuwa. These accessions are organoleptically different from the cultivated melons. Resistance to race 1,2 is complex, and appears to be controlled by multiple recessive genes, except in breeding line BIZ, where it was reported to be controlled by two recessive genes. To date, QTL associated with resistance to race 1,2 have been reported only in the lines Isabelle and BIZ. The availability of the melon genome sequence (Garcia-Mas et al. 2012) and the use of newer technologies

such as DNA microarrays will accelerate genome mapping and tagging of new QTL associated with resistance to race 1,2. These QTLs could be used to transfer the resistance into high yielding melon genotypes and combine different QTL with major resistance genes to the other races.

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