Scientia Horticulturae 220 (2017) 160–167

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Mapping of powdery mildew resistance genes in melon (*Cucumis melo* L.) by bulked segregant analysis

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ARTICLE INFO

Keywords:
Bulked segregant analysis
Powdery mildew
Resistance gene
Melon

ABSTRACT

The fungus *Podosphaera xanthii* causes powdery mildew, a disease that has serious negative effects on the production of melon (*Cucumis melo* L.) worldwide. In this study, an F_2 melon population, derived from a cross between a resistant (MR-1) and a susceptible cultivar (Top Mark), was used for excavation of major powdery mildew resistance genes by bulked segregant analysis (BSA). The resistant and susceptible DNA bulks were constructed using 30 plants selected from the F_2 population. Next-generation sequencing (NGS) was applied for the resequencing of the parental materials and two bulks. A total of 237.6 million paired reads were obtained that were used to calculate the Δsingle nucleotide polymorphisms (SNP)-index. A 0.6-Mb region on chromosome 12 was identified and further narrowed by 424 identified SNPs and quantitative trait locus (QTL) mapping in the F_2 progeny with 25 screened polymorphic cleaved amplified polymorphic sequences (CAPS) markers. A major effective QTL named *BPm12.1* was detected between the CAPS markers *BSA12-LI3ECORI* and *BSA12-LI4HINFI* which was tightly linked to the resistance gene for powdery mildew with genetic distances 0.02 cM and 0.28 cM. Seventeen candidate genes were identified, seven of which were predicted as candidate genes related to the resistance of melon to powdery mildew. The results of candidate gene analysis provided a potential target for further cloning and functional identification of the resistance gene in MR-1.

1. Introduction

Melon (*Cucumis melo* L., Cucurbitaceae) is an economically important vegetable crop. In 2013, global production of melon reached 29.3 million tons (http://faostat3.fao.org). Powdery mildew, a fungal disease occurring in both field and greenhouse conditions worldwide, reduces the productivity of all Cucurbitaceae crops. Researchers have previously reported many pathogens in melons; however, the two most commonly reported were *Podosphaera xanthii* (*P. xanthii*; initially referred to as *Sphaerotheca fuliginea*) and *Golovinomyces cichoracearum* (*G. cichoracearum*; originally named *Erysiphe cichoracearum*) (Křístková et al., 2009). Infestations by *G. cichoracearum, P. xanthii* have been documented to occur more frequently in areas with relatively high temperature and humidity worldwide (Mccreight, 2003; Mohamed et al., 1995). A case of *P. xanthii* infestation was first reported in 1925 in California. Since then many physiological races have been identified according to the various reactions after infection of different melon lines in *P. xanthii*, such as races 0, 1, 2 US, 2 France, 3, 4, 5, N1 (race 6), N2 (race 7), N3, and N4 (Hosoya et al., 1999). More recently, based on the results of 30 melon accessions inoculations, more races of *P. xanthii* in melon have been identified as eight variants of race 1, six variants of race 2 along with a newly discovered race N5, a variant of race 5 (Mccreight, 2006). Geographical location, melon cultivar, and weather conditions are the three main factors that influenced the major races of powdery mildew. Cases of incidence of races 1, 2, and 3 were reported mostly in America, whereas incidence of races 0, 4, and 5 were identified in France (Bardin et al., 1999a). In China, the most prevalent races of *P. xanthii* are races 1 and 2F (Hao et al., 2015; Wang et al., 2011), but which race of them is the predominant in the current study and still needs to be elucidated.

Identifying molecular markers and possible candidate genes related to the trait of resistance to powdery mildew is a necessary step toward making progress in molecular breeding directed at obtaining resistance to this plant disease (Varshney et al., 2012). Marker-assisted breeding has been extensively used in the search for molecular markers that are linked to this specific trait, especially when considering that resistance

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http://dx.doi.org/10.1016/j.scienta.2017.04.001
Received 20 December 2016; Received in revised form 3 March 2017; Accepted 4 April 2017
Available online 12 April 2017

is controlled by a single gene (Teixeira et al., 2008). Genetic linkage analysis is usually used in QTL derived from crosses between populations of bi-parents with contrasting phenotypes. Consequently, DNA markers were obtained based on the variation in the sequences of the parents, resulting in the linkage of markers with the trait of interest by analyzing the phenotype data. In marker-assisted selection (MAS), DNA markers that are closely linked with the target trait in the elite plant are mapped. Then, the target gene region is introduced into the donor plant by crossing of the elite and donor plants. Next, during the progress of a backcross, the desirable QTL are selected through the use of DNA markers until the target gene is stably inherited in the resulting plant cultivar (Ashikari and Matsuoka, 2006).

Currently, a total of 12 major genes have been reported that are related to resistance to powdery mildew in melon (Liu et al., 2010), and many studies have established the presence of resistance genes corresponding to *P. xanthii* races 1 and 2F. *Pm-1*, the gene responsible for resistance to *P. xanthii* race 1 is associated with LG IX (Teixeira et al., 2008); two loci (2.1cM) from Edisto 47 that confer resistance to *P. xanthii* race 1 have been mapped on LGII and LGV, respectively (Ning et al., 2014). The accession PI 414723 carries several genes bearing resistance as follows: *Pm-7* for resistance to race 1 (Pitrat, 2006); *Pm-x* resistant to race 2F (Pitrat, 1990); and two loci (on LGII) denote *Pm-x1*, *5* and *Pm-x3* for resistance to races 1, 5, and 3, respectively (Fazza et al., 2013); WMR 29 carries *Pm-w* resistant to races 1 and 2 associated with LGV; one major QTL (*Pm-R*) on LGV for resistance to races 1, 2, and 5 was found in the cultivar TGR-1551 (Yuste-Lisbona et al., 2011); *Pm-AN* carried by Ano2 is located in LGV with distances 1.4–1.8cM and 1.6–2cM (Wang et al., 2011); PI 124112 is resistant to *P. xanthii* races 1, 2, 3, 5 and *G. cichoracearum* race 1. *Pm-4* and *Pm-5* in PI 124112 were reported on LGV, and two independent genes (*PmV.1* and *PmXII.1*) were identified on LGXII (Perchepied et al., 2005); The resistance to *P. xanthii* race 2F in melon K7-1 was found to be controlled by a dominant gene *Pm-2F* on LGII (Zhang et al., 2012). In addition, earlier investigations revealed that the resistance gene *Pm-y* on LGXII from VA435 was responsible for resistance to *P. xanthii* race 2 (Périn et al., 2002). The breeding lines MR-1 and PI 124111F derived from PI 124111 present a high level of nonspecific resistance to powdery mildew. PI 124111F is resistant to *P. xanthii* races 1 and 2 (Cohen and Eyal, 1987). However, one resistance gene, MR-1, has never been reported previously.

In general, the traditional QTL mapping method is ineffective and time-consuming in screening polymorphic markers and genotyping (Abe et al., 2012). A previous study found bulked segregant analysis (BSA) was one of the most important methods employed to locate the gene of interest, and provided a convenient and a rapid method for identifying genes by generating two DNA bulks with a contrasting extreme target trait (Michelmore et al., 1991). In the BSA procedure, screening work was carried out among parents, F_1 and extreme phenotype bulks, and the markers showing genotypes that were identical in the bulks and their related parents were identified as polymorphic and used in the genotyping of the progeny (Semagn et al., 2010).

Over the past several years, the BSA approach has been widely used in the mapping of genes for the given phenotype in melon (Gao et al., 2015). Recently, the rapid development of next-generation sequencing (NGS) technologies (high-throughput sequencing) has accelerated the pace of research of gene mapping in a cost-effective manner (Schneeberger et al., 2009). NGS-based BSA that allows whole genome sequencing in two bulk DNA samples and analyzes single nucleotide polymorphism (SNP) is largely applied in the identification of target genes and the fine mapping of many traits, such as the gene for resistance to rice blast (Zheng et al., 2016), lethal gametophyte mutations (Uchida et al., 2015), and early flowering in cucumber (Lu et al., 2014). Furthermore, many related technologies, including MutMap (Abe et al., 2012; Schneeberger et al., 2009), SHOREmap (Schneeberger et al., 2009), etc., have taken full advantage of the rapidly declining cost of genome sequencing and were expected to significantly contribute to efforts to find more effective solutions to the breeding problem. However, no previous reports were found for the application of whole-genome sequencing-based BSA for the identification of the gene for resistance to powdery mildew in melon.

MR-1 is a differential host and is known to be responsible for resistance to many races of powdery mildew, but no resistance gene for MR-1 has been identified. The present study identified the inheritance and resistance to *P. xanthii* race 1 in MR-1. This study employed an F_2 population comprised of 346 individuals derived from a cross of MR-1 and Top Mark. BSA was coupled with NGS to detect the region of resistance genes of interest. Validation and narrow mapping were performed by detecting SNPs and using classic QTL analysis to identify the candidate gene related to the resistance of melon to powdery mildew.

2. Materials and methods

2.1. Plant materials

The cultivars MR-1 is highly resistant to powdery mildew, whereas Top Mark is susceptible to many races of powdery mildew. An F_2 generation of 346 individuals was obtained from a cross between MR-1 (P_1) and Top Mark (P_2). To identify the race of the *P. xanthii* isolated from the infected plants, thirteen melon accessions (Iran H, Top Mark, Védrantais, PMR 45, PMR 5, WMR 29, Edisto 47, PI 414723, MR-1, PI 124111, PI 124112, PMR 6, and Nantais Oblong) were required; these accessions have been commonly applied for identifying powdery mildew (Hosoya et al., 1999). Seeds of all melon accessions used in this study were provided by the College of Horticulture (Northeast Agricultural University, Harbin, China).

In the summer of 2015, individuals of P_1 ($n = 15$), P_2 ($n = 15$), F_1 ($n = 15$), and F_2 ($n = 346$), and 13 differential hosts were planted in a plastic greenhouse at the Horticultural Station of the Northeast Agricultural University, Harbin, China (44°04′N, E125°42′). Further, the individuals were separated by genotype and managed by using standard horticultural procedures (irrigation, hand-weeding, and pathogen prevention and control) typical of the climatic conditions in Harbin.

2.2. Phenotyping for powdery mildew resistance

Powdery mildew isolate was collected from a seriously infected melon field in 2015. Inoculations with the spore suspensions (10^6 spores per milliliter) were performed with a hand sprayer while plants were blooming (Zhang et al., 2011). The reaction of the infected parental lines, F_1, F_2 individuals, and 13 melon accessions were evaluated 10 days after inoculation with powdery mildew. Photographs were taken, and each individual was evaluated by observing 10 true leaves from the base to the top of the plant; the infection intensity with powdery mildew was categorized on a scale of 0–5 as previously described (Bardin et al., 1999b) and defined as follows: Class 0, no infection; Class 1, no visible infection; classes 2, 3, and 4 indicating low, medium, and severe levels of infection, respectively; Class 5, infection of entire leaves (Fig. 1). The percent disease index (PDI) was obtained to reliably identify the phenotype data using Eq. (1):

$$PDI = \sum \frac{\text{Sum of numerical disease ratings}}{\text{No. of plants evaluated} \times \text{Maximum disease rating scale}} \times 100 \quad (1)$$

Individuals were considered resistant, having a medium level of resistance, or susceptible to powdery mildew if PDI < 40, 40 ≤ PDI ≤ 60, or PDI > 60, respectively. A Chi-square test was performed to check the goodness of fit of resistance to powdery mildew. In addition, the race of the *P. xanthii* pathogen was determined according to the inoculation reaction of the 13 melon lines.