Conversion of an AFLP fragment into one dCAPS marker linked to powdery mildew resistance in melon

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Abstract

Resistance to powdery mildew races 1, 2 and 5 has been evaluated in F₂/F₃ families from a cross between the resistant line ‘TGR-1551’ and the susceptible Spanish cultivar ‘Bola de oro’. Resistance is controlled by two independent genes, one dominant and one recessive, that both confer resistance to races 1, 2 and 5 of Podosphaera xanthii. Bulked segregant analysis (BSA) and amplified fragment length polymorphism (AFLP) technique have been used for the identification of markers linked to powdery mildew resistance. The AFLP marker E38M82 has been converted into a derived cleaved amplified polymorphic sequences (dCAPS) marker. The marker developed was codominant and the estimated distance with the dominant gene for powdery mildew resistance was 5.7 cM.

INTRODUCTION

Marker assisted selection (MAS) is a powerful tool for breeding programs, since it provides significant advantages to traditional phenotypic screening. MAS is rapid, relatively inexpensive and is not hampered by pathogen unavailability. There is no environmental limitation, since MAS can be performed off season, and it allows simultaneous screening for many diseases. The combination of bulked segregant analysis [BSA (Michelmore et al. 1991)] and highly polymorphic PCR-based markers permits the identification and mapping of useful molecular markers for breeding purposes. Although the amplified fragment length polymorphism (AFLP) technique is powerful and reliable in identifying markers closely linked to genes of interest, it has some disadvantages. AFLP markers are mostly dominant, relatively costly, and laboriously, as well as not amenable for routine and quick screening. Hence, AFLP markers need to be converted into sequence-specific PCR markers for their application in breeding programs.

Powdery mildew is a major problem in melon production worldwide (McCreight 2006). Two fungal species, Podosphaera xanthii (DC.) VP Gelyuta and Golovinomyces cichoracearum (Castagne) U. Braun & N. Shishkoff, have been reported as the most common agents of powdery mildew in melon, although P. xanthii is the species usually found in regions with a temperate climate (Bertrand and Pitrat 1989; Kenigsbuch and Cohen 1992; Vakalounakis et al. 1994). To date, many

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races of \textit{P. xanthii} have been described (McCreight 2006). The predominant race of powdery mildew populations changes depending on the melon cultivar, the growing season, and the geographical area (Hosoya et al. 2000). Races 1, 2, and 5 are reported to be the most extensive in Southern European regions (Bertrand 1991; Bardin et al. 1997; Olalla 2001; Del Pino et al. 2002). Protective fungicides are currently used to control powdery mildew in spite of the usual loss of their efficiency (Hollomon and Wheeler 2002). The use of genetically resistant melon lines is a safe alternative to control this pathogen.

Genetics of powdery mildew resistance in melon has been profusely studied by several authors. Nevertheless, the inheritance of resistance remains somewhat confusing, due to many factors, including the environmental conditions which highly affect resistance expression (Cohen et al. 1996, 2002). Furthermore, the number of genes involved differs according to the study (Kenigsbuch and Cohen 1992; Epinat et al. 1993) and to the strain used (McCreight et al. 1987). The melon line ‘TGR-1551’ is resistant to races 1, 2 and 5 of \textit{P. xanthii} (Gómez-Guillamón et al. 1995, 1998). Resistance seems to be controlled by a dominant and a recessive gene (Gómez-Guillamón et al. 2006). The race specificity of each resistance factor is still not clear. In this paper, the genetic model of powdery mildew resistance in ‘TGR-1551’ is discussed. The identification of AFLP markers linked to powdery mildew resistance by using a BSA methodology and the development of a sequence-specific PCR marker are also reported.

**MATERIAL AND METHODS**

The powdery mildew resistant genotype ‘TGR-1551’ and the susceptible Spanish cultivar ‘Bola de oro’ were crossed to get the \textit{F}_1, \textit{F}_2 and \textit{F}_3 populations. Inoculations with \textit{P. xanthii} race 1, 2 and 5 were carried out by depositing a small amount of conidia on the second true leaf (Ferriere and Molot 1988). Two hundred and ninety five \textit{F}_2 plants were inoculated on the second true leaf with races 1 (isolate 27), 2 (isolate P-15.0), and 5 (isolate C8). \textit{F}_3 plants of nine \textit{F}_2 plants, which exhibited resistance to only one or two of the three races, were inoculated with each race separately. Fifteen plants of each \textit{F}_3 family and plants of the parental lines and the \textit{F}_1 hybrid were tested in the same conditions. Plants were maintained in a growth chamber at 32°C - 22°C with a 16:8h (light: dark) photoperiod and scored 12 days post-inoculation as either resistant (no apparent fungal development) or susceptible (profuse sporulation).

Total genomic DNA was isolated from young leaves of individual \textit{F}_2 plants as described by Doyle and Doyle (1990). For BSA, equal quantities of DNA from 10 resistant and 10 susceptible \textit{F}_2 individuals were pooled to establish the resistant and susceptible bulks, respectively (Michelmore et al. 1991).

The AFLP analysis was performed following the procedure described by Vos et al. (1995) and genomic DNA was digested with \textit{MseI} and \textit{EcoRI} enzymes. DNAs from the two parental lines, the \textit{F}_1 hybrid, and the resistant and susceptible bulks were simultaneously screened with 106 AFLP primer combinations. The primer combinations that generated polymorphic bands between the bulks were tested on the bulked individuals to eliminate false positive markers prior the screening of the whole \textit{F}_2 population. AFLP markers were named according to the standard list for AFLP

Target AFLP bands on the autoradiograph were matched to the corresponding area in the gel and the appropriated AFLP fragment was excised from the dried gel. The amplified fragment was purified according to Qu et al. (1998) and the DNA isolated was re-amplified by PCR, following the corresponding AFLP selective amplification using non-labeled primers. The resulting PCR products were purified by size-exclusion chromatography, using the GenElute PCR Clean-up kit (Sigma). DNA fragment was cloned into pGEM-T (Promega, USA) and transformed into Escherichia coli strain DH5α, according to the manufacture instructions. DNA sequences of clones were obtained from an ABIPRISM 377 Sequencer (Applied Biosystem) and used to design PCR primers from both ends of the AFLP fragment.

For linkage analysis, the recombination frequencies were estimated using the maximum likelihood equations of Allard (1956), whereas map distances were based on Kosambi’s mapping function (Kosambi 1944).

RESULTS

Plants of ‘TGR-1551’ and of the F1 hybrid were resistant to races 1, 2 and 5 of P. xanthii and plants of ‘Bola de oro’ were severely infected. Inoculation test of the F2 population was previously described in Gómez-Guillamón et al. 2006. The 295 F2 plants were either resistant or susceptible to the three races, except nine of them, which seemed to be recombinant (resistant to one race and susceptible to another one). F3 progenies obtained from these nine F2 plants were tested towards each race separately. The 15 plants of 8 F3 families evaluated were resistant to races 1, 2 and 5 while all plants of one F3 families were susceptible to the three races. No segregation was observed within the F3 families evaluated and selected from their respective apparently segregant F2. These results suggest that the same genetic factors control the resistance to the three powdery mildew races in TGR-1551. Finally, we can consider that the F2 population segregated 248 resistant plants to all three races: 47 plants susceptible to all three races, which is consistent with a 13 R: 3 S ratio corresponding to the segregation of two independent genes, a dominant and a recessive one (χ² = 1.54; P= 0.21), each of them conferring the resistance.

To identify markers associated with powdery mildew resistance, a total of 106 AFLP primer combinations were used to screen DNA from the two parental lines, the F1 hybrid and two bulks constructed for the BSA analysis. PCR analyses resulted in the amplification of 6850 AFLP fragments approximately, of which 1390 (20 %) were polymorphic between the parental lines. This polymorphism found between the parental lines corresponded with the rates of polymorphism found by Périn et al. (2002) between ‘Védra’ and ‘PI 161375’ (28.9 %) and by Perchepied et al. (2005) between ‘Védra’ and ‘PI 124112’ (29.8 %).

Nine AFLP primer combinations generated a total 16 polymorphic bands between the bulks. These combinations were tested on the bulked individuals to eliminate false positive markers. Finally, four primer combinations that generated five fragments linked to powdery mildew resistance were used to screen the F2 population to calculate the recombination frequencies and estimate the genetic distances. The AFLP fragments selected were E42M50 (8.4 cM), E42M80 (8.4 cM), E38M82 (9.0 cM), E38M43-1 (10.1 cM), and E38M43-2 (11.1 cM).
So far, only the fragment E38M82 has been transformed into PCR-based marker. Based on the sequence information of the E38M82 AFLP fragment, forward and reverse primers were designed for PCR amplification. The PCR products generated one monomorphic band between parental lines, and therefore bands from both parents were cloned and sequenced. The two fragments had the same length [109 bases pairs (bp)] and sequences of the two parental lines differed by a single nucleotide. New primers were designed to generate a derived cleaved amplified polymorphic sequences (dCAPS) marker. Polymorphism detected by this marker was codominant. The marker developed was screened in the whole F2 population and the estimated distance was 5.7 cM.

**DISCUSSION**

The absence of F2 individuals showing different response to the three races of powdery mildew have been demonstrated by the evaluation of F2/F3 recombinant families. Furthermore, Gómez-Guillamón et al. (2006) reported that the segregations ratios observed in the F2 populations were 13:3 resistant: susceptible for races 1, 2 and 5 of *P. xanthii*, with highly significant Chi-square values. According to these results, the resistance for powdery mildew race 1, 2 and 5 seems to be controlled by two independent genes, one dominant gene and one recessive gene (which could be designated as *A* and *b*, respectively) that both confer resistance to the three races. In accordance with this hypothesis, *A*--- and *--bb* individuals would show resistance, while *aab* individuals would be susceptible. Recent evaluations have shown that some susceptible F2 originated resistant and susceptible F3 plants. It could be assumed that those F2 individuals, had a genotype corresponding to *aabB* (susceptible); the segregation for resistance observed in their correspondent F3 [ *aaBB* and *aabB* (susceptible) and *aab* (resistant)], confirms the genetic model proposed.

According to this genetic model, the molecular markers identified would likely be linked to the dominant gene, and the genetic distances should be then overestimated because of the existence of some resistant F2 plants *aabb*. Currently, new bulks including resistant F3 plants of *aabb* genotype are under construction in order to identify molecular markers linked to the recessive gene. These markers should allow a more accurate estimation of the genetic distances among all these molecular markers and the powdery mildew resistance genes.

Markers linked to powdery mildew in melon are scarce. JianShe et al. (2005) reported a dominant molecular marker (RAPD-S329) linked to *P. xanthii* resistance gene on a F2 population from the cross between ‘1A15l’ and ‘Hengjin RRS’, and the distance between them was 6.8 cM. Fukino et al. (2006) established that the powdery mildew resistance gene in ‘PMAR No5’ was flanked by two SSR markers, and the map distance between these markers was estimated to be 6 cM. Perchepied et al. (2005) reported a linkage map based on a recombinant inbred line (RIL) population derived from the cross between ‘Védrantais’ and ‘PI 124112’, which allowed the localization of the resistance genes to *P. xanthii*, but nearby markers for breeding programs were not found. In this paper, a codominant marker linked to powdery mildew resistance has been developed and the linkage distance between them is of 5.7 cM, which is the closest molecular marker to powdery mildew resistance described until now. The difficulty of work with this pathogen and the low number of markers...
linked to this resistance makes that the marker developed could be useful in breeding programs for the selection of powdery mildew resistance to race 1, 2 and 5.

Currently, the conversion of AFLP fragments linked to the dominant resistance gene to powdery mildew into sequence-specific PCR markers, the identification of markers linked to the recessive gene and the construction of a genetic map are in progress.

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