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Er3 gene, conferring resistance to powdery mildew in pea, is located in pea LGIV

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Abstract Three genes for resistance to *Erysiphe pisi*, named *er1*, *er2* and *Er3* have been described in pea so far. *er1* gene is located in pea linkage group VI, while *er2* gene has been mapped in LGIII. SCAR and RAPD markers tightly linked to *Er3* gene have been identified, but the position of these markers in the pea genetic map was unknown. The objective of this study was to localize *Er3* gene in the pea genetic map. Towards this aim, the susceptible pea cv. Messire (*er3er3*) and a resistant near isogenic line of Messire (cv. Eritreo, *Er3Er3*) were surveyed with SSRs with known position in the pea map. Three SSRs were polymorphic between "Messire" and "Eritreo" and further surveyed in two contrasting bulks formed by homozygous *Er3Er3*/*er3er3* individuals obtained from a F_2 population derived from the cross C2 (*Er3Er3*) × Messire (*er3er3*). A single marker, AA349, was polymorphic between the bulks. Subsequently, other ten markers located in the surrounding of AA349 were selected and analysed in *Er3Er3* and *er3er3* plants. As a results, another SSR, AD61, was found to be polymorphic between *Er3Er3* and *er3er3* plants. Further linkage analysis confirmed that SSRs AA349 and AD61 were linked to *Er3* and to the RAPD and SCAR markers previously reported to be linked to this gene. *Er3* gene was located in pea LGIV at 0.39 cM downstream of marker AD61. The location of *Er3* gene in the pea map is a first step toward the identification of this gene.

Keywords *Er3* gene · Mapping · Pea powdery mildew · *Erysiphe pisi*

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Introduction

Pea powdery mildew is an air-borne disease of worldwide distribution, being particularly important in climates with warm dry days and cool nights (Smith et al. 1996). This disease can cause up to 50% yield losses (Ram and Prasad 1994; Warkentin et al. 1996), reducing total yield biomass, number of pods per plant, number of seeds per pod, plant height and number of nodes (Gritton and Ebert 1975). *Erysiphe pisi* was thought to be the only causal agent of this disease, but, recently, *Erysiphe trifolii* and *Erysiphe baeumleri* have been also found infecting pea in USA,

Spain, India and Czech Republic (Ondřej et al. 2005; Attanayake et al. 2010; Fondevilla et al. 2013).

The use of resistant cultivars is the most efficient and ecological method to control this disease. Three genes conferring resistance to *E. pisi*, named *er1*, *er2* and *Er3*, have been identified so far (Harland 1948; Heringa et al. 1969; Fondevilla et al. 2010). *er2* and *Er3* genes are, in addition, also effective against *E. trifolii* (Fondevilla et al. 2013). While *er1* and *er2* were identified in *Pisum sativum* accessions, *Er3* derives from *Pisum fulvum* and has been successfully introduced into adapted *P. sativum* material by crossing (Fondevilla et al. 2010). *er1* gene, also called *PsMLO1*, is a member of the *mlo* gene family and confers resistance to *E. pisi* penetration (Humphry et al. 2011; Fondevilla et al. 2006). The gene has been sequenced and mapped in pea LGVI (Humphry et al. 2011; Timmerman et al. 1994). Resistance to *E. pisi* mediated by *er2* gene is influenced by temperature and leaf age and based mainly on post-penetration cell death, complemented by a reduction of percentage penetration success in mature leaves (Fondevilla et al. 2006). By contrast, *er2* resistance to *E. trifolii* is not temperature dependent (Fondevilla et al. 2013). Molecular markers linked to this gene have been reported, locating the gene in LGIII (Tiwari et al. 1999; Katoch et al. 2010). Resistance to *E. pisi* governed by *Er3* resistance is effective at all temperatures and expressed as a strong hypersensitive response (Fondevilla et al. 2007), while resistance conferred by *Er3* to *E. trifolii* is temperature dependent, being overcome at high temperatures (Fondevilla et al. 2013). RAPD (Random amplified polymorphic DNA) and SCAR (Sequence Characterized Amplified Region) markers tightly linked to *Er3* gene have been identified, but the position of these markers in the pea genetic map is unknown (Fondevilla et al. 2008). Locating *Er3* gene in the pea linkage map would be a relevant step for identifying and characterizing this gene.

Materials and methods

Toward the objective of locating *Er3* gene in the pea genetic linkage map, in the present study 79 SSR markers distributed along the pea linkage map (Loridon et al. 2005) were surveyed in the susceptible cv. Messire (*er3er3*) and a near isogenic line of cv. Messire homozygous for the *Er3* allele conferring the resistance (Cv. Eritreo, *Er3Er3*). PCR reaction mix and amplification conditions were as described in Loridon et al. (2005). Amplification products were first subjected to electrophoresis performed in 2% agarose with Tris–borate EDTA Buffer gels for 3 h at 90 V. Gel green-stained gels were visualized on an ultraviolet light transilluminator and photographed. As not any visible polymorphism was detected using this method, in order to increase polymorphism power detection, 47 of these SSRs were analysed using capillary-based fragment analysis technique. Forward primers were labeled with fluorophores 6FAM or HEX (Sigma-Genosys Ltd.) at the 5’ ends. Amplification products were separated in an automated capillary sequencer (ABI 3130 Genetic Analyzer, Applied Biosystems/HITACHI) at the Genomics Unit of the Central Service for Research Support at the University of Córdoba (Spain). The size of the amplified bands was calculated based on an internal standard DNA (400HD-ROX, Applied Biosystems) with GeneScan software (v 3.x Applied Biosystems) and the results analyzed using the Genotyper software (v 3.7, Applied Biosystems).

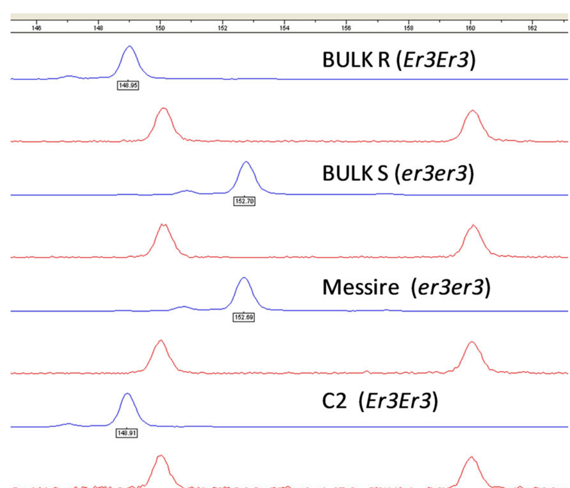

Fig. 1 Results of polymorphism detection by fragment analysis for SSR marker AA349 in two contrasting bulks formed by homozygous *Er3Er3* (Bulk R) and *er3er3* (Bulk S) F_2 individuals obtained from C2 × Messire population, the susceptible cultivar Messire and the resistant breeding line C2. Numbers indicate the estimated band size (pb) in the different samples analysed

SSRs showing polymorphisms between cv. Messire and cv. Eritreo were further surveyed in two contrasting bulks formed each by eight homozygous *Er3Er3*/

Fig. 2 Results of polymorphism detection by fragment analysis for SSR marker AD61 in a resistant (*Er3Er3*) and susceptible (*er3er3*) F_2 individuals derived from the cross C2 × Messire, the susceptible cultivar Messire and the resistant breeding line C2. Numbers indicate the estimated band size (bp) in the different samples analysed

er3er3 F_2 individuals. This F_2 population derived from the cross between the resistant breeding line C2 (*Er3Er3*) and cv. Messire (*er3er3*), which had previously been used by us to identify molecular markers linked to *Er3* gene (Fondevilla et al. 2008) and also used in this study for linkage analysis. One marker was polymorphic between the bulks and, subsequently, in order to identify other markers linked to gene *Er3*, ten additional markers located in the surroundings of this polymorphic marker, according to different pea maps, were selected to be analysed in cv. Eritreo, cv. Messire, breeding line C2 and the two contrasting F_2 bulks *Er3Er3*/*er3er3*. These markers were the SSRs AD61, AD186, AA122, AD171, AC22, AA315, AB52, AA378 and AB45 (Loridon et al. 2005) and *cwi2* gene (Carrillo et al. 2014). The SSRs markers were analysed using the fragment analysis technique, as described above. To identify polymorphism for *cwi2*, the gene was amplified and the resulting amplicon sequenced. Primers used to amplify *cwi2* gene were: Fw: CACTAGATCCAGCCATCTTT; Rv: TGAATCGAAAGGGTGCTTAG. To analyse this gene, PCR assay was performed in 15 µl containing 1xPCR buffer with 1.5 mM $MgCl_2$, 0.125 mM of each dNTP, 0.3 µM of reverse and forward primers, 0.375 U Taq polymerase (Bioline) and 40–50 ng of template DNA. PCR conditions were 5 min at 95 °C followed by 30 cycles of denaturation at 95 °C for 1 min, annealing for 1 min at 60 °C, and 2 min elongation at 72 °C, with a final extension step at 72 °C for 5 min.

Finally, a linkage analysis was performed using C2 × Messire F_2 population and combining the amplification profile of the markers found to be polymorphic between *Er3Er3*/*er3er3* plants in this

study and those of the RAPD and SCAR markers previously found to be linked to *Er3* gene (Fondevilla et al. 2008). The genetic map was constructed using the software MAPMAKER ver. 2.0 (Lander et al. 1987) with a LOD value of 5 and recombination fraction < 0.30 as thresholds for considering significant linkage. The most likely order of markers was established using the command 'compare'. Recombination fractions were converted to centiMorgans (cM) using the Kosambi mapping function (Kosambi 1944).

Results and discussion

Three SRRs, AA332 (LGII), AA349 (LGIV) and AB23 (LGV) were found to be polymorphic between cv. Messire and cv. Eritreo. To further check the association of these markers with *Er3* gene, the three candidate markers were analysed in two contrasting bulks formed by homozygous *Er3Er3*/*er3er3* F_2 individuals derived from the cross C2 (*Er3Er3*) × Messire (*er3er3*). Only the marker AA349 was polymorphic between the bulks (Fig. 1). Subsequently, in order to identify other markers linked to gene *Er3*, ten additional markers located in the surroundings of AA349 marker, according to different pea maps, were selected to be analysed in cv Eritreo, cv Messire, breeding line C2 and the two contrasting F_2 bulks. Of them, only the SSR AD61 marker was polymorphic between the resistant and susceptible lines and the bulks formed by homozygous *Er3Er3* or *er3er3* F_2 individuals (Fig. 2). Further linkage analysis performed using C2 × Messire F_2 population and combining the amplification profile of markers AA349 and AD61 (analysed in this study) and those of the RAPD and SCAR markers previously found to be linked to *Er3* gene (analysed in Fondevilla et al. 2008) showed that marker AA349 and AD61 were linked to *Er3* and to the markers previously reported to be linked to this gene (Fig. 3). *Er3* was located in the final part of pea LGIV at 0.39 cM downstream of marker AD61.

In this study we report, for the first time, the position of *Er3* in the pea linkage map. We also identified a SSR marker tightly linked to this gene, AD61, that is common to other pea maps and could be useful in marker assisted selection. Knowledge of the position of *Er3* gene in the pea linkage map could allow the postulation of candidate genes making use of

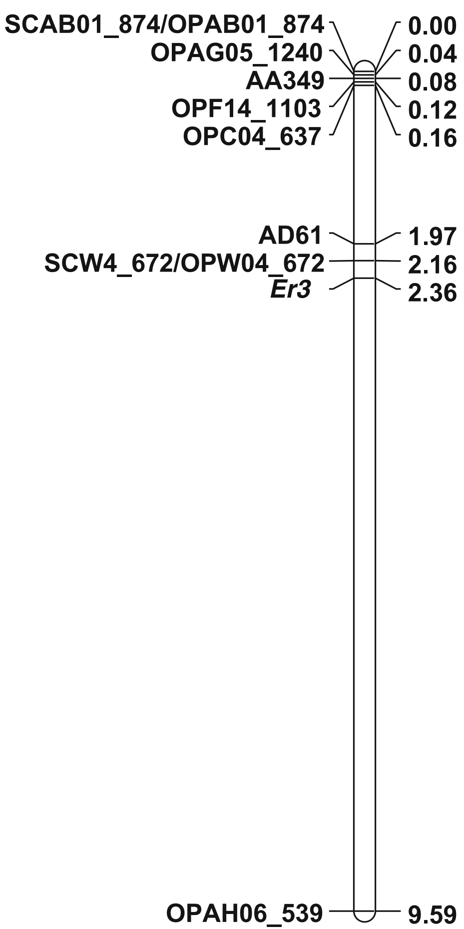

Fig. 3 Linkage map of the pea C2 × Messire F_2 population showing the position of *Er3* gene vis-à-vis various LGIV markers. Marker names are shown on the left and estimated map distances between markers are shown on the right

the conserved synteny existing between pea and other legume species with genomes sequenced or gene-based maps (reviewed in Tayed et al. 2015). Furthermore, pea genome sequencing is in progress and draft genome sequence is expected to be released soon. Therefore, knowledge of the position of *Er3* gene in the pea map is a first step towards the characterization of this dominant powdery mildew resistance gene.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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