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Two major *er1* alleles confer powdery mildew resistance in three pea cultivars bred in Yunnan Province, China

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ABSTRACT

Powdery mildew, caused by *Erysiphe pisi* D.C., is an important disease of pea (*Pisum sativum* L.). The use of cultivars carrying powdery mildew resistance alleles at the *er1* locus is the most effective and economical means of controlling this disease. The objectives of this study were to screen Chinese elite pea cultivars for resistance to *E. pisi* and to identify the responsible gene at the *er1* locus. Among the 37 pea cultivars tested, three (Yunwan 8, Yunwan 21, and Yunwan 23) were immune to *E. pisi* infection in phenotypic evaluations. The full-length cDNA sequences of the *er1* candidate gene, *PsMLO1*, from the three resistant cultivars and control plants were analyzed. Comparison of the cDNA sequences of 10 clones revealed differences among the powdery mildew-resistant cultivars, susceptible controls, and wild-type cultivar Sprinter. The observed resistance in Yunwan 8 plants resulted from a point mutation (C → G) at position 680 of *PsMLO1* that introduced a stop codon, leading to premature termination of protein synthesis. The responsible resistance allele was identified as *er1–1*. Powdery mildew resistance in Yunwan 21 and Yunwan 23 plants was caused by identical insertions or deletions in *PsMLO1*. Three distinct *PsMLO1* transcripts were observed in Yunwan 21 and Yunwan 23 plants. These transcripts were characterized by a 129-bp deletion and 155- and 220-bp insertions, respectively. The responsible resistance allele was identified as *er1–2*. We have characterized two important *er1* alleles in three *E. pisi*-resistant pea cultivars bred in Yunnan Province, China. These cultivars represent important genetic resources for the breeding of powdery mildew-resistant pea cultivars.

1. Introduction

Powdery mildew, caused by *Erysiphe pisi* D.C., is one of the most serious threats to pea (*Pisum sativum* L.) production, causing yield losses of 25%–50% [1–3]. To control this disease, the use of genetically resistant cultivars is the most efficient, economical, and environmentally friendly method [4]. Researchers have focused on screening for *E. pisi* resistance and genetic analyses of powdery mildew resistance in pea. Three genes (*er1*, *er2*, and *Er3*) have been reported to be associated

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with powdery mildew (*E. pisi*) resistance in pea germplasm [5–7]. Previous studies revealed that *er1* and *er2* are single recessive genes located in pea linkage groups (LGs) VI and III, respectively [8–10]. *Er3* is a newly identified dominant gene from a wild relative of pea (*Pisum fulvum*) that was recently incorporated into the genome of cultivated pea [3,7]. Although *Er3* has been localized between the sequence-characterized amplified region (SCAR) marker $Scw4_{637}$ and the random amplified polymorphic (RAPD) marker OPAG05_1240, its exact location in the pea genome is unknown [11].

The mechanisms of the three known resistance genes (*er1*, *er2*, and *Er3*) have been studied at the cellular level [12–14]. The *er1* gene confers immunity or high-level resistance by preventing *E. pisi* from penetrating pea epidermal cells. In contrast, the disease resistance provided by *er2* and *Er3* is mediated by a post-penetration hypersensitive response [7,12,13]. *er2* expression is strongly influenced by temperature and leaf age. Complete disease resistance conferred by *er2* occurs only at high temperatures (e.g., 25 °C) or in mature leaves. Effective *er2*-regulated resistance to *E. pisi* has been observed only in specific geographic regions [6,12,15,16].

Several genetic analyses of *E. pisi* resistance have revealed that *er1* is the gene responsible for conferring stable and durable resistance in most cases [15–19]. Thus, *er1* has been used for decades in pea breeding programs. *er2* is present only in a few *E. pisi*-resistant pea accessions, including SVP951, SVP952, and JI2480 [15]. Recently, it was reported that resistance conferred by *er1* is caused by loss-of-function mutations in a powdery mildew susceptibility gene, *PsMLO1*, belonging to the mildew resistance locus O (MLO) gene family [20,21].

To date, nine *er1* alleles (*er1*–1, *er1*–2, *er1*–3, *er1*–4, *er1*–5, *er1*–6, *er1*–7, *er1mut1*, and *er1mut2*) have been characterized in pea accessions resistant to *E. pisi*, according to differences in mutations in *PsMLO1* [20–27]. Each *er1* allele corresponds to a different *PsMLO1* mutation produced by natural or artificial mutagenesis, except for *er1*–1 and *er1mut1*, which carry the identical mutation [20–22]. All *er1* alleles but *er1*–5, *er1mut1*, and *er1mut2* were generated by natural mutations [21,25–27]. Seven alleles (*er1*–1, *er1*–3, *er1*–4, *er1*–5, *er1*–6, *er1mut1*, and *er1mut2*) were the result of point mutations in *PsMLO1* [20–22,25–27]. The *er1*–2 allele was generated by an insertion or deletion of a DNA fragment of unknown size and identity into *PsMLO1*, resulting in abnormal *PsMLO1* transcription [20–24]. We recently detected *er1*–7 in the resistant pea cultivar DDR-11, and determined that this allele harbors a 10-bp deletion in exon 1 of *PsMLO1* [26].

In China, powdery mildew caused by *E. pisi* has reduced pea quality and yields since 1991 [28]. Thus, pea germplasm continues to be screened to detect genes conferring resistance to *E. pisi* [23–26,28–32]. The results of these studies indicated that several Chinese pea accessions were immune or highly resistant to the Chinese *E. pisi* isolates EPBJ and EPYN. To identify disease resistance genes, genetic analyses and investigations of the *PsMLO1* sequence were performed using the Chinese pea cultivar Xucai 1, pea line X9002, and several Chinese pea landraces resistant to *E. pisi* [23–25]. Wang et al. [24] and Sun. et al. [23] reported that the disease resistance in Xucai 1 and X9002 was conferred by an *er1* allele, *er1*–2, a commonly used resistance gene in breeding programs. Recently, Sun. et al. [25] identified and characterized a novel *er1* allele (*er1*–6) in 15 Chinese pea landraces resistant to *E. pisi*. Using a high-resolution melting technique, they developed and validated a functional marker specific to *er1*–6, SNP1121, which can be used in pea breeding by marker-assisted selection [25]. These results suggest that there are various sources of resistance in Chinese pea germplasm that carry novel *er1* alleles that may be useful for breeding *E. pisi*-resistant pea cultivars. The objectives of this study were to screen Chinese pea cultivars for resistance to *E. pisi* and to characterize the powdery mildew resistance alleles at the *er1* locus.

2. Materials and methods

2.1. Plant materials

Thirty-six Chinese elite pea cultivars developed in eight provinces or autonomous regions (Beijing, Gansu, Hebei, Jiangsu, Qinghai, Sichuan, Tibet, and Yunnan), and preserved in the China National Genebank were evaluated for resistance to powdery mildew (*E. pisi*) (Table 1). Two susceptible cultivars, Bawan 6 [23,24,31] and Longwan 1 [23,25,26], harboring the susceptibility gene *Er1*, were used as susceptible controls. These two cultivars were kindly provided by Mr. Dongxu Xu of the Zhangjiakou Academy of Agricultural Sciences, China, and Dr. Xiaoming Yang of the Gansu Academy of Agricultural Sciences, China, respectively. Two resistant pea cultivars, Xucai 1 [23] and YI (JI1591) [21], harboring *er1*–2 and *er1*–4, respectively, were also used as resistant controls. These cultivars were kindly provided by Prof. Fengbao Wang of the Hebei Normal University of Science & Technology, China, and Prof. Weidong Chen of Washington State University, USA, respectively.

2.2. E. pisi isolates

Two highly virulent *E. pisi* isolates, EPBJ (NCBI accession number: KR912079) and EPYN (NCBI accession number: KR957355), collected from Beijing and Yunnan, China, respectively, were used as inocula [23–26,31,32]. The isolates were maintained on Longwan 1 seedlings. The inocula were reproduced by continuously transferring *E. pisi* conidia to healthy Longwan 1 seedlings by gently shaking diseased plants. The inoculated plants were incubated in a growth chamber at 10 ± 1 °C with a 12-h photoperiod.

2.3. Phenotypic evaluation

The seeds of 37 Chinese pea cultivars and susceptible (Bawan 6 and Longwan 1) and resistant (Xucai 1 and YI) controls were planted in 15-cm-diameter paper pots (five seeds per pot) filled with a mixture of vermiculite and peat moss (1:1). Twenty-five seeds of each pea cultivar and control were planted for all experiments, which were replicated five times. Seeded pots were placed in a greenhouse at 18 °C–26 °C. Fourteen days after planting, seedlings at the fourth or fifth leaf stage were inoculated with the two *E. pisi* isolates (EPBJ and EPYN), using conidia collected by gently shaking infected Longwan 1 plants [23,24]. The treated plants were incubated in a growth chamber at 15 ± 1 °C with a 12-h photoperiod. Ten days later, disease severity was rated on a 0–4 scale based