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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

Table 1 – Phenotypic reactions of Chinese elite pea cultivars to *Erysiphe pisi* isolates EPBJ and EPYN, and the *er1* alleles harbored by the analyzed cultivars.

Cultivar name	Source origin	Resistance reaction		*er1* allele	Reference
		EPBJ	EPYN		
Bawan 6	Hebei	S	S	*Er1*	Sun et al. [23]
Longwan 1	Gansu	S	S	*Er1*	Sun et al. [23]
Xucai 1	Hebei	I	I	*er1–2*	Sun et al. [23]
YI	Guangdong	I	I	*er1–4*	Humphry et al. [20]
Zhongwan 5	Beijing	S	S	*Er1*	This study
Zhongwan 6	Beijing	S	S	*Er1*	This study
Dingwan 1	Gansu	S	S	*Er1*	This study
Dingwan 2	Gansu	S	S	*Er1*	This study
Dingwan 3	Gansu	S	S	*Er1*	This study
Dingwan 4	Gansu	S	S	*Er1*	This study
Dingwan 6	Gansu	S	S	*Er1*	This study
Dingwan 7	Gansu	S	S	*Er1*	This study
Qianjin 1	Hebei	S	S	*Er1*	This study
Jizhangwan 2	Hebei	S	S	*Er1*	This study
Bawan 1	Hebei	S	S	*Er1*	This study
Suwan 1	Jiangsu	S	S	*Er1*	This study
Suwan 2	Jiangsu	S	S	*Er1*	This study
Suwan 3	Jiangsu	S	S	*Er1*	This study
Suwan 4	Jiangsu	S	S	*Er1*	This study
Caoyuan 6	Qinghai	S	S	*Er1*	This study
Caoyuan 276	Qinghai	S	S	*Er1*	This study
Caoyuan 23	Qinghai	S	S	*Er1*	This study
Caoyuan 24	Qinghai	S	S	*Er1*	This study
Wuxudoujie 1	Sichuan	S	S	*Er1*	This study
Fengyou 1	Sichuan	S	S	*Er1*	This study
Chengwan 8	Sichuan	S	S	*Er1*	This study
Chengwan 9	Sichuan	S	S	*Er1*	This study
Bomi 1	Tibet	S	S	*Er1*	This study
Bomi 23–1	Tibet	S	S	*Er1*	This study
Bomi 071	Tibet	S	S	*Er1*	This study
Linzhi 1	Tibet	S	S	*Er1*	This study
Yata 1	Tibet	S	S	*Er1*	This study
Yata 2	Tibet	S	S	*Er1*	This study
Yata 3	Tibet	S	S	*Er1*	This study
Yata 23	Tibet	S	S	*Er1*	This study
Xinong 2	Tibet	S	S	*Er1*	This study
Qingdou 1	Tibet	S	S	*Er1*	This study
Yunwan 8	Yunnan	I	I	*er1–1*	This study
Yunwan 21	Yunnan	I	I	*er1–2*	This study
Yunwan 23	Yunnan	I	I	*er1–2*	This study

on infected foliage area, macroscopic and microscopic observations of mycelial growth, and sporulation [16,23,24,32,33]. Plants with a score of 0 were considered immune, while those with scores of 1 or 2 were classified as resistant and those with 3 or 4 as susceptible. Cultivars identified as immune or resistant to *E. pisi* infection were retested.

2.4. RNA extraction and PsMLO1 sequence analysis

With the aim of identifying the resistance alleles at the *er1* locus in immune or resistant pea cultivars, total RNA was extracted from their young leaves using an RNAprep Pure Plant Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. First-strand cDNA was synthesized using a BioRT Two-Step RT-PCR Kit (Hangzhou Bioer Technology, Hangzhou, China) with oligo(dT) primers.

To amplify the full-length cDNA of *PsMLO1*, a polymerase chain reaction (PCR) was performed using *PsMLO1*-specific primers (PsMLO1F: 5′-AAAATGGCTGAAGAGGGAGTT-3′; PsMLO1R: 5′-TCCACAAATCAAGCTGCTACC-3′) [21]. The PCR program was as follows: 95 °C for 5 min; 35 cycles of 94 °C for 30 s, 58 °C for 45 s, and 72 °C for 1 min; 72 °C for 10 min. The amplicons were purified using a PCR Purification Kit (Qiagen) and then ligated into the pEasy-T5 vector (TransGen Biotech, Beijing, China). Recombinant plasmids were cloned in *Escherichia coli* TOP 10 competent cells and recovered using the QIAprep Spin Miniprep kit (Qiagen). Ten clones for each cultivar were sequenced at the Beijing Genomics Institute. To confirm that the amplified *PsMLO1* fragments contained the correct sequences, the susceptible (Bawan 6 and Longwan 1) and resistant (Xucai 1 and YI) controls were similarly analyzed. The resulting sequences were compared with *PsMLO1* cDNA sequences from the controls and wild-type pea cultivar Sprinter (susceptible to *E. pisi*; NCBI accession number: FJ463618.1) using DNAMAN software [20].

3. Results

3.1. Phenotypic evaluation

All Bawan 6 and Longwan 1 plants (susceptible controls) were severely infected by the EPBJ and EPYN *E. pisi* isolates, with a disease severity of 4 (Fig. 1; Table 1). In contrast, no disease symptoms were observed on the resistant controls, Xucai 1 and YI. These results are consistent with those of previous studies [15,23,31]. Of the 37 pea cultivars analyzed, 34 were heavily infected by the two *E. pisi* isolates, with masses of *E. pisi* conidia and mycelia observed on plants (with disease ratings of 3 and 4). These 34 cultivars were considered susceptible to powdery mildew infection. Yunwan 8, Yunwan 21, and Yunwan 23, which were developed in Yunnan, China, remained healthy and symptom-free, similarly to the resistant controls (Xucai 1 and YI). These three cultivars appeared to be immune to both *E. pisi* isolates (with disease ratings of 0) (Fig. 1; Table 1).

3.2. Analysis of PsMLO1 cDNA sequence

The *PsMLO1* cDNA sequences of Bawan 6 and Longwan 1 plants were identical to that of the wild-type pea cultivar Sprinter, indicating that Bawan 6 and Longwan 1 plants carry the *Er1* gene (Table 1). The *PsMLO1* cDNA sequence of YI plants matched the sequence of *er1–4*, which harbors a single-base deletion mutation (ΔA) at position 91. [20]. The *PsMLO1* cDNA sequence of Xucai 1 plants was identical to that of *er1–2* [23]. These results confirm the accuracy of the *PsMLO1* cDNA sequences of the three identified resistant cultivars, Yunwan 8, Yunwan 21, and Yunwan 23.

The *PsMLO1* cDNA sequences of Yunwan 8, Yunwan 21, and Yunwan 23 plants differed from those of Bawan 6, Longwan 1, and Sprinter plants. The *PsMLO1* cDNA from Yunwan 8 plants had a point mutation (C → G) at position 680, matching that in the corresponding sequence in the Mexican pea cultivar Mexique 4 (JI1559), which carries *er1–1* [20]. Thus, the resistance gene present in Yunwan 8 plants

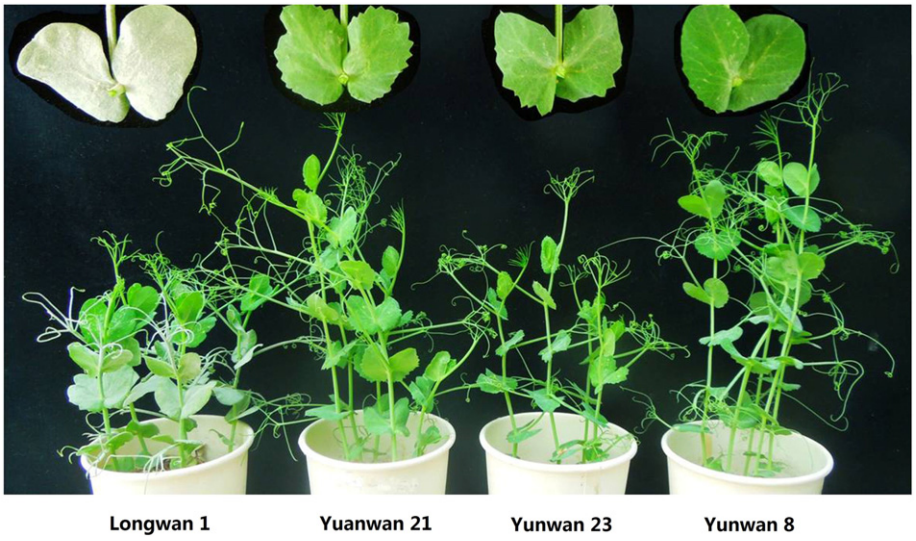

Fig. 1 – Phenotypic reactions of Yunwan 21, Yunwan 23, and Yunwan 8, and susceptible control Longwan 1 to *Erysiphe pisi* isolate EPYN. All plants of Longwan 1 were severely infected by *E. pisi*, with masses of *E. pisi* conidia and mycelia observed (disease rating 4). In contrast, there were no disease symptoms on the resistant pea cultivars Yunwan 21, Yunwan 23, and Yunwan 8.

was identified as *er1–1* (Fig. 2; Table 1). The mutations in the Yunwan 21 and Yunwan 23 *PsMLO1* cDNA sequences were identical to those in Xucai 1 *PsMLO1* sequences. There are three distinct *PsMLO1* transcripts in Xucai 1 plants, characterized by a 129-bp deletion between positions 1171 and 1299, and 155-bp and 220-bp insertions at position 1263 of the coding sequence [23,24] (Fig. 3). The sequencing results of *PsMLO1* from Yunwan 21 and Yunwan 23 plants indicated that they carried *er1–2* (Fig. 3; Table 1). Additionally, the *PsMLO1* cDNA sequences in Yunwan 21 and Yunwan 23 plants contained a frame shift associated with a 5-bp deletion.

4. Discussion

Powdery mildew of pea is an important disease that results in considerable yield and quality losses [1–3]. The most efficient strategy to control this disease involves the use of resistant cultivars, given that host resistance permits sustainable pea production without potential safety risks for the environment and consumers. In the present study, the phenotypes of two susceptible and two resistant controls were consistent with those described in previous studies [20,23,24], indicating the

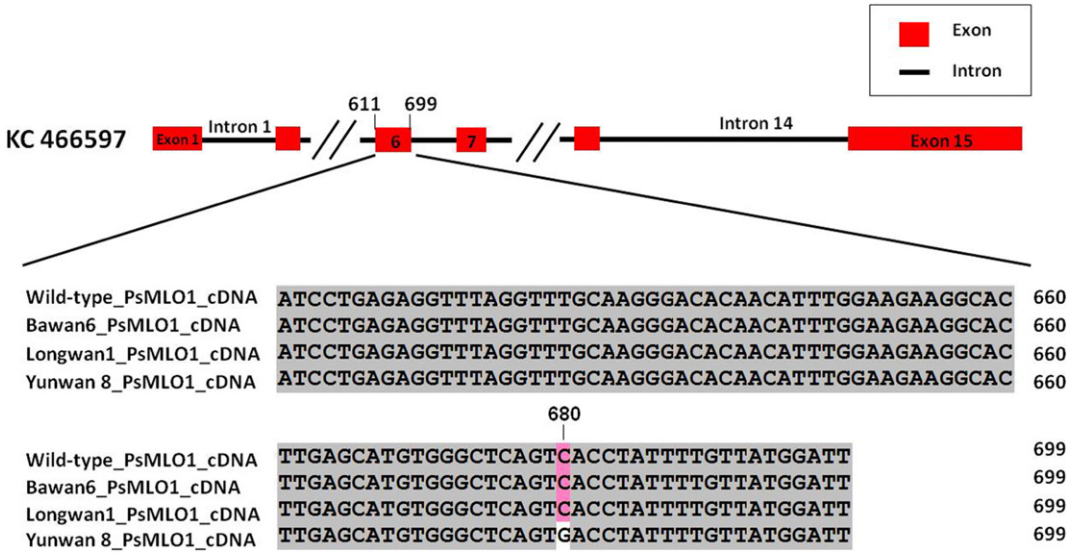

Fig. 2 – Comparison of *PsMLO1* cDNA sequence from powdery mildew-resistant Yunwan 8 plants and susceptible Bawan 6, Longwan 1, and wild-type Sprinter (NCBI accession number: FJ463618.1) plants. The *PsMLO1* cDNA sequence from Yunwan 8 plants harbors a base substitution (C → G) at position 680 in exon 6. The mutation site is indicated in the cDNA sequences.

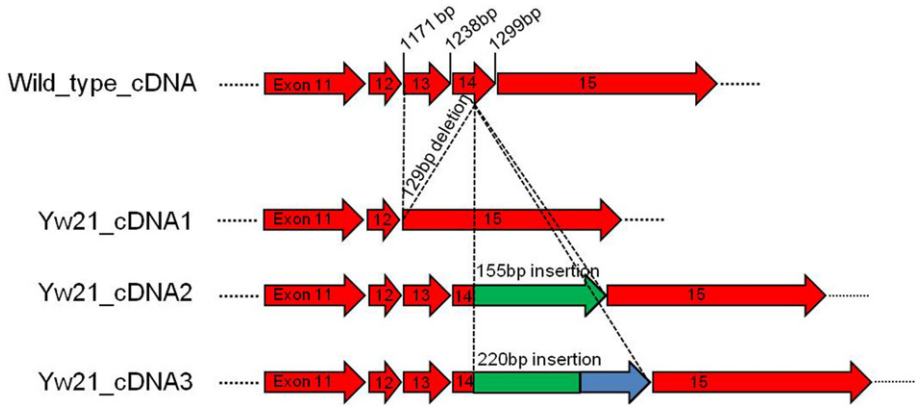

Fig. 3 – Three distinct *PsMLO1* transcripts are present in Yunwan 21 plants. The transcripts are differentiated by a deletion (129 bp, cDNA1) or insertions (155 bp, cDNA2; 220 bp, cDNA3). Yw21 refers to Yunwan 21.

accuracy and reliability of the phenotypes observed for the tested pea cultivars. The cultivars Yunwan 8, Yunwan 21, and Yunwan 23 were immune to two *E. pisi* isolates from China. This result suggests that pea accessions from Yunnan, China, may be viable sources of resistance to *E. pisi*, in agreement with our previous findings [25]. These three pea cultivars may be useful for the breeding of new powdery mildew-resistant cultivars in China.

The *E. pisi* resistance gene at the *er1* locus in Yunwan 8 plants was identified as *er1–1*. The base substitution (C → G) at position 680 of *PsMLO1* introduces a stop codon (UGA) that results in premature termination of protein synthesis. This observation is consistent with the results of previous studies by Humphry et al. [20] and Fu et al. [32]. Humphry et al. [20] detected *er1–1* in Mexique 4 plants, and Fu et al. [32] subsequently identified this allele in two powdery mildew-resistant pea cultivars, Tara from Canada and Cooper from the Netherlands. Thus, Yunwan 8 is the fourth pea cultivar identified as carrying *er1–1* but the first Chinese pea cultivar confirmed to carry this allele.

Powdery mildew resistance was conferred by *er1–2* (Table 1) in Yunwan 21 and Yunwan 23 plants, as in other pea cultivars/lines including Stratagem (JI2302), Franklin, ROI3/02, Dorian, Nadir, X9002, and Xucai 1 [20–24]. The pea line X9002 and pea cultivar Xucai 1 were recently confirmed to carry *er1–2*, according to genetic mapping and sequence analyses of *PsMLO1* [23,24]. In this study, we detected three distinct *PsMLO1* transcripts in Yunwan 21 and Yunwan 23 plants, characterized by a 129-bp deletion or 155-bp and 220-bp insertions. The 129-bp deletion eliminated exons 13 and 14 from *PsMLO1*, resulting in the removal of 43 amino acids from the final protein (Fig. 3). The 155-bp and 220-bp insertions were highly similar to five regions of a pea genomic BAC clone (accession number: CU655882). Interestingly, the 220-bp insert was also very similar (approximately 87% identical) to part of a giant retroelement that forms a major component of the pea genome (accession number: AY299395) [34,35].

The three pea cultivars identified as resistant to *E. pisi* were bred by researchers at the Yunnan Academy of Agricultural Sciences, China. This province is an important center of field pea diversity because it has a complex landform, which results in multiple geographical and climatic features and a variable environment [36–38]. Zong et al. [36] reported that pea accessions originating in Yunnan exhibit high genetic diversity. This province is located in a low-latitude plateau region and experiences extreme biotic and abiotic stresses [38,39]. The presence of a rich stock of germplasm exhibiting disease resistance is likely the result of specific adaptations to local climates and high selection pressure. Recently, Sun. et al. [25] discovered a novel *er1* allele, *er1–6*, in several Chinese pea landraces originating in Yunnan, suggesting that the variable climate in this province caused mutations in *PsMLO1* that were specific to this geographic region.

Two single recessive genes (*er1* and *er2*) and one single dominant gene (*Er3*) have been identified as influencing resistance to *E. pisi* [5–7]. Previous studies have concluded that the resistance in most pea germplasm is controlled by *er1*, which is the most widely deployed gene in contemporary pea cultivars because of its highly effective broad-spectrum resistance to *E. pisi* [15]. The *er1* resistance phenotype is induced by loss-of-function mutations in *PsMLO1*. Nine *er1* alleles have been identified as conferring resistance to *E. pisi* in pea germplasm [20–27]. Among the reported alleles, *er1–1* and *er1–2* are commonly used in pea breeding programs for their ability to provide stable and durable resistance to *E. pisi* [20,21,23,24,32]. In this study, we identified these two major *er1* alleles in three powdery mildew-resistant Chinese pea cultivars, which represent valuable genetic resources for breeding *E. pisi*-resistant cultivars.

5. Conclusions

Three powdery mildew-resistant pea cultivars bred in Yunnan Province were confirmed to be immune to two Chinese *E. pisi* isolates, EPBJ and EPYN. Two (*er1–1* and *er1–2*) of seven *er1* alleles for pea powdery mildew resistance were characterized in the three resistant cultivars. These results are relevant to the breeding of *E. pisi*-resistant pea cultivars in China.

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