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Resistance to powdery mildew in the pea cultivar Xucai 1 is conferred by the gene *er1*

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ABSTRACT

Powdery mildew, caused by *Erysiphe pisi* D.C., is a major constraint to pea production worldwide. The pea cultivar Xucai 1 has shown high resistance to *E. pisi* under greenhouse and field conditions. The objectives of this study were to identify and characterize genes conferring resistance to powdery mildew in Xucai 1. Three crosses, Qizhen 76 × Xucai 1, Bawan 6 × Xucai 1, and Xucai 1 × Bawan 6, were made to generate populations for genetic analysis. The resistance to *E. pisi* and segregation ratios in the F_1, F_2, and $F_{2:3}$ populations suggested a single recessive gene conferring the resistance of Xucai 1. Bulked segregant analysis was used to map the resistance gene using two F_2 populations. The resistance gene was close to markers AD60 and c5DNAmet on linkage group VI with genetic distances of 9.9 cM and 15.4 cM in the Xucai 1 × Bawan 6 F_2 population and 8.7 cM and 8.1 cM in the Qizhen 76 × Xucai 1 F_2 population, respectively, suggesting that the resistance gene was an *er1* allele. This hypothesis was confirmed by comparison of the cDNA sequences of the *PsMLO1* gene between the parents and the *PsMLO1* wild type. Three distinct types of transcripts in Xucai 1, characterized by a 129-bp deletion and 155- and 220-bp insertions, were detected, consistent with the structure of the *er1*-2 allele. We concluded that resistance in Xucai 1 was conferred by *er1*-2 and that its linked markers will be useful in pea breeding programs.

1. Introduction

Pea (*Pisum sativum* L.) is widely distributed and has been cultivated in China for more than two thousand years [1]. According to the latest report, China ranks second only to Canada in dry pea production [2]. Pea frequently suffers from biotic and abiotic stresses throughout the lifecycle of the plant. Powdery mildew, caused by *Erysiphe pisi* D.C., is one of the most important pea diseases worldwide. The disease severely affects yield and quality of peas [3,4]. It is of serious concern in temperate and subtropical regions with warm, dry days and cool nights [4]. Powdery mildew caused by *E. pisi* results in 25–50% yield losses in pea production [5–7]. Severe infection by *E. pisi* can result in yield loss of up to 80% in susceptible cultivars [8]. In pea production, agronomic practices, chemical prevention, and genetic resistance strategies

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have been applied to control the disease. However, the control efficacy of chemical and agronomic practices is limited by many factors. Genetic resistance has become the first choice not only for its efficiency, low cost, and environmental friendliness [8,9] but also for qualitative disease resistance that are relatively easy to use in breeding programs.

As early as 1948, Harland [10] identified powdery mildew resistance in the pea landrace Huancabamba and showed that it carried a single recessive gene. Since then, screening and genetic analysis of resistance to pea powdery mildew have been performed for more than 60 years [9]. Many resistant pea accessions have been identified and characterized their gene(s) for resistance to *E. pisi*. Two recessive genes (*er1* and *er2*) and one dominant gene (*Er3*) have been identified for powdery mildew resistance in pea germplasm [10–12]. Genetic analyses of resistance to *E. pisi* indicated that the vast majority of resistant pea accessions carried the resistance gene *er1* [13]. The resistance gene *er2* is harbored by only a few resistant pea accessions [13]. *Er3* is a newly identified dominant gene from a wild relative of pea (*P. fulvum*) that has recently been successfully introduced into cultivated pea (*P. sativum*) [7,12].

With respect to their resistance mechanisms, the modes of action at the cellular level of the two single recessive genes, *er1* and *er2*, have been revealed by histological studies [14,15]. *er1* confers complete immunity or high levels of durable broad-spectrum resistance to powdery mildew by inhibiting *E. pisi* invasion of pea epidermal cells. The expression of resistance gene *er2* is influenced primarily by temperature and leaf age. The complete resistance conferred by *er2* is revealed only at high temperature (25 °C) or in mature leaves and is based mainly on post-penetration cell death, mediated by a hypersensitive response [14]. The *Er3* gene confers effective resistance to all *E. pisi* isolates tested to date [7]. On *Er3* plants, most *E. pisi* conidia are able to penetrate pea epidermal cells and form secondary hyphae, but growth of these established colonies is prevented by a strong hypersensitive response [12,16].

DNA marker technologies and their application in genetic analysis have developed rapidly over the last decade. In peas, molecular markers linked to the three resistance genes (*er1*, *er2*, and *Er3*) for powdery mildew have been developed by several groups [17–25]. The single recessive genes *er1* and *er2* have been located on pea linkage groups (LGs) VI and III, respectively [18,22,26], whereas *Er3* has been mapped between the sequenced characterized amplified region (SCAR) marker $Scw4_{637}$ and random amplified polymorphic DNA (RAPD) marker OPAG05_1240, located on an uncertain pea LG [27].

The recessively inherited resistance gene *er1* has been used successfully for decades in pea breeding programs [13]. Recently, the resistance of *er1* in pea has been found to be similar to that of *MLO* (mildew resistance locus *O*) genes in barley (*Hordeum vulgare* L.), *Arabidopsis thaliana*, and tomato (*Lycopersicon esculentum* Mill.) [28,29]. The *er1* resistant phenotype is caused by loss-of-function mutations of a pea *MLO* homolog, known as *PsMLO1* [28,29]. Thus far, five *er1* alleles (*er1*-1 to *er1*-5) have been characterized in pea resistant accessions, according to the mutation site and pattern of *PsMLO1*, each corresponding to a different *PsMLO1* mutation [28–30]. Among the reported five *er1* alleles, *er1*-1 and *er1*-2 are used commonly in pea breeding programs [28,29].

In China, powdery mildew is one of the most important diseases among more than 20 diseases infecting peas and severely affects the yield and quality of peas [31]. The incidence of pea powdery mildew has reached 100% in some regions of Sichuan province [32]. To date, the pathogen *E. pisi* is thought to be the only causal agent of pea powdery mildew in China [33–35], although two other species, *E. baeumleri* and *E. trifolii*, have been reported to cause powdery mildew on peas and showed the same symptoms as *E. pisi* in the Czech Republic [36] and the USA [37]. Ondřej et al. [36] and Attanayake et al. [37] showed that *E. baeumleri* and *E. trifolii* were able to overcome the *er1* gene conferring resistance to *E. pisi*. Fondevilla et al. [38] further confirmed that *E. trifolii* had also overcome resistance gene *Er3*, but not *er2*. In China, screening for resistance to *E. pisi* in pea germplasms has been performed in greenhouses and in fields in recent years [32–35]. Several pea cultivars/landraces were highly resistant to *E. pisi* isolates EPBJ (NCBI, accession number KR912079) and EPYN (NCBI, accession number KR957355) [34,35]. Both *E. pisi* isolates were identified by morphological and molecular characterization [39]. However, the genetic basis of their resistance and the characteristics of their resistance gene(s) are unknown.

Xucai 1, derived from a cross between Cuipimi and Baofeng 2, is the first semi-leafless pea cultivar (cv.) in China. Xucai 1 has desirable traits including high yield and resistance to lodging and drought, root rot, and powdery mildew [40]. Recently, Zeng et al. [33] showed that Xucai 1 was stably resistant to pea powdery mildew both in the greenhouse and in fields in 3 consecutive years. The present study was designed to clarify the genetic basis of this resistance and identify the resistance gene for powdery mildew present in Xucai 1. With this aim, we performed an inheritance study using three crosses between resistant cv. Xucai 1 and two susceptible cultivars (cvs.), Qizhen 76 and Bawan 6. We further characterized the resistance gene as *er1* by sequencing cDNA sequences of the *PsMLO1* gene in the parents of the crosses and the pea cv. Sprinter [28], which is susceptible to *E. pisi* and carries the *PsMLO1* wild-type allele.

2. Materials and methods

2.1. Plant materials and inoculum

Three pea cultivars, Xucai 1 (resistant to *E. pisi*), Qizhen 76 (susceptible to *E. pisi*), and Bawan 6 (susceptible to *E. pisi*) were used in the experiment. Xucai 1 was kindly provided by Prof. Fengbao Wang of the Hebei Normal University of Science & Technology, Qinghuangdao, Hebei province, China; Bawan 6 was provided by Mr. Dongxu Xu of the Zhangjiakou Academy of Agricultural Sciences, Zhangjiakou, Hebei province, China; and Qizhen 76 was from Dr. Xin Chen of Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu province, China. Three crosses were produced including reciprocal crosses between Bawan 6 and Xucai 1 and Qizhen 76 × Xucai 1. Derived F_1, F_2, and $F_{2:3}$ populations of each cross were subjected to phenotypic evaluation and inheritance analysis. The F_1 plants from the crosses were self-pollinated to produce F_2 progeny. Each F_2 plant produced seeds for the $F_{2:3}$ families by self-pollination and was threshed individually. The two F_2 populations derived from the crosses of Qizhen 76 × Xucai 1, and Xucai

1 × Bawan 6 and comprising 91 and 161 individuals, respectively, were used for genotypic evaluation and genetic analysis.

The *E. pisi* isolate EPBJ (NCBI accession number KR912079), a highly virulent isolate collected from a greenhouse in Beijing, was used as the inoculum in the present study [34,35]. The isolate was maintained on the seedlings of susceptible pea cv. "Longwan 1." Reproduction of inocula was achieved by continuous transfer to new healthy seedlings of pea cv. "Longwan 1" by gently shaking plants containing masses of *E. pisi* conidia. The inoculated plants were incubated in a growth chamber at 10 ± 1 °C with a 12 h light regime.

2.2. Inoculation with *E. pisi* and disease assessment

2.2.1. Parental phenotypic evaluation

Seeds of Xucai 1, Bawan 6, and Qizhen 76 were planted in 15-cm-diameter paper pots (six seeds per pot) filled with a mixture of vermiculite and peat moss (1:1). Each parent was planted in five replications using a total of 30 seeds per cultivar. The potted plants were placed under controlled conditions in a greenhouse at 18–26 °C. Ten to fourteen days after planting, the plants at the fourth- or fifth-leaf stage were inoculated with *E. pisi* isolate EPBJ, using conidia shed by shaking heavily infected plants of pea cv. Longwan 1 over them. Ten days after inoculation, disease severity was scored on a 0–4 scale, according to the infected foliage area, macroscopic and microscopic density of mycelia and sporulation on the lower part of the plant [41,42]. Plants with scores of 0–2 were classified as resistant (R) and those with scores of 3–4 as susceptible (S) [41,42].

2.2.2. Phenotypic evaluation of F_1 and F_2 populations

Planting was performed in a propagation greenhouse to generate offspring seeds. Plants were grown under the same conditions as described above for the parents. Inoculation was conducted under controlled conditions using the detached leaf method, together with susceptible and resistant parents as the controls. This inoculation method is effective for the evaluation of resistance to *E. pisi* in peas [14,16]. The third or fourth leaf along with petiole from the bottom of the plant was detached from each F_1 and F_2 seedling at the fourth- or fifth-leaf stage. The cut leaves were inserted into 9-cm-diameter Petri dishes containing 2% water agar and 50 mg L^{-1} benzimidazole to enhance longevity [43]. The detached leaves were then inoculated with *E. pisi* isolate EPBJ using the method described above for the inoculation of the parental plants. After inoculation, the plates containing the inoculated leaves were sealed tightly with Parafilm and placed in a growth chamber at 20 °C with a 14 h photoperiod. The detached leaves of the three parents Xucai 1, Qizhen 76, and Bawan 6 were treated in the same way with and without inoculation as controls. The disease severity was determined using the 0–4 scale 10 days after inoculation. The plants scored as resistant to *E. pisi* were subjected to repeated inoculation.

2.2.3. Phenotypic evaluation of $F_{2:3}$ populations

Twenty-four seeds selected randomly from each $F_{2:3}$ family were grown as described above. Disease was scored 10 days after inoculation using the 0–4 scale. $F_{2:3}$ families classified as either homozygous resistant or segregating for resistance to *E. pisi* were subjected to repeated inoculation. A chi-squared (χ^2) test was used to determine the goodness-of-fit to Mendelian segregation patterns of phenotypes of all F_2 and $F_{2:3}$ populations.

2.3. DNA extraction and pooling for bulk segregation analysis (BSA)

Genomic DNA was isolated from healthy young leaves collected from seedlings of the three pea parents, Xucai 1, Qizhen 76, and Bawan 6, and of two F_2 mapping populations derived from Qizhen 76 × Xucai 1, and Xucai 1 × Bawan 6 using the cetyl trimethylammonium bromide (CTAB) extraction method with minor modifications [44]. The DNA concentration was determined using an ND-1000 Nano-Drop instrument (NanoDrop Technologies Inc., Wilmington, DE, USA). The DNA solution was diluted to a working concentration of 50 ng μL^{-1} with Tris–EDTA buffer (pH 8.0) and maintained at −20 °C. For the bulked segregant analysis (BSA), resistant and susceptible bulks were prepared from equal amounts of DNA from 10 each of resistant and susceptible individual F_2 plants [45].

2.4. Molecular marker analysis

Initially 148 simple sequence repeat (SSR) markers that were approximately evenly distributed over a high-density pea genetic linkage map [46] were selected to screen for polymorphisms between the resistant and susceptible parents and pooled bulks. PCR amplification of SSR markers was performed in a total volume of 20 μL, containing 50 ng of genomic DNA, 2.5 μL 10× PCR reaction buffer (20 mmol L^{-1} $MgCl_2$), 0.2 mmol L^{-1} of each dNTP, 1.5 U of *Taq* DNA polymerase, and 0.2 μmol L^{-1} of primer mixture. PCR reactions were performed at 94 °C for 5 min; followed by 35 cycles of 94 °C for 45 s, 49–60 °C (depending on the primer-specific annealing temperature according to Loridon et al. [46]) for 45 s and 72 °C for 1 min; with a final extension at 72 °C for 10 min, using a thermal cycler (Biometra, Goettingen, Germany). The amplified PCR products were separated on a 6% polyacrylamide sequencing gel after mixing with 4 μL of 6× loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol FF, and 40% sucrose).

SSR markers that were polymorphic between the contrasting bulks were further tested in the full F_2 populations derived from the crosses Qizhen 76 × Xucai 1, and Xucai 1 × Bawan 6. Based on the results of preliminary genetic linkage analysis, markers near the linked markers were further selected from a consensus functional map [47] to identify polymorphisms and analyze genetic linkage and were amplified using the method described above for SSR markers.

Four SCAR markers, ScOPD10-650 [22], ScOPE16-1600 [23], ScOPO18-1200 [23], and ScOPX04-880 [25] linked to resistance gene *er1*, were also used to identify polymorphisms between the two parents and contrasting bulks and to analyze genetic linkage. PCR amplification and reaction were performed as described previously [34].

2.5. Data analysis and genetic linkage map construction

Linkage analysis was performed with MAPMAKER/EXP version 3.0b [48]. Genetic distances were computed using the Kosambi mapping function [49]. Linkage groups were determined using

a logarithm of odds (LOD) threshold of 3.0, with a maximum distance of 50 cM. The segregation of molecular markers in the F_2 populations was evaluated for goodness-of-fit to Mendelian segregation ratios by χ^2 test. The genetic linkage map was drawn with molecular markers linked to the resistance gene using MapDraw [50].

2.6. Sequence analysis of *PsMLO1* gene

Total RNA was extracted from the young leaves of three parents Xucai 1, Bawan 6, and Qizhen 76 using an RNAprep pure Plant kit (Tiangen Biotech, Co., Ltd., Beijing, China), according to the manufacturer's instructions.

First-strand cDNAs were synthesized using a BioRT Two Step RT-PCR Kit (Hangzhou Bioer Technology Co., Ltd, Hangzhou, China) including an oligo (dT) primer. The PCRs were performed to amplify the full-length cDNA of the target gene, *PsMLO1*, using the *PsMLO1*-specific primer pair PsMLO1F/PsMLO1R (F: 5′-AAAAT GGCTGAAGAGGGAGTT-3′; R: 5′-TCCACAAATCAAGCTGCTACC-3′) [29]. The amplification reaction was performed under the following conditions: 5 min at 95 °C; 35 cycles of 30 s at 94 °C, 45 s at 58 °C (annealing temperature), and 70 s at 72 °C; with a final extension of 10 min at 72 °C. Amplicons were purified with a PCR Purification Kit (Qiagen) and ligated into the pEasy_T5 vector (TransGen Biotech, Beijing, China). Sequencing reactions were performed using universal M13F or M13R primers at the Beijing Genomics Institute (BGI). The resulting cDNA sequences of the *PsMLO1* gene in the parents were compared with the reported cDNA sequence of the *PsMLO1* gene in the susceptible pea cv. Sprinter, having the wild-type *PsMLO1* sequence (NCBI, accession number FJ463618.1) [28], using ClustalX2 [51].

2.7. Amplification of the molecular marker er1-2/MGB

PCR amplification of marker er1-2/MGB linked to *er1-2* in the parents was performed in a total volume of 20 μL reaction, containing 50 ng of genomic DNA, 2.5 μL of 10× PCR reaction buffer (containing 20 mmol L^{-1} $MgCl_2$), 0.8 mmol L^{-1} of each dNTP, 2.5 U of long-range TaKaRa LA *Taq* (TaKaRa Bio Inc., Otsu, Japan), and 0.2 mmol L^{-1} of primer mixture. PCR reactions were performed in a thermal cycler (Biometra, Goettingen, Germany), at 94 °C for 5 min; followed by 35 cycles of 94 °C for 45 s, 54 °C for 3 min, and 72 °C for 1 min; with a final extension at 72 °C for 15 min. The amplified PCR products were separated on a 6% polyacrylamide gel.

3. Results

3.1. Phenotypic evaluation of resistance to *E. pisi*

3.1.1. Parental phenotypic evaluation

The two susceptible parents Qizhen 76 and Bawan 6 showed severe infection by *E. pisi* isolate EPBJ 10 days after inoculation under greenhouse conditions. All plants of Qizhen 76 and Bawan 6 were covered by masses of conidia and mycelium of *E. pisi*, showing a disease severity of 4 and confirming their susceptibility (Fig. 1; Table 1). By contrast, there were no symptoms on the resistant parent Xucai 1, which appeared to be completely resistant to *E. pisi* isolate EPBJ (Fig. 1; Table 1). These results are consistent with previous observations [31,33].

3.1.2. Phenotypic evaluations of F_1, F_2, and $F_{2:3}$ populations

The segregation pattern of pea resistance to powdery mildew in the F_1, F_2, and $F_{2:3}$ populations derived from the three crosses of Qizhen 76 × Xucai 1, Bawan 6 × Xucai 1, and Xucai 1 × Bawan 6 are presented in Table 1.

The cross of Qizhen 76 × Xucai 1 generated four F_1 plants that were susceptible to *E. pisi* (Table 1). One of four F_1 plants produced 91 F_2 plants, of which 17 were resistant and 74 susceptible. Thus, the segregation of susceptibility and resistance in F_2 population fitted a ratio of 3:1 ($\chi^2 = 1.94$; $P = 0.16$), indicating monogenic recessive inheritance. Moreover, the segregation ratio in the $F_{2:3}$ population gave a good fit to a 1:2:1 ratio ($\chi^2 = 2.41$; $P = 0.30$) (Table 1), confirming that the resistance was controlled by a single recessive gene in Xucai 1.

The cross between the susceptible parent Bawan 6 and the resistant parent Xucai 1 also generated four F_1 plants susceptible to *E. pisi*. The F_2 population comprised 58 plants

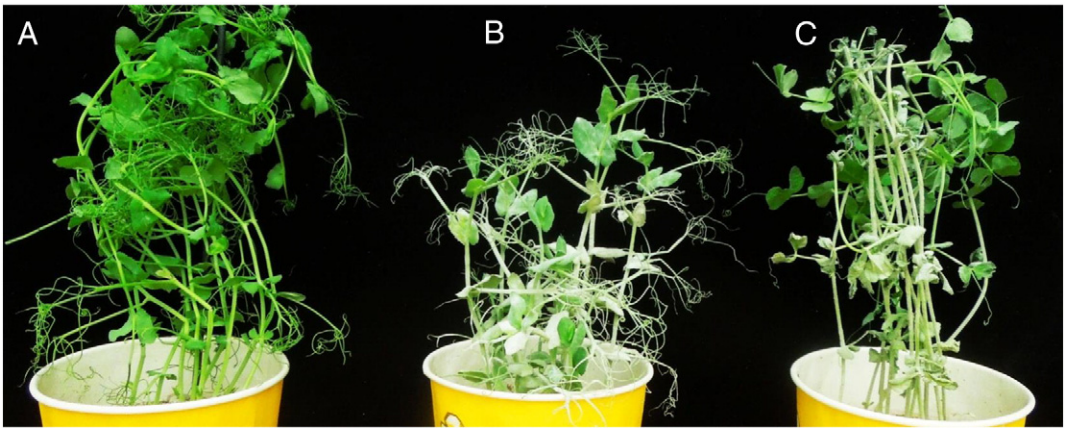

Fig. 1 – Phenotypic evaluation of the resistant pea cultivar Xucai 1 (A) and susceptible cultivars Bawan 6 (B) and Qizhen 76 (C) inoculated with *E. pisi* isolate EPBJ.

Table 1 – The segregation patterns of pea resistance to powdery mildew in the F_1, F_2, and $F_{2:3}$ populations derived from the crosses of Qizhen 76 × Xucai 1, Bawan 6 × Xucai 1, and Xucai 1 × Bawan 6.

Cultivars and crosses	Generation	Number of plants	Observed ratio			Expected ratio and goodness of fit		
			S	Rs	R	(R:Rs:S)	χ^2	P
Xucai 1	P_1	30	–	–	30	–	–	–
Bawan 6	P_2	30	30	–	–	–	–	–
Qizhen 76	P_3	30	30	–	–	–	–	–
Qizhen 76 × Xucai 1	F_1	4	4	0	0	–	–	–
	F_2	91	74	–	17	3:1	1.94	0.16
	$F_{2:3}$	91	22	52	17	1:2:1	2.41	0.30
Bawan 6 × Xucai 1	F_1	4	4	0	0	–	–	
	F_2	58	47	–	11	3:1	1.13	0.28
Xucai 1 × Bawan 6	F_1	8	8	0	0	–	–	
	F_2	161	126	–	35	3:1	0.91	0.34
	$F_{2:3}$	161	48	78	35	1:2:1	2.26	0.32

R: Resistant plant or family; Rs: Family segregated for resistance and susceptibility; S: Susceptible plant or family.

that segregated into 47 susceptible and 11 resistant, with a good fit to a 3:1 ratio ($\chi^2 = 1.13$; $P = 0.28$), as expected for single recessive gene inheritance (Table 1).

The cross between the resistant parent Xucai 1 and the susceptible parent Bawan 6 generated eight susceptible F_1 plants. In the F_2 population of 161 plants, 35 were resistant and 126 susceptible, fitting a 3:1 ratio ($\chi^2 = 0.91$; $P = 0.34$). Moreover, in the $F_{2:3}$ population (161 families), 35 were homozygous resistant, 48 were homozygous susceptible, and 78 segregated in response to isolate *E. pisi* EPBJ (Table 1), fitting the genetic model ratio 1:2:1 ($\chi^2 = 2.26$; $P = 0.32$) for a single recessive gene.

These results indicated that the observed segregation patterns fitted 3 (susceptible):1 (resistant) and 1 (homozygous susceptible):2 (segregating):1 (homozygous resistant) ratios in all F_2 and $F_{2:3}$ populations derived from the three crosses, respectively, suggesting that the resistance to *E. pisi* in Xucai 1 was controlled by a single recessive gene.

3.2. Molecular marker analysis

Among the 148 SSR markers, 59 were polymorphic between the parents Qizhen 76 and Xucai 1 and 74 between Xucai 1 and Bawan 6. These polymorphic SSR markers were further used to screen for polymorphism between the resistant and susceptible DNA pools. Only SSR marker AD60 in pea LG VI was polymorphic between the two contrasting DNA bulks differing in their response to *E. pisi* [46] and was thus likely to be linked to the resistance gene. SSR marker AD60 was verified as a marker linked to the *er1* locus by Ek et al. [19]. This marker was accordingly genotyped in both F_2 populations. The marker was codominant and its segregation in both F_2 populations fitted the expected Mendelian ratio of 1:2:1. Further linkage analysis confirmed that the SSR marker AD60 was linked to the resistance gene (Fig. 2-A), showing that the gene is located on LG VI, which harbors the *er1* locus [19].

In addition, according to this primary mapping result, molecular markers on LG VI adjacent to SSR AD60 were selected from a genetic map [47] and analyzed in the contrasting DNA bulks. The codominant marker c5DNAmet (cytosine-5 DNA-methyltransferase) was polymorphic between the bulks and thus likely to be linked to the resistance gene. Its segregation in both F_2 populations fitted the expected Mendelian ratio of 1:2:1. Further linkage analysis confirmed that c5DNAmet was linked to the resistance gene (Fig. 2-B).

The four known SCAR markers ScOPD10-650 [22], ScOPE16-1600 [23], ScOPO18-1200 [23], and ScOPX04-880 [25] linked to *er1* were used to identify polymorphism between the parents Xucai 1 and Bawan 6 and between Xucai 1 and Qizhen 76. The results of amplification by SCAR markers in the three parents are shown in Table 2. Marker ScOPD10-650

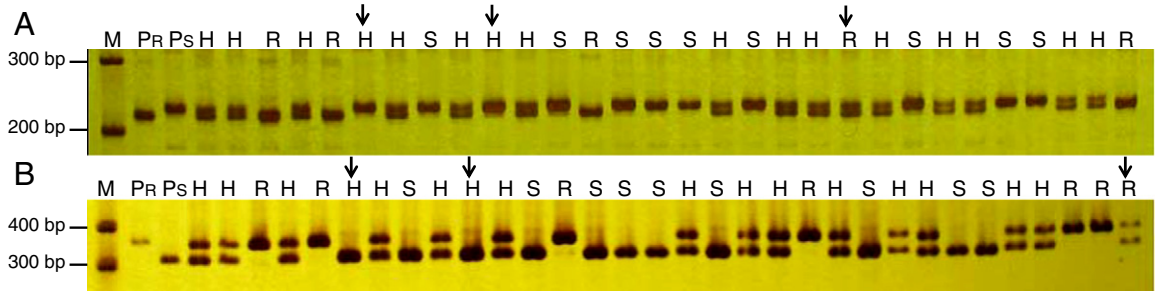

Fig. 2 – The segregation patterns of molecular markers AD60 (A) and c5DNAmet (B) linked to the resistance gene in some of the F_2 progeny derived from the cross of Qizhen 76 × Xucai 1. M: 100 bp marker ladder; P_R: resistant parent Xucai 1; P_S: susceptible parent Qizhen 76; R: resistant families; H: families segregating for resistance and susceptibility; S: susceptible families. Arrows indicate recombinants between the markers and the resistance gene.

Table 2 – Amplification of four SCAR markers known to be linked to resistance gene *er1* in resistant parent Xucai 1 and susceptible parents Bawan 6 and Qizhen 76 ("–" indicates no amplification).

Parents	Markers and amplified PCR fragments (bp)			
	ScOPD10-650	ScOPE16-1600	ScOPO18-1200	ScOPX04-880
Xucai 1	650	–	–	880
Bawan 6	–	–	1200	880
Qizhen 76	650	1600	–	–

amplified a fragment of expected size (650-bp) in the resistant Xucai 1 and the susceptible Qizhen 76, but there was no amplification in the other susceptible cultivar Bawan 6. Marker ScOPE16-1600 amplified a 1600-bp fragment in the susceptible Qizhen 76, but there was no amplification in either resistant Xucai 1 or susceptible Bawan 6. Marker ScOPX04-880 amplified an 880-bp fragment in both resistant Xucai 1 and susceptible Bawan 6, but produced no fragment in susceptible Qizhen 76. Marker ScOPO18-1200 amplified a 1200-bp fragment only in susceptible Bawan 6. Amplifications in the contrasting bulks pooled from F_2 plants were then performed using the SCAR markers polymorphic in the parents. However, these markers did not form different fragment patterns in the contrasting bulks.

3.3. Genetic mapping of the resistance gene

Linkage analysis of segregation data allowed construction of genetic maps for both F_2 mapping populations derived from the crosses of Xucai 1 × Bawan 6 and Qizhen 76 × Xucai 1, respectively. Maps comprising two SSR markers and the resistance gene for both F_2 populations were constructed with MapDraw [50]. Linked markers AD60 and c5DNAmet were located on the same side of the resistance gene. For the F_2 population derived from Qizhen 76 × Xucai 1, markers c5DNAmet and AD6 were linked to the resistance gene at genetic distances of 8.1 and 8.7 cM, respectively (Fig. 3-A). For the F_2 population derived from Xucai 1 × Bawan 6, the resistance gene was linked to markers AD60 and c5DNAmet at genetic distances of 9.9 and 15.4 cM, respectively (Fig. 3-B). These results indicated that the powdery mildew resistance gene in Xucai 1 is an allele of *er1*, in agreement with a previous study [19].

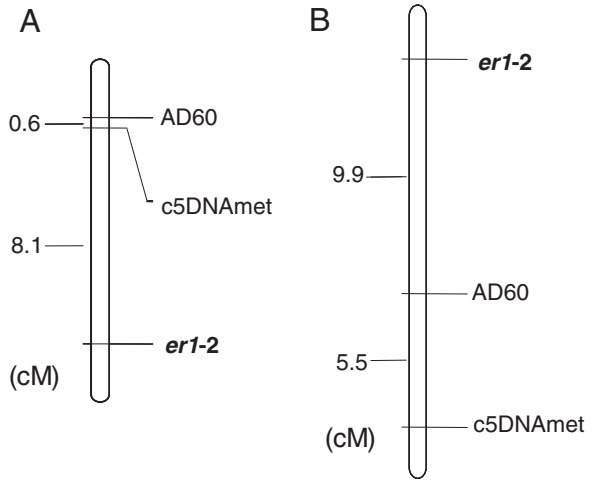

Fig. 3 – Genetic linkage maps of F_2 populations derived from Qizhen 76 × Xucai 1 (A) and Xucai 1 × Bawan 6 (B) showing the position of the gene conferring resistance to powdery mildew and the molecular markers AD60 and c5DNAmet. Map distances and locus orders were determined with MAPMAKER 3.0 (Lander et al. 1993). Estimated genetic distances between loci are shown on the left in centiMorgans (cM).

3.4. *PsMLO1* cDNA sequence analysis

We determined the nucleotide sequences of homologous *PsMLO1* cDNAs of the contrasting parents to confirm which allele of *er1* is present in Xucai 1. *PsMLO1* full-length coding sequences of the three parents were obtained by cloning and sequencing of PCR products amplified by the specific primers PsMLO1F and PsMLO1R [29]. On the basis of 10 clones, the homologous cDNA sequences of the *PsMLO1* gene from Bawan 6 and Qizhen 76 were identical to that of *PsMLO1* from the wild-type pea cv. Sprinter (susceptible to *E. pisi*; NCBI accession number FJ463618). Thus, Bawan 6 and Qizhen 76 carry the susceptibility gene *Er1* (Fig. 4). In contrast, the cDNA sequences of *PsMLO1* from Xucai 1 were markedly different from that of wild-type pea cv. Sprinter and the two susceptible parents. The cDNAs from Xucai 1 comprised three distinct types of mis-spliced transcripts that were not present in the wild-type pea or the two susceptible parents. On the basis of 10 clones from Xucai 1, we obtained three *PsMLO1* transcripts that were characterized by a 129-bp deletion and 155- and 220-bp insertions, respectively, in the *PsMLO1* gene of wild-type Sprinter (Fig. 4). Compared to the *PsMLO1* transcript of the susceptible cv. Sprinter, Bawan 6, and Qizhen 76, cDNA1 from Xucai 1 showed a 129-bp deletion between positions 1171 and 1299 of the *PsMLO1* coding sequence, and the cDNA2 and cDNA3 from Xucai 1 had larger insertions of 155- and 220-bp at position 1263 in *PsMLO1* coding sequence, respectively (Fig. 4). These distinct transcripts were attributed to alternative splicing during transcription. The cDNA1 sequence also showed a 5-bp deletion from positions 770–774 of the *PsMLO1* coding sequence (Fig. 5), which was attributed to an aberrant splicing event. The transcripts of the *PsMLO1* gene in Xucai 1 are consistent with those in pea cv. Stratagem (JI 2302) and Franklin which harbor the *er1*-2 alleles of resistance gene *er1*, indicating that Xucai 1 carries the *er1*-2 allele for resistance to powdery mildew [28,29].

4. Discussion

In this study, the pea cv. Xucai 1 was confirmed to have high resistance (immunity) to powdery mildew, and no colonies developed on the whole plants after artificial inoculation with

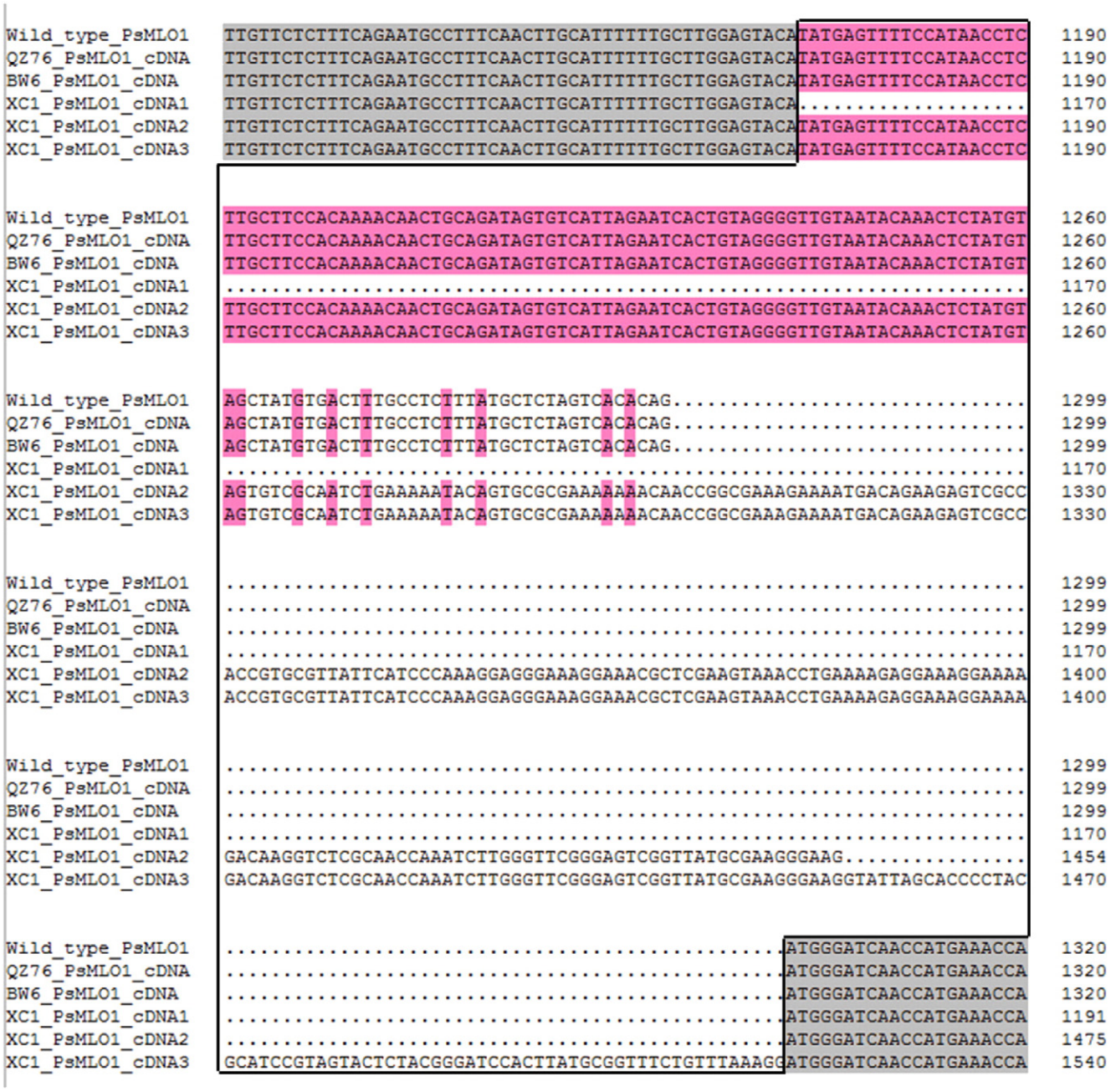

Fig. 4 – Comparisons of cDNA sequence of the *PsMLO1* gene from the wild-type pea cultivar Sprinter (Wild_type_PsMLO1) with those identified in three parents, including two susceptible cultivars, Qizhen 76 (QZ76_PsMLO1_cDNA) and Bawan 6 (BW6_PsMLO1_cDNA), and a resistant cultivar, Xucai 1 (XC1_PsMLO1_cDNA1, 2 and 3). Mutation sites with the deletion or insertion fragments in *PsMLO1* coding sequence are boxed in black.

E. pisi (Fig. 1, Table 1). The genetic basis of resistance to *E. pisi* in Xucai 1 was investigated using three F_2 and two $F_{2:3}$ populations derived from three crosses. Phenotypic observations of the populations showed that resistance in Xucai 1 is conferred by a single recessive gene.

To date, only two recessive genes (*er1* and *er2*) and one dominant gene (*Er3*) have been identified as conferring resistance to *E. pisi* [10–12]. A majority of pea germplasm resistant to *E. pisi* carries the recessive gene *er1*, which provides durable broad-spectrum resistance to powdery mildew disease caused by *E. pisi* [13,52].

To aid marker-assisted selection (MAS) in pea breeding programs, studies have been performed to identify different types of molecular markers, including RFLP (restriction fragment length polymorphism), RAPD, SCAR, and SSR, linked to the *er1* locus [17–25,53]. In these studies, various markers linked to the resistance gene *er1* were reported by different researchers; however, the markers were located at different genetic distances in different mapping populations. First, Sarala [17] and Timmerman et al. [22] placed the *er1* gene on pea LG VI. Dirlewanger et al. [18] found the *er1* gene 9.8 cM distant from a RFLP marker, *p236*, whereas Timmerman et al.

Fig. 5 – Alignment showing a 5-bp deletion from 770 to 774 bp, compared to the wild type (Wild_type_PsMLO1), in one of the transcripts derived from Xucai 1 (XC1_PsMLO1_cDNA1). Transcripts "QZ76_PsMLO1_cDNA" and "BW6_PsMLO1_cDNA" derived from the two susceptible cultivars Qizhen 76 and Bawan 6, respectively.

[22] placed the RAPD marker $OPD10_{650}$ at a distance of 2.1 cM from *er1*. This linked RAPD marker ($OPD10_{650}$) was converted to a SCAR marker, $ScOPD10_{650}$, which was mapped at a distance of 3.4 cM from *er1* [20]. However, Tiwari et al. [23] found that $ScOPD10_{650}$ was not useful to identify Canadian pea resistant germplasm and related F_3 progeny derived from "Highlight (*er1*) × Radley." Tiwari et al. [23] obtained three additional linked RAPD markers: $OPO18_{1200}$, $OPE16_{1600}$, and $OPL6_{1900}$. The first two of these were converted into SCAR markers ($ScOPE16_{1200}$ and $ScOPL6_{1600}$) and used successfully to identify resistant pea germplasm. Janila and Sharma [20] found that of the three SCAR markers, only $ScOPD10_{650}$ was linked to *er1*, at a genetic distance of 3.4 cM. Later, Ek et al. [19] discovered five SSR markers (PSMPSAD51, PSMPSA5, PSMPSAD60, PSMPSAA374e, and PSMPSAA369) linked to *er1*, and PSMPSAD60 (named AD60 in this study) was identified as the most closely linked marker, at 10.4 cM distance from the *er1* locus. Pereira et al. [21] identified two mutated genes, *er1mut1* and *er1mut2*, that induced powdery mildew resistant mutants in pea, via ethylnitrosourea mutagenesis. The *er1mut1* and *er1mut2* loci were mapped to a pea LG containing 16 DNA markers including six linked markers. Pereira et al. [21] also attempted to remap all previously reported SCARs and five SSR markers linked to *er1*. Only the reported SCAR marker $ScOPL6_{1600}$ and SSR marker PSMPSA5 were linked to the *er1mut2* locus, at a longer distance. Recently, Santo et al. [54] developed specific molecular markers SCAR and a sequence-tagged site (STS) for the mutation *er1mut1*. Srivastava et al. [25] also developed another coupling-phase SCAR marker, $ScOPX04_{880}$, linked to *er1*. In their study, a previous repulsion-phase marker, $ScOPD10_{650}$, and a coupling-phase marker, $ScOPX04_{880}$, were mapped flanking *er1* at 2.2 and 0.6 cM distance, respectively. Taken together, these studies identified several markers linked to the *er1* locus, with various degrees of utility, in different plant materials. In our study, in addition to the previous SCAR marker AD60, gene marker c5DNAmet was newly identified as linked to resistance gene *er1*. This novel marker can be used in MAS for powdery mildew resistance breeding in pea.

In this study, the resistance gene in Xucai 1 was mapped at distances of 8.7 and 9.9 cM from the linked marker AD60 in the respective F_2 populations (Fig. 3-A and 3-B). Our result is comparable with that reported by Ek et al. [19]. The parents were then tested with four previous SCAR markers linked to the *er1* locus, $ScOPD10_{650}$, $ScOPO18_{1200}$, $ScOPE16_{1600}$, and $ScOPX04_{880}$ using the methods described by Wang et al. [34]. However, the four markers were useless among the three tested parents because they were unable to distinguish the resistant parent from the susceptible ones and showed the same single band in the contrasting bulks (Table 2). This result was consistent with those of previous studies [20,21,23]. We then selected molecular markers distributed in the vicinity of the linked marker AD60 on a pea linkage map [47] to identify additional markers linked to the resistance gene in our populations, and found that gene marker c5DNAmet was linked to the resistance gene in Xucai 1. Thus, we further confirmed that powdery mildew resistance gene in Xucai 1 resides on LG VI of the pea genetic map. The marker c5DNAmet mapped at 8.1 cM and 15.4 cM distances from the resistance gene in the two F_2 populations derived from Xucai 1 × Bawan 6 and Qizhen 76 × Xucai 1, respectively (Fig. 3). Markers AD60 and c5DNAmet were mapped to the same side of the resistance gene on LG VI in the two F_2 populations (Fig. 3). The mapping of the resistance gene in LG VI linked to AD60 and c5DNAmet markers suggested that the resistance gene is an *er1* allele.

Recently, *er1* resistance was demonstrated to be caused by a loss-of-function mutations of a powdery mildew susceptibility gene, *PsMLO1*, belonging to the *MLO* gene family [28,29]. To date, five *er1* alleles, each corresponding to a different *PsMLO1* mutation, have been reported in pea accessions resistant to *E. pisi* [30]. Humphry et al. [28] identified four *er1* resistance alleles, *er1*-1 (JI1559), *er1*-2 (JI2302), *er1*-3 (JI210), and *er1*-4 (JI1951), in naturally occurring *PsMLO1* loss-of function mutations in resistant pea accessions. Pavan et al. [29] identified another *er1* allele, *er1*-5, in pea line ROI3/02 following a mutagenesis program. Four of the five *er1* alleles (excluding *er1*-2) are caused by point mutations in the *PsMLO1* gene. Allele *er1*-2 comprises a large DNA insertion of unknown size and identities in the *PsMLO1* gene, leading to aberrant *PsMLO1* transcription [28,29]. Allele *er1*-2 has been identified in a few pea powdery mildew resistant cultivars/lines, including Stratagem (JI 2302), Franklin, Dorian, and Nadir [28,29]. Stratagem (JI 2302) is the earliest identified pea cultivar resistant

to *E. pisi* and has been widely used in pea breeding, owing to its stable resistance [52].

In the present study, the mutation mode that occurred in Xucai 1 is consistent with that in the pea cv. Stratagem (JI 2302) carrying the *er1* resistance allele, *er1*-2 [28,29]. Thus, the resistance gene in cv. Xucai 1 is an *er1* allele, *er1*-2. The inserted fragments of 155- and 220-bp were compared against the NCBI database by BLASTN. The result revealed that, as reported for *er1*-2 allele by Humphry et al. [28], the 155- and 220-bp insertions were very similar (96% and 95%, respectively) to a sequence repeated five times in the genomic BAC clone (GenBank accession number, CU655882). Interestingly, the 220-bp insertion is also highly similar (~87% identity) to part of the sequence of the pea *Ogre* retrotransposon (GenBank accession number AY299395). The *Ogre* is a giant retroelement of more than 22 kb that makes up at least 5% of the pea genome [54,55].

Recently, Pavan et al. [30] developed different types of functional markers corresponding to five *er1* alleles, including an STS dominant marker *er1*-2/MGB targeting the *er1*-2 allele, according to the sequence difference. *er1*-2/MGB was used to test our parents in the present study, but showed no amplification in either resistant or susceptible parents. To date, *er1*-2/MGB has not been tested in other resistance cultivars or in derived populations carrying *er1*-2, except Franklin [30]. Our finding may have been due to the large difference in genetic background between Xucai 1 and Franklin. Such difference can be dissected with obtaintion of complicated genome sequence of pea carrying resistance gene *er1*-2 after developing a codominant marker for *er1*-2. Four foreign pea cultivars or lines, including Stratagem (JI2302), Franklin, Dorian, and Nadir, have been shown to harbor the resistance gene *er1*-2 [28,29]. In our study, we identified another recessive resistance source harboring *er1*-2: the Chinese pea cultivar Xucai 1.

The desirable agronomic traits of Xucai 1, including high yield and tolerance to lodging and drought [31], would be an advantage for the use of this cultivar in breeding programs. Our study revealed that resistance to *E. pisi* in the Xucai 1 is conferred by the gene *er1*-2. This result provides valuable genetic information about Xucai 1 that will improve its use in pea breeding for resistance to *E. pisi*.

5. Conclusions

Powdery mildew resistance in Xucai 1 was governed by a recessive gene that was mapped in pea LG VI, close to markers AD60 and c5DNAmet. These results suggested that the resistance gene is an allele of *er1*. The analysis of *PsMLO1* coding sequence in Xucai 1 revealed that the resistance gene was generated by a large insertion or deletion in the *PsMLO1* gene. Thus, the resistance gene in Xucai 1 was identified as an *er1* allele, specifically the *er1*-2 allele.

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REFERENCES

[1] L.J. Lang, Z.J. Zheng, Production of faba bean and pea in China, in: R.J. Summerfield (Ed.), World Crops: Cool Season Food Legumes, Current Plant Science and Biotechnology in Agriculture, Kluwer Academic Publisher, Dordrecht, Netherlands 1988, pp. 1135–1152.
[2] F.A.O. Statistical Database, Food and Agriculture Organization (FAO) of the United Nations, Rome, 2014 (http://faostat3.fao.org/).
[3] E.T. Gritton, R.D. Ebert, Interaction of planting date and powdery mildew on pea plant performance, Am. Soc. Horticult. Sci. 100 (1975) 137–142.
[4] P.H. Smith, E.M. Foster, L.A. Boyd, J.K.M. Brown, The early development of *Erysiphe pisi* on *Pisum sativum* L, Plant Pathol. 45 (1996) 302–309.
[5] R.L. Munjal, V.V. Chenulu, T.S. Hora, Assessment of losses due to powdery mildew (*Erisiphe polygoni*) on pea, Indian Phytopathol. 19 (1963) 260–267.
[6] T.D. Warkentin, K.Y. Rashid, A.G. Xue, Fungicidal control of powdery mildew in field pea, Can. J. Plant Sci. 76 (1996) 933–935.
[7] S. Fondevilla, J.I. Cubero, D Rubiales, Confirmation that the *Er3* gene, conferring resistance to *Erysiphe pisi* in pea, is a different gene from *er1* and *er2* genes, Plant Breed. 130 (2011) 281–282.
[8] A. Ghafoor, K. McPhee, Marker assisted selection (MAS) for developing powdery mildew resistant pea cultivars, Euphytica 186 (2012) 593–607.
[9] S. Fondevilla, D. Rubiales, Powdery mildew control in pea: a review, Agron. Sustain. Dev. 32 (2012) 401–409.
[10] S.C. Harland, Inheritance of immunity to mildew in Peruvian forms of *Pisum sativum*, Heredity 2 (1948) 263–269.
[11] R.J. Heringa, A. van Norel, M.F. Tazelaar, Resistance to powdery mildew (*Erysiphe polygoni* D. C.) in peas (*Pisum sativum* L.), Euphytica 18 (1969) 163–169.
[12] S. Fondevilla, A.M. Torres, M.T. Moreno, D. Rubiales, Identification of a new gene for resistance to powdery mildew in *Pisum fulvum*, a wild relative of pea, Breed. Sci. 57 (2007) 181–184.
[13] K.R. Tiwari, G.A. Penner, T.D. Warkentin, Inheritance of powdery mildew resistance in pea, Can. J. Plant Sci. 77 (1997) 307–310.
[14] S. Fondevilla, T.L.W. Carver, M.T. Moreno, D. Rubiales, Macroscopic and histological characterisation of genes *er1* and *er2* for powdery mildew resistance in pea, Eur. J. Plant Pathol. 115 (2006) 309–321.
[15] R. Iglesias-García, D. Rubiales, S. Fondevilla, Penetration resistance to *Erysiphe pisi* in pea mediated by *er1* gene is associated with protein cross-linking but not with callose apposition or hypersensitive response, Euphytica 201 (2015) 381–387.
[16] S. Fondevilla, T.L.W. Carver, M.T. Moreno, D. Rubiales, Identification and characterisation of sources of resistance to *Erysiphe pisi* Syd. in *Pisum* spp. Plant Breed. 126 (2007) 113–119.

[17] K. Sarala, Linkage studies in pea (*Pisum sativum* L.) with reference to *er* gene for powdery mildew resistance and other genesPh.D. thesis Indian Agricultural Research Institute, New Delhi, India, 1993.
[18] E. Dirlewanger, P.G. Isaac, S. Ranade, M. Belajouza, R. Cousin, D. Vienne, Restriction fragment length polymorphism analysis of loci associated with disease resistance genes and developmental traits in *Pisum sativum* L, Theor. Appl. Genet. 88 (1994) 17–27.
[19] M. Ek, M. Eklund, R. von Post, C. Dayteg, T. Henriksson, P. Weibull, A. Ceptilis, P. Issac, S. Tuvesson, Microsatellite markers for powdery mildew resistance in pea (*Pisum sativum* L.), Hereditas 142 (2005) 86–91.
[20] P. Janila, B. Sharma, RAPD and SCAR markers for powdery mildew resistance gene *er* in pea, Plant Breed. 123 (2004) 271–274.
[21] G. Pereira, C. Marques, R. Ribeiro, S. Formiga, M. Dâmaso, T. Sousa, M. Farinhó, J.M. Leitão, Identification of DNA markers linked to an induced mutated gene conferring resistance to powdery mildew in pea (*Pisum sativum* L.), Euphytica 171 (2010) 327–335.
[22] G.M. Timmerman, T.J. Frew, N.F. Weeden, Linkage analysis of *er1*, a recessive *Pisum sativum* gene for resistance to powdery mildew fungus (*Erysiphe pisi* D.C.), Theor. Appl. Genet. 88 (1994) 1050–1055.
[23] K.R. Tiwari, G.A. Penner, T.D. Warkentin, Identification of coupling and repulsion phase RAPD markers for powdery mildew resistance gene *er1* in pea, Genome 41 (1998) 440–444.
[24] M. Tonguc, N.F. Weeden, Identification and mapping of molecular markers linked to *er1* gene in pea, J. Plant Mol. Biol. Biotechnol. 1 (2010) 1–5.
[25] R.K. Srivastava, S.K. Mishra, K. Singh, T. Mohapatra, Development of a coupling-phase SCAR marker linked to the powdery mildew resistance gene *er1* in pea (*Pisum sativum* L.), Euphytica 186 (2012) 855–866.
[26] V. Katoch, S. Sharma, S. Pathania, D.K. Banayal, S.K. Sharma, R. Rathour, Molecular mapping of pea powdery mildew resistance gene *er2* to pea linkage group III, Mol. Breed. 25 (2010) 229–237.
[27] S. Fondevilla, D. Rubiales, M.T. Moreno, A.M. Torres, Identification and validation of RAPD and SCAR markers linked to the gene *Er3* conferring resistance to *Erysiphe pisi* D.C. in pea, Mol. Breed. 22 (2008) 193–200.
[28] M. Humphry, A. Reinstädler, S. Ivanov, T. Bisseling, R. Panstruga, Durable broad-spectrum powdery mildew resistance in pea *er1* plants is conferred by natural loss-of-function mutations in *PsMLO1*, Mol. Plant Pathol. 12 (2011) 866–878.
[29] S. Pavan, A. Schiavulli, M. Appiano, A.R. Marcotrigiano, F. Cillo, R.G.F. Visser, Y. Bai, C. Lotti, L. Ricciardi, Pea powdery mildew *er1* resistance is associated to loss-of-function mutations at a *MLO* homologous locus, Theor. Appl. Genet. 123 (2011) 1425–1431.
[30] S. Pavan, A. Schiavulli, M. Appiano, C. Miacola, R.G.F. Visser, Y. Bai, C. Lotti, L. Ricciardi, Identification of a complete set of functional markers for the selection of *er1* powdery mildew resistance in *Pisum sativum* L, Mol. Breed. 31 (2013) 247–253.
[31] X.M. Wang, Z.D. Zhu, C.X. Duan, X.X. Zong, Identification and Control Technology of Disease and Pest on Faba Bean and Pea, Chinese Agricultural Science and Technology Press, Beijing, 2007.
[32] H.X. Peng, G. Yao, R.L. Jia, H.Y. Liang, Identification of pea germplasm resistance to powdery mildew, J. Southwest Agric. Univ. 13 (1991) 384–386 (in Chinese with English abstract).
[33] L. Zeng, M.Q. Li, X.M. Yang, Identification of resistance of peas resources to powdery mildew, Grass & Turf 32 (2012) 35–38 (in Chinese with English abstract).
[34] Z.Y. Wang, S.Y. Bao, C.X. Duan, X.X. Zong, Z.D. Zhu, Screening and molecular identification of resistance to powdery mildew in pea germplasm, Acta Agron. Sin. 39 (2013) 1030–1038 (in Chinese with English abstract).
[35] H.N. Fu, S.L. Sun, Z.D. Zhu, C.X. Duan, X.M. Yang, Phenotypic and genotypic identification of powdery mildew resistance in pea cultivars or lines from Canada, J. Plant Genet. Resour. 15 (2014) 1028–1033 (in Chinese with English abstract).
[36] M. Ondřej, R. Dostálová, L. Odstrčilová, Response of *Pisum sativum* germplasm resistant to *Erysiphe pisi* to inoculation with *Erysiphe baeumleri*, a new pathogen of peas, Plant Prot. Sci. 41 (2005) 95–103.
[37] R.N. Attanayakea, D.A. Glaweab, K.E. McPheec, F.M. Dugand, W. Chend, *Erysiphe trifolii*—a newly recognized powdery mildew pathogen of pea, Plant Pathol. 59 (2010) 712–720.
[38] S. Fondevilla, C. Chattopadhyay, N. Khare, D. Rubiales, *Erysiphe trifolii* is able to overcome *er1* and *Er3*, but not *er2*, resistance genes in pea, Eur. J. Plant Pathol. 136 (2013) 557–563.
[39] P. Baiswar, S.V. Ngachan, V.K. Verma, R. Kumar, A.K. Jha, S. Chandra, Molecular evidence of *Erysiphe pisi* on pea and *E. trifoliorum* on white clover in northeast India, Aust. Plant Dis. Notes 10 (2015) 12.
[40] L.F. Dong, F.B. Wang, J.F. Fu, Development of a new semi-leafless sweet pea Xucai No. 1, J. Changjiang Veget. (2008) 45–46 (in Chinese with English abstract).
[41] A. Vaid, P.D. Tyagi, Genetics of powdery mildew resistance in pea, Euphytica 96 (1997) 203–206.
[42] J.C. Rana, D.K. Banyal, K.D. Sharma, K. Sharma Manish, S.K. Gupta, S.K. Yadav, Screening of pea germplasm for resistance to powdery mildew, Euphytica 189 (2013) 271–282.
[43] D. Rubiales, J.K.M. Brown, A. Martín, *Hordeum chilense* resistance to powdery mildew and its potential use in cereal breeding, Euphytica 67 (1993) 215–220.
[44] M. Shure, S. Wessler, N. Fedoroff, Molecular-identification and isolation of the waxy locus in maize, Cell 35 (1983) 225–233.
[45] R.W. Michelmore, I. Paran, R.V. Kesseli, Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 9828–9832.
[46] K. Loridon, K. McPhee, J. Morin, P. Dubreuil, M.L. Pilet-Nayel, G. Aubert, C. Rameau, A. Baranger, C. Coyne, I. Lejeune-Henaut, J. Burstin, Microsatellite marker polymorphism and mapping in pea (*Pisum sativum* L.), Theor. Appl. Genet. 111 (2005) 1022–1031.
[47] A. Bordat, V. Savois, M. Nicolas, J. Salse, A. Chauveau, M. Bourgeois, J. Potier, H. Houtin, C. Rond, F. Murat, P. Marget, G. Aubert, J. Burstin, Translational genomics in legumes allowed placing in silico 5460 unigenes on the pea functional map and identified candidate genes in *Pisum sativum* L, Genes Genom. Genet. 1 (2011) 93–103.
[48] E.S. Lander, M.J. Daly, S.E. Lincoln, Constructing Genetic Linkage Maps with MAPMAKER/EXP Version 3.0: A Tutorial And Reference Manual, Third EditionA Whitehead Institute for Biomedical Research Technical Report, Cambridge, MA, USA, 1993.
[49] D.D. Kosambi, The estimation of map distances from recombination values, Ann. Eugen. 12 (1944) 172–175.
[50] R.H. Liu, J.L. Meng, MapDraw: a Microsoft excel macro for drawing genetic linkage maps based on given genetic linkage data, Hereditas 25 (2003) 317–321 (in Chinese with English abstract).
[51] M.A. Larkin, G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson, D.G. Higgins, Clustal W and Clustal X version 2.0, Bioinformatics 23 (2007) 2947–2948.

[52] R. Cousin, Resistance to powdery mildew in pea, Ann. Amélior. Plantes 15 (1965) 93–97.
[53] M. Nisar, A. Ghafoor, Linkage of a RAPD marker with powdery mildew resistance *er1* gene in *Pisum sativum* L, Russ. J. Genet. 47 (2011) 300–304.
[54] T. Santo, M. Rashkova, C. Alabaca, J. Leitao, The ENU-induced powdery mildew resistant mutant pea (*Pisum sativum* L.) lines S (*er1mut1*) and F (*er1mut2*) harbour early stop codons in the *PsMLO1* gene, Mol. Breed. 32 (2013) 723–727.
[55] P. Neumann, D. Pozarkova, J. Macas, Highly abundant pea LTR retrotransposon *Ogre* is constitutively transcribed and partially spliced, Plant Mol. Biol. 53 (2003) 399–410.