Mol Breeding (2017) 37: 151
https://doi.org/10.1007/s11032-017-0740-7

SHORT COMMUNICATION

Development and validation of breeder-friendly KASPar markers for *er1*, a powdery mildew resistance gene in pea (*Pisum sativum* L.)

Yu Ma · Clarice J. Coyne · Dorrie Main · Stefano Pavan · Suli Sun · Zhendong Zhu · Xuxiao Zong · José Leitão · Rebecca J. McGee

Received: 26 June 2017 / Accepted: 15 October 2017 / Published online: 21 November 2017

Abstract Powdery mildew of pea is caused by *Erysiphe pisi* DC and is a serious threat to pea (*Pisum sativum* L.) production throughout much of the world. Development and utilization of genetic resistance to powdery mildew is considered an effective and sustainable strategy to manage this disease. One gene, *er1*, conferring powdery mildew resistance, was previously cloned and sequenced, and the functional markers for each resistance allele were reported. Allele-specific DNA markers are efficient and powerful tools to facilitate crop improvement and new cultivar development in breeding programs. However, extensive application of these markers is limited by gel-associated obstacles. In this study, eight breeder-friendly kompetitive allele-specific PCR (KASPar) markers were developed to overcome the problems of gel-based markers and increase the efficiency of genotypic screening. In order to identify additional pea germplasm with powdery mildew resistance, these KASPar markers were deployed and used to genotype a pea collection derived from the USDA pea single-plant (PSP) collection. Simultaneously, a phenotypic screening and a genotypic validation using the corresponding gel-based functional markers were conducted on the PSP collection. One pea accession, PI 142775, was identified by both phenotyping and genotyping to carry the allele *er1-1* for powdery mildew resistance, indicating that the KASPar assay is an efficient and robust tool for breeding for powdery mildew resistance.

Keywords *er1* · KASPar assay · Marker-assisted breeding · Powdery mildew · *Pisum sativum*

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11032-017-0740-7) contains supplementary material, which is available to authorized users.

Y. Ma · D. Main
Department of Horticulture, Washington State University, Pullman, WA 99164, USA

C. J. Coyne
Plant Germplasm Introduction and Testing, USDA ARS, Pullman, WA 99164, USA

S. Pavan
Section of Genetics and Plant Breeding, Department of Soil, Plant and Food Science, University of Bari, Via Amendola 165/A, 70126 Bari, Italy

S. Sun · Z. Zhu · X. Zong
National Key Facility for Crop Gene Resources and Genetic Improvement, Institute of Crop Science, Chinese Academy of Agricultural Sciences, 12 Zhongguancun South Street, Beijing 100081, China

J. Leitão
Laboratory of Genomics and Genetic Improvement, BioFIG, FCT, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

R. J. McGee (✉)
Grain Legume Genetics and Physiology Research Unit, USDA ARS, Pullman, WA 99164, USA
e-mail: rebecca.mcgee@ars.usda.gov

Powdery mildew is a common disease of pea (*Pisum sativum* L.) with worldwide distribution and can result in yield losses up to 50% (Warkentin et al. 1996). It is

particularly prevalent in areas with a warm, humid climate. The first symptoms usually appear on the lowest part of the plant, with small, but distinct, spots on the leaflets. Later, these lesions grow, covering the plant surfaces and affecting the growth of the plant and the quality of harvested seeds (Fondevilla and Rubiales 2012). Given the high cost and detrimental environmental impacts associated with fungicide use, the use of powdery mildew-resistant cultivars is a sustainable, efficient, and environmentally friendly method to control this disease.

Three monogenic sources of powdery mildew resistance were identified in pea named *er1*, *er2*, and *Er3* (Harland 1948; Heringa et al. 1969; Fondevilla et al. 2007). Recessive *er1* resistance alleles have been successfully cloned and shown to carry loss-of-function mutations of the pea *MLO* homolog *PsMLO1* (Humphry et al. 2011; Pavan et al. 2011). To date, nine *er1* alleles have been characterized, with each of them corresponding to a different *PsMLO1* mutation (Pavan et al. 2013; Sun et al. 2015; Pavan et al. 2011; Humphry et al. 2011; Sun et al. 2016a; Sun et al. 2016b; Santo et al. 2013; Sudheesh et al. 2015). Allele names from *er1-1* to *er1-7* were previously assigned (Pavan et al. 2011 and 2013; Sun et al. 2016a; Sun et al. 2016b). In this study, the two alleles corresponding to the *PsMLO1* mutations described by Santo et al. (2013) and Sudheesh et al. (2015) were designated as *er1-10* and *er1-11*, respectively (the names of *er1-8* and *er1-9* are applied by Dr. Zhendong Zhu through personal communication with him).

Marker-assisted breeding (MAB), based on DNA markers linked to loci or genes controlling phenotypes of interest, is routinely used in many crops to select for desired characteristics in the early stages of plant growth and facilitate cultivar improvement. Among DNA marker types, SNP markers are considered the ideal choice for geneticists and breeders with advantages of co-dominance, low cost, high throughput, and automation (Gupta et al. 2001; Singh and Singh 2015). Several studies targeted the identification of markers suitable for the selection of *er1* resistance, which is commonly used in pea breeding. Recently, functional markers, specifically designed on different *er1* alleles, were developed (Pavan et al. 2013; Sun et al. 2015; Pavan et al. 2011; Humphry et al. 2011; Sun et al. 2016a; Sun et al. 2016b; Santo et al. 2013; Sudheesh et al. 2015). However, most of these markers are based on gel detection, which is costly and time-consuming. In addition to the limits mentioned above, gel-based markers are also associated with issues of gel-based scoring, such as blurry bands, incomplete digestion of restriction enzymes, and insufficient segregation (Holdsworth and Mazourek 2015). The kompetitive allele-specific PCR (KASPar) assay is a SNP genotyping system based on fluorescence and is flexible, accurate, and inexpensive (Semagn et al. 2014). It has been successfully applied in pepper (Holdsworth and Mazourek 2015), wheat (Neelam et al. 2013), weedy rice (Rosas et al. 2014), soybean (Rosso et al. 2011), and pea (Boutet et al. 2016). To facilitate the current genotyping system for *er1* alleles, the objectives of this study were to develop eight KASPar markers, KASPar-*er1-1*, KASPar-*er1-3*, KASPar-*er1-4*, KASPar-*er1-5*, KASPar-*er1*-6, KASPar-*er1*-7, KASPar-*er1*-10, and KASPar-*er1-11* and use them to genotype a pea collection to identify germplasm carrying the *er1* alleles.

The pea germplasm accessions characterized in this study included the 246 accessions (Table S1) of the USDA PSP collection (Western Regional Plant Introduction Station, USDA-ARS, Pullman, WA, USA), nine lines carrying *er1* resistance alleles, and one susceptible check. In more detail, the nine resistant lines, their *er1* allele and source were as follows: JI 1559 (*er1-1*, John Innes Centre); JI 210 (*er1-3*, John Innes Centre); JI 1951 (*er1-4*, John Innes Centre); ROI3/02 (*er1-5*, University of Bari, Italy); G0001778 (*er1-6*, Chinese Academy of Agricultural Sciences, China); DDR-11 (*er1-7*, Chinese Academy of Agricultural Sciences, China); F(*er1mut2*) (*er1-10*, Universidade do Algarve, Portugal); and ps1771 and Yarrum (*er1-11*, Centre for AgriBioscience, Australia). The susceptible check was JI 502 (John Innes Centre).

Eight KASPar markers (KASPar-*er1-1*, KASPar-*er1-3*, KASPar-*er1-4*, KASPar-*er1-5*, KASPar-*er1-6*, KASPar-*er1-7*, KASPar-*er1-10*, and KASPar-*er1-11*) were designed by LGC KBioscience (KBioscience, Hoddesdon, UK) from the *PsMLO1* sequence, based on the mutation information of the *er1* alleles. Sequence information of the two allele-specific forward primers and the common reverse primer for each marker set are shown in Table 1. The KASPar markers were amplified in 10 μl reaction volumes with 5 μl of 20 ng/μl DNA, 5 μl 2× KASP master mix, and 0.14 μl KASP assay primer mix (KBioscience, Hoddesdon, UK). The PCR amplifications were performed on a GeneAmp 9700 thermal cycler (Applied Biosystems, Forster City, CA, USA) using the following cycling program: template DNAs were initially denatured at 94 °C for 15 min; followed by 10 cycles, when the annealing temperature dropped by 0.6 °C per cycle: 94 °C for 20 s, annealing

for 60 s (Table 1); and followed by 26 cycles of 94 °C for 10 s and 55 °C for 60 s finally cooling to 4 °C. Fluorescent endpoint reading was performed on a Bio-Rad CFX96 Real-Time PCR System (Hercules, CA, USA) with 37 °C for 60 s, and the genotypes were called using the accompanying Bio-Rad CFX Manger 2.0 software (Bio-Rad Laboratories Inc., Hercules, CA, USA).

Co-segregation of the KASPar markers with the previously reported *er1* functional markers, *er1-1*_S (*er1mut1*)_STS, *er1-3/XbaI*_dCAPS, *er1-4/AgsI*_CAPS, *er1-5/HRM54*_HRM, *er1-6*_SNP1121_HRM, *er1-7*_InDel111-120_STS, *er1-10*_F(*er1mut2*)_STS, and *er1-11*_SCAR, was assessed using modified protocols (Pavan et al. 2013; Sun et al. 2016a; Sun et al. 2016b; Sudheesh et al. 2015). The PCR amplifications for the *er1-1*, *er1-3*, and *er1-4* functional markers were performed in 25 μl reaction volumes with 40 ng of template DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.16 mM dNTPs, 0.5 μl of each forward and reverse primer (10 pmol/μl), and 1 U Taq DNA polymerase. The PCR amplifications were performed on a GeneAmp 9700 thermal cycler (Applied Biosystems, Forster City, CA, USA) using the following cycling program: template DNA was initially denatured at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s for STS_ S (*er1mut1*), 53 °C for 30 s for *er1-3/XbaI*_dCAPS and *er1-4/AgsI*_CAPS, and 72 °C for 1 min, with a final 10 min at 72 °C, finally cooling to 4 °C. The PCR products from CAPS were digested with *AgsI* (SibEnzyme Ltd., Novosibirsk, Russia), while the PCR products from dCAPS were digested with *XbaI* (NewEngland Biolabs, Beverly, MA, USA) according to the manufacturer's instructions. The PCR products were discriminated on a 2% MetaPhor agarose gel (Lonza, Rockland, ME, USA) and visualized with ethidium bromide. For the *er1-5* and *er1-6* HRM

Table 1 Sequence information of the eight KASPar markers

Markers	Primers	Sequence information (5′-3′)	Annealing Tm
KASPar-*er1-1*	Forward-C	CCCTTACAATCCATAACAAAATAGGTG	61–55 °C touchdown
	Forward-G	CCCTTACAATCCATAACAAAATAGGTC	
	Common reverse	TTTGCAAGGGACACAACATTTGGAAGAA	
KASPar-*er1-3*	Forward-G	GTATATTTAATCTTAAGTCACACCTTATTCC	61–55 °C touchdown
	Forward-N/A	AGTATATTTAATCTTAAGTCACACCTTATTCT	
	Common reverse	AGATCAATTGAAGAGGATTTTAAAGTTGTT	
KASPar-*er1-4*	Forward-A	GTGTCTTGTGTTGCTAGCTGTTTCAA	61–55 °C touchdown
	Forward-N/A	GTGTCTTGTGTTGCTAGCTGTTTCAT	
	Common reverse	TAGAACGAACCATGCTTAGCTTACCTTT	
KASPar-*er1-5*	Forward-G	ATTCAACTGTTCTTGTCTCATCTTCC	61–55 °C touchdown
	Forward-A	GATATTCAACTGTTCTTGTCTCATCTTCT	
	Common reverse	TTTCTTCAGATGAGGAAGTGGAAGACTT	
KASPar-*er1-6*	Forward-T	TTGAAGTTACCTGAAAGAGAACAA	68–62 °C touchdown
	Forward-C	CTTTGAAGTTACCTGAAAGAGAACAG	
	Common reverse	GTCCTCACCTTCTTCTCTTCACGAT	
KASPar-*er1-7*	Forward-TCATGTTATT	AGCTGTTTCAATCTTAATTGAACATATTATT	57 °C
	Forward-N/A	AGCTGTTTCAATCTTAATTGAACATATTATG	
	Common reverse	ATAGAACGAACCATGCTTAGCTTACCTTT	
KASPar-*er1-10*	Forward-G	TACAATTAGTGGAAGAAATGGAAGC	68–62 °C touchdown
	Forward-A	GCTTACAATTAGTGGAAGAAATGGAAGT	
	Common reverse	GTTATATGGGCAGGGTGGTATTCTTATTA	
KASPar-*er1-11*	Forward-N/A	ATGCAAATCTCATGCGCGTGTGTA	61–55 °C touchdown
	Forward-GA	GCAAATCTCATGCGCGTGTGTG	
	Common reverse	TCAGGATTCAAGATGAGATTCATGTACAAA	

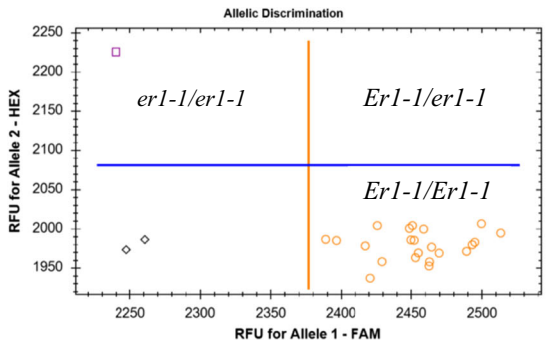
markers, PCR amplifications were performed in a total volume of 10 μl including 40 ng of template DNA, 500 nM of each forward and reverse primer, and 1× Sso Fast EvaGreen Supermix (Bio-Rad). The PCR reactions for HRM curve acquisition were conducted on a Light-Cycler Roche 480 II Real-Time PCR instrument (Roche applied science, Indianapolis, Ind.) using the following cycling program: template DNA were initially denatured at 98 °C for 2 min, followed by 50 cycles of 98 °C for 10 s, 59 °C (*er1-5*), and 56 °C (*er1-6*) for 10 s; then 1 cycle of 95 °C for 10 s; followed by increasing the temperature from 65 °C to 95 °C with an increment of 0.19 °C per minute; and finally cooling down to 37 °C for 30 s. A web-based tool, uAnalyze (Dwight et al. 2012), was used for data evaluation with thermodynamic prediction from specific melting curves. For *er1-7*_InDel111-120_STS, *er1-10*_F(*er1mut2*)_STS, and *er1-11*_SCAR markers, PCR amplifications were performed with 4 ng genomic DNA; 1× PCR buffer; 1.5 mM $MgCl_2$; 0.2 mM dNTPs; 0.05 μM forward primer; 0.25 μM reverse primer; 0.2 μM M13 primers with dyes of FAM, VIC, NED, PET, and 0.6 U BIOLASE™ DNA polymerase (Bioline); and 6.76 μl ddH_2O in a total volume of 12 μl. The template DNAs were initially denatured at 95 °C for 5 min, followed by 42 cycles, each of which consisted of 95 °C for 1 min, 52 °C for 1 min, and 72 °C for 1 min. The final extension was at 72 °C for 10 min. These PCR products were analyzed on an ABI 3730 DNA analyzer (Applied Biosystems), and data were scored using GeneMarker software version 2.2.0 (SoftGenetics).

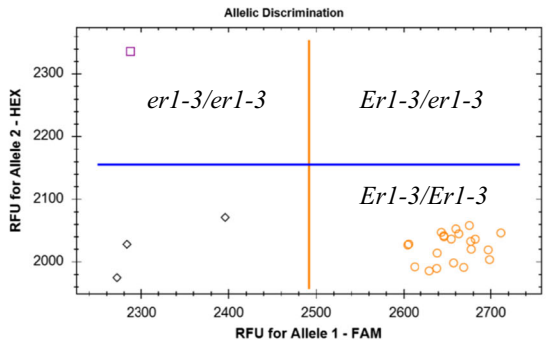
To evaluate the response of the pea collection to powdery mildew, the 246 pea accessions were planted in a naturally infected disease nursery at the Oregon State University Horticulture Farm, Corvallis, OR in 2015. The reaction to powdery mildew was rated at the flat pod stage through visual assessment according to the disease severity key with modifications of methods from the study of Falloon et al. (1995), where 1 = 0–20% of leaflets covered by lesions, 2 = 20–40%, 3 = 40–60%, 4 = 60–80%, and 5 = 80–100%. In order to determine the species of *Erysiphe* present, six infected plants were collected from the nursery. Isolates were developed from the powdery mildew on each plant, and the internal transcribed spacer (ITS) regions were sequenced. Total DNA was extracted from the powdery mildew conidia and mycelia using the DNeasy Mini Plant Kit according to the manufacturer's protocol (Qiagen, Valencia, CA). PCR amplifications were performed as described in Attanayake et al. (2009) with the total genomic DNA using ITS1 (5′-TCCG TAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCT CCGCTTATTGATATGC-3′) primers (White et al. 1990) or EryF (5′-TACAGAGTGCGAGGCTCAGT CG-3′) and EryR (5′-GGTCAACCTGTGATCCATGT GACTGG-3′) (Attanayake et al. 2010). The PCR products were purified using QIAquick PCR Purification Kit (Qiagen, Valencia, CA), and purified products were sequenced using the BigDye Terminator Sequence Kit (Life Technologies, Grand Island, NY, USA) on an ABI PRISM 377 sequencer at the Genomics Core Facility, Washington State University. Pairwise comparison with the BLAST algorithm (BLASTN, E value < 1e−10) in NCBI was used to identify most similar sequences.

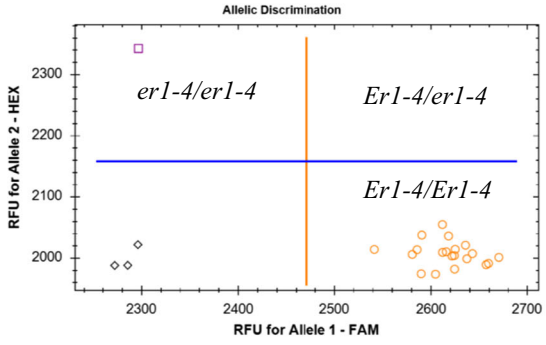
The KASPar assay separated homozygous resistant and homozygous susceptible accessions into different clusters, corresponding to the HEX-labeled cluster and FAM-labeled cluster (Fig. 1). They were easily visualized through Bio-Rad CFX Manger software. The eight KASPar markers developed in this study were successfully tested on 246 pea accessions. The results indicated that only one pea accession PI 142775 was homozygous for the *er1-1* allele, and none carried any of the other *er1-3*, *er1-4*, *er1-5*, *er1-6*, *er1-7*, and *er1-10* alleles. A total of 195 accessions were *er1-11*/*er1-11* homozygotes (Table S2); however, 182 of which were susceptible based on the phenotypic evaluation (Table S2). Thus, this allele may be population specific and would need to be confirmed in the specific germplasm under investigation.

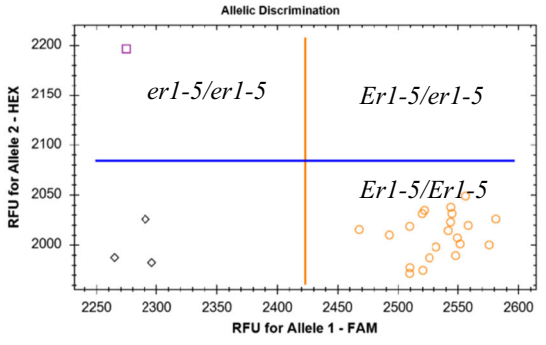
The KASPar markers were tested for co-segregation with the corresponding functional markers (Fig. 2) and the phenotypic characterization. All the functional markers, except for *er1-6*_SNP1121_HRM, *er1-10*_F(*er1mut2*)_STS, and *er1-11*_SCAR, showed complete co-segregation with the corresponding KASPar markers (Table S2). The *er1-6*_SNP1121_HRM and *er1-10*_F(*er1mut2*)_STS markers showed no

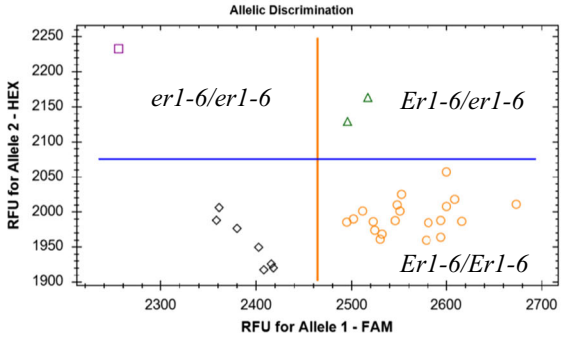
Fig. 1 Genotypic data of eight KASPar markers on 18 pea accessions, 9 positive controls, and 1 negative control. *X*-axis indicates relative FAM fluorescence units, and *Y*-axis indicates relative HEX fluorescence units. Individuals clustered in the upper left (purple) are homozygous for HEX-labeled *er1/er1* powdery mildew resistance. Individuals clustered in lower right (orange) are homozygous for FAM-labeled *Er1/Er1* powdery mildew susceptibility. Individuals clustered in the lower left (black) are no template controls and samples failed to identify (color figure online)

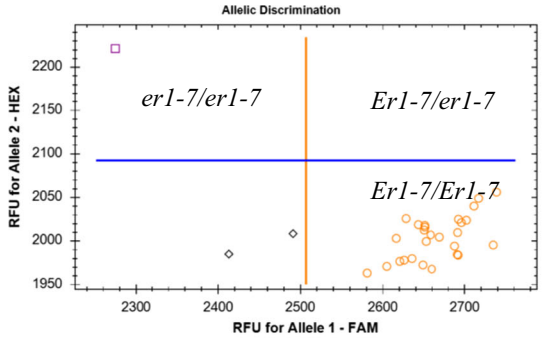

Allelic Discrimination
er1-1/er1-1
Er1-1/er1-1
Er1-1/Er1-1
RFU for Allele 2 - HEX
RFU for Allele 1 - FAM

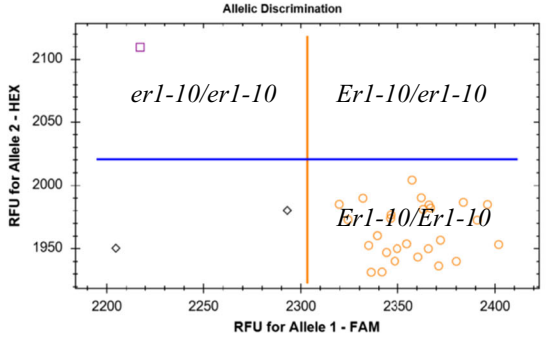

Allelic Discrimination
er1-3/er1-3
Er1-3/er1-3
Er1-3/Er1-3
RFU for Allele 2 - HEX
RFU for Allele 1 - FAM

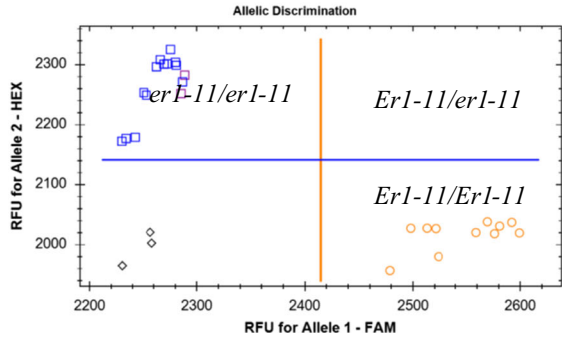

Allelic Discrimination
er1-4/er1-4
Er1-4/er1-4
Er1-4/Er1-4
RFU for Allele 2 - HEX
RFU for Allele 1 - FAM

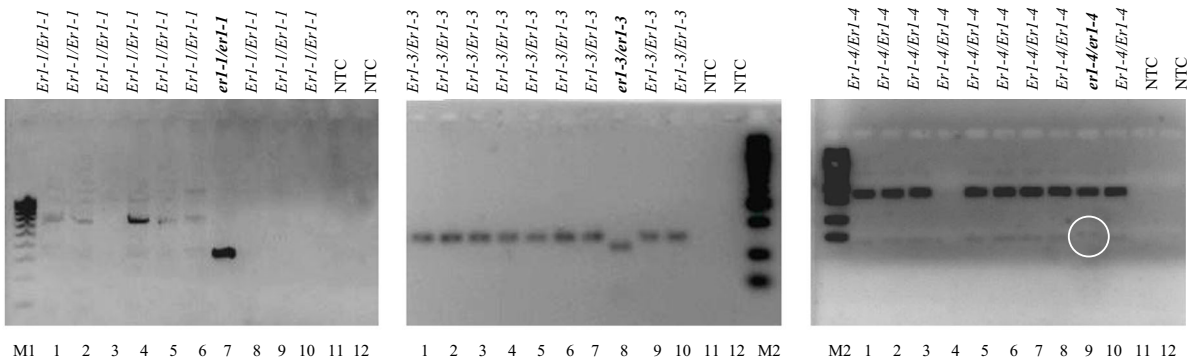

Allelic Discrimination
er1-5/er1-5
Er1-5/er1-5
Er1-5/Er1-5
RFU for Allele 2 - HEX
RFU for Allele 1 - FAM

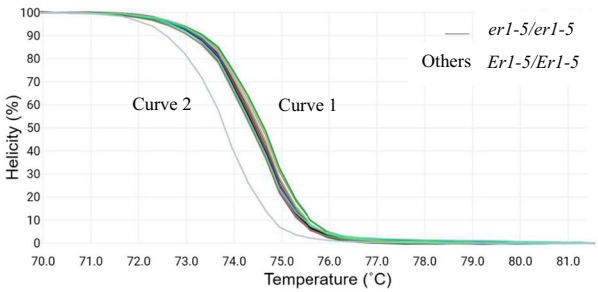

Allelic Discrimination
er1-6/er1-6
Er1-6/er1-6
Er1-6/Er1-6
RFU for Allele 2 - HEX
RFU for Allele 1 - FAM


Allelic Discrimination
er1-7/er1-7
Er1-7/er1-7
Er1-7/Er1-7
RFU for Allele 2 - HEX
RFU for Allele 1 - FAM


Allelic Discrimination
er1-10/er1-10
Er1-10/er1-10
Er1-10/Er1-10
RFU for Allele 2 - HEX
RFU for Allele 1 - FAM


Allelic Discrimination
er1-11/er1-11
Er1-11/er1-11
Er1-11/Er1-11
RFU for Allele 2 - HEX
RFU for Allele 1 - FAM





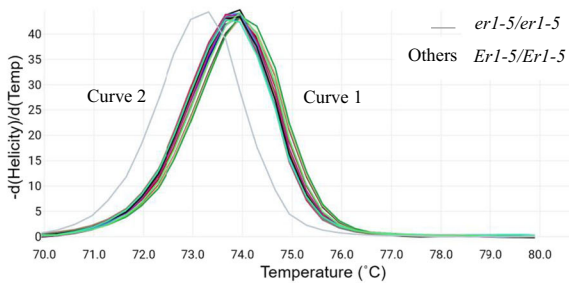


Fig. 2 Gel images of *er1-1*_S (*er1mut1*)_STS (upper left), *er1-3/XbaI*_dCAPS (upper middle), and *er1-4/AgsI*_CAPS (upper right). M1, Bioline HyperLadder™ 100 bp; M2, Bioline HyperLadder™ 50 bp; 1, PI 163126, homozygous susceptible; 2, PI 261671, homozygous susceptible; 3, PI 393488, homozygous susceptible; 4, PI 269762, homozygous susceptible; 5, PI 314795, homozygous susceptible; 6, PI 356984, homozygous susceptible; 7, JI 1559, homozygous *er1-1* resistant; 8, JI 210, homozygous *er1-3* resistant; 9, JI 1951, *er1-4* resistant; 10, JI 502, homozygous susceptible; 11–12, no template control (NTC). The lower graphs show HRM curve profiles (*er1-5/HRM54*_HRM). The lower left graph indicates the normalized melting curves, while the lower right graph displays derivative melting curves. The HRM profiles show two distinct melting curves in the pea accessions, corresponding to susceptible homozygotes (curve 1) and *er1-5*-resistant homozygote (ROI3/02) (curve 2)

polymorphism among the pea accessions used in this study. The *er1-11*_SCAR showed 183 *er1-11*/*er1-11* homozygotes while KASPar-*er1-11* indicated 195 *er1-11*/*er1-11* homozygotes (Table S2). According to the phenotypic evaluation, 17 pea accessions were found to be highly resistant to powdery mildew with a score value of 1, namely Stirling, W6 17293, PI 102888, PI 116944, PI 142775, PI 179451, PI 183467, PI 207508, PI 220174, PI 220189, PI 222071, PI 222117, PI 273605, PI 274307, PI 307666, PI 486131, and PI 628276. Among them, PI 142775 was identified as an *er1-1/er1-1* homozygote and 12 accessions were identified as *er1-11/er1-11* homozygotes (Table S2). The identification of susceptible *er1-11/er1-11* individuals indicated that both *er1-11*_SCAR and KASPar-*er1-11* cannot be regarded as functional markers, which by definition are designed on DNA sequence polymorphisms responsible for phenotypic variation (Andersen and Lubberstedt 2003). Four pea accessions were shown to be resistant to powdery mildew in the disease nursery but were rated as susceptible from the genotypic results from the KASPar markers and the functional markers. A possible explanation is that these pea accessions might carry other *er1*, *er2*, or *Er3* alleles, which were not tested in this study. To verify the species identity of the pathogen from the disease nursery, ITS sequencing was performed on six powdery mildew isolates. The sequences are listed in Table S3. From the BLAST search, all the isolates have 99 to 100% identity with *Erysiphe pisi* (accession number in NCBI: FJ378867.1). In conclusion, we predict that KASPar markers developed in this study might be powerful and valuable tools for use in pea breeding to help develop cultivars with resistance to powdery mildew.

Acknowledgments The authors thank Drs. Shimna Sudheesh, Sukhjiwan Kaur, and John W. Foster from the Centre for AgriBioscience, Bundoora, Australia for their contributions in this research.

Funding information The authors gratefully acknowledge the funding from the Washington State Department of Agriculture Specialty Crop Block Grant K1273 and USA Dry Pea and Lentil Council Research grant.

References

Andersen JR, Lubberstedt T (2003) Functional markers in plants. Trends Plant Sci 8(11):554–560

Attanayake R, Glawe D, Dugan F, Chen W (2009) *Erysiphe trifolii* causing powdery mildew of lentil (*Lens culinaris*). Plant Dis 93:797–803

Attanayake R, Glawe D, McPhee K, Dugan F, Chen W (2010) *Erysiphe trifolii*—a newly recognized powdery mildew pathogen of pea. Plant Pathol 59(4):712–720

Boutet G, Carvalho SA, Falque M, Peterlongo P, Lhuillier E, Bouchez O, Lavaud C, Pilet-Nayel ML, Rivière N, Baranger A (2016) SNP discovery and genetic mapping using genotyping by sequencing of whole genome genomic DNA from a pea RIL population. BMC Genomics 17(1):121

Dwight ZL, Palais R, Wittwer CT (2012) uAnalyze: web-based high-resolution DNA melting analysis with comparison to thermodynamic predictions. IEEE/ACM Trans Comput Biol Bioinf 9(6):1805–1811

Falloon R, Viljanen-Rollinson S, Coles G, Poff J (1995) Disease severity keys for powdery and downy mildews of pea, and powdery scab of potato. N Z J Crop Hortic Sci 23(1):31–37

Fondevilla S, Rubiales D (2012) Powdery mildew control in pea. A review. Agron Sustain Dev 32(2):401–409

Fondevilla S, Torres AM, Moreno MT, Rubiales D (2007) Identification of a new gene for resistance to powdery mildew in *Pisum fulvum*, a wild relative of pea. Breed Sci 57(2): 181–184

Gupta P, Roy J, Prasad M (2001) Single nucleotide polymorphisms: a new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. Curr Sci 80(4):524–535

Harland S (1948) Inheritance of immunity to mildew in Peruvian forms of *Pisum sativum*. Heredity 2(Pt 2):263

Heringa R, van Norel A, Tazelaar M (1969) Resistance to powdery mildew (*Erisyphe polygoni* D.C.) in peas (*Pisum sativum* L.) Euphytica 18(2):163–169

Holdsworth WL, Mazourek M (2015) Development of user-friendly markers for the *pvr1* and *Bs3* disease resistance genes in pepper. Mol Breed 35(1):1–5

Humphry M, Reinstaedler A, Ivanov S, Bisseling T, Panstruga R (2011) Durable broad-spectrum powdery mildew resistance in pea er1 plants is conferred by natural loss-of-function mutations in *PsMLO1*. Mol Plant Pathol 12(9):866–878

Neelam K, Brown-Guedira G, Huang L (2013) Development and validation of a breeder-friendly KASPar marker for wheat leaf rust resistance locus *Lr21*. Mol Breed 31(1):233–237

Pavan S, Schiavulli A, Appiano M, Marcotrigiano AR, Cillo F, Visser RG, Bai Y, Lotti C, Ricciardi L (2011) Pea powdery mildew *er1* resistance is associated to loss-of-function mutations at a *MLO* homologous locus. Theor Appl Genet 123(8):1425–1431

Pavan S, Schiavulli A, Appiano M, Miacola C, Visser RG, Bai Y, Lotti C, Ricciardi L (2013) Identification of a complete set of functional markers for the selection of *er1* powdery mildew resistance in *Pisum sativum* L. Mol Breed 31(1):247–253

Rosas JE, Bonnecarrère V, Pérez de Vida F (2014) One-step, codominant detection of imidazolinone resistance mutations in weedy rice (*Oryza sativa* L.) Electron J Biotechnol 17(2): 95–101

Rosso ML, Burleson SA, Maupin LM, Rainey KM (2011) Development of breeder-friendly markers for selection of *MIPS1* mutations in soybean. Mol Breed 28(1):127–132

Santo T, Rashkova M, Alabaça C, Leitão J (2013) 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(3):723–727

Semagn K, Babu R, Hearne S, Olsen M (2014) Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement. Mol Breed 33(1):1–14

Singh B, Singh AK (2015) Marker-assisted plant breeding: principles and practices. Springer, pp 77–122

Sudheesh S, Lombardi M, Leonforte A, Cogan NO, Materne M, Forster JW, Kaur S (2015) Consensus genetic map construction for field pea (*Pisum sativum* L.), trait dissection of biotic and abiotic stress tolerance and development of a diagnostic marker for the *er1* powdery mildew resistance gene. Plant Mol Biol Report 33(5):1391–1403

Sun S, Deng D, Wang Z, Duan C, Wu X, Wang X, Zong X, Zhu Z (2016a) A novel *er1* allele and the development and validation of its functional marker for breeding pea (*Pisum sativum* L.) resistance to powdery mildew. Theor Appl Genet 129(5): 909–919

Sun S, Fu H, Wang Z, Duan C, Zong X, Zhu Z (2016b) Discovery of a novel *er1* allele conferring powdery mildew resistance in Chinese pea (*Pisum sativum* L.) landraces. PLoS One 11(1): e0147624

Sun S, Wang Z, Fu H, Duan C, Wang X, Zhu Z (2015) Resistance to powdery mildew in the pea cultivar Xucai 1 is conferred by the gene *er1*. The Crop Journal 3(6):489–499

Warkentin T, Rashid K, Xue A (1996) Fungicidal control of powdery mildew in field pea. Can J Plant Sci 76(4):933–935

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: Guide Meth Appl 18:315–322