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Molecular Markers for Powdery Mildew in Pea (*Pisum sativum* L.): A Review

Reginah Pheirim, Noren Singh Konjengbam, Mayurakshee Mahanta

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ABSTRACT

Powdery mildew is caused by an obligate parasite *Erysiphe pisi* and considered as one of the most important constraints causing yield reductions in pea. Development and utilization of genetic resistance is acknowledged as the most effective, economic and environmental friendly method of control. Therefore, development of cultivars with improved resistance to biotic stresses is a primary goal of plant breeding programs throughout the world. Three monogenic sources *er1*, *er2* and *Er3* have been described to govern the powdery mildew disease resistance. Several markers have been reported linked to resistant genes at varying distances in different mapping populations. Genetic markers linked to the disease resistance gene make the breeding process more efficient for the use of Marker Assisted Selection (MAS) strategy to aid in obtaining a complete powdery mildew resistance in pea.

Key words: *er* genes, *Erysiphe pisi* in getting, Marker assisted selection, Molecular markers, Pea.

Field pea (*Pisum sativum* L.) is one of the most important pulse crops grown worldwide. It was considered to be one of the most thoroughly studied genetic systems in crops, next only to maize. However, in the recent era of genomics, there has been a distinct shift in the research priorities to cereals such as rice and wheat. Accelerating research in field pea is a pressing need as it is a rich source of protein and also is capable of fixing atmospheric nitrogen for enriching the soil like other pulses crops.

Field pea has several biotic and abiotic stresses related constraints in achieving potential production. Powdery mildew is one among the major constraints to pea production affecting yield and quality by causing yield reductions up to 26-47% (Munjal *et al.,* 1963). The disease not only reduces seed yield but also seed quality (Tiwari *et al*., 1997a,b). Dixon (1987) identified powdery mildew as the greatest threat to dry peas. The disease is particularly damaging when the seeds are sown late or in late maturing varieties. The earlier the disease occurs the more severe is the damage (Fondvilla and Rubiales, 2012). Furthermore, release of spores of the fungus can cause allergic and breathing problems (Ek *et al.,* 2005). Conventional method uses fungicides and cultural practice such as early planting of crop to stop the spread of disease. However, the control efficacy of chemicals and agronomic practices is limited and causes environmental pollution and health hazard. Therefore, development of powdery mildew resistant cultivars is necessary. The fungus evolves continuously to overcome host resistance and keeps plant breeder to the endless task of developing new crop varieties (Collard and Mackill, 2008). Further, introgression of genes for diseases from wild species into adapted cultivars is complicated (Foolad, 2007). For all these reasons and other problems associated with the use of conventional breeding methods, successful improvement of pea demand the employment of techniques that have higher potential for resolution like Marker Assisted Selection (MAS).

College of Post-Graduate Studies in Agricultural Sciences, (Central Agricultural University, Imphal), Umiam-793 103, Meghalaya, India.

Corresponding Author: Noren Singh Konjengbam, College of Post-Graduate Studies in Agricultural Sciences, (Central Agricultural University, Imphal), Umiam-793 103, Meghalaya, India.
Email: norensingh27@gmail.com

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Molecular markers are the key in order to implement MAS in breeding program (Choudhary *et al*., 2019). Identification of DNA markers linked to major genes such as many disease resistance genes have permitted the identification of tightly linked DNA tags for use as diagnostic tools in breeding (Vignesh *et al*., 2011). MAS has several advantageous over conventional breeding such as assisting genetic selection in early generations and accelerate the breeding process (Jha *et al*., 2021), allowing rapid introgression of disease resistance genes into susceptible varieties as well as the incorporation of multiple genes into individual lines for durable resistance. This review discusses to understand pea powdery mildew resistance genes and importance of use of molecular markers that may lead to the successful breeding for developing lines with broad resistance against powdery mildew.

Causal organism of powdery mildew

Erysiphe pisi causes powdery mildew in field pea (Fondevilla and Rubiales, 2012). Two other species *Erysiphe trifolii* (Attanayake *et al.,* 2010) and *Erysiphe baeumleri* (Ondřej *et al.,* 2005) also have been identified to infect pea plants in some regions. It is an obligate parasite and depends on the

photosynthetic status of the host and cannot develop on photosynthetically inactive tissue (Carver and Jones, 1988). *Erysiphe pisi* overwinters on infected pea debris or on alternative hosts (Falloon and Viljanen-Rollinson, 2001) and wind-dispersed the conidia locally and over long distance (Warkentin *et al*., 1996). The manifestations of powdery mildew not only depend upon the inherent qualities of resistance or susceptibility of the different genotypes, but also depend upon other physical and physiological properties such as age (Dixon, 1987), temperature (Banyal and Tyagi,1997) and duration of maturity (Singh *et al*., 1995). The conidia of *Erysiphe pisi* can germinate at wide range of relative humidity and limited temperature (Singh *et al*., 2020). Temperature plays an important role in determining powdery mildew epidemics with an ideal temperature at 20-24°C for conidia germination (Smith, 1970).

Powdery mildew usually first appears on the lowest part of the plants as small, diffuse spots on leaflets and stipules. These lesions grow and became white powdery, talcum like growth over entire aerial parts of the plant (Fig 1). In severe infection, the fungus penetrates to reach seeds causing discolouration (Chupp and Shref, 1960) and may affect the entire plant leading to premature drying (Singh *et al*., 1995). High rate of respiration and decrease in the rate of photosynthesis occurs due to reduced exposure of leaves to light under dense mycelial growth (Singh and Singh, 1983).

Genetics of powdery mildew

Genotypes resistant to powdery mildew was first described by Hammarlund (1925) from his pea collections. However, the mode of inheritance of powdery mildew resistance has been reported by Harland (1948) as a monogenic recessive trait and designated the gene as *er1* (*Erysiphe* resistance). In contrary, Heringa *et al*. (1969) reported the resistance gene *er2* in Pervuian material that was confined to leaves of pea. Sokhi *et al*. (1979) observed involvement of two different recessive genes *er1* and *er2*. Gene *er1* was reported to confer a high level of protection to all plant parts while the *er2* resistance was reported to express complete resistance only at 25°C or in mature leaves (Fondevilla *et al*., 2006, Smıkal *et al*., 2008). Genotypes exhibiting resistance with *er1* gene is reported to be complete and durable under field and controlled environments. Gene *er2* alone might be unable to provide resistance in pea whereas in combination with *er1* might enhance resistance during the growth cycle (Su *et al*., 2004). Fondevilla *et al*. (2007) reported a new dominant gene (*Er3*) in *Pisum fulvum* that segregates independently from *er1* and *er2* genes. This gene leads to high rates of cell death as a prompt response to attempted infection.

(A) (B)

Fig 1: Powdery mildew symptoms in pods (A) and in stems and stipules (B).

Resistance by *er1* is the most widely used for breeding purposes because of its complete and durable resistance (Sun *et al*., 2016). Humphry *et al*. (2011) reported that resistance by *er1* is due to a loss of function by mutation in PsMLO1, a MLO (Mildew Resistance Locus O) gene family. Many carriers of the powdery mildew resistance trait differ in their phenotype and expression indicating existence of multiple alleles at the locus (Sharma, 2016). To date, eleven *er1* alleles (*er1*-1 to *er1*-11) have been identified and characterized in pea resistant germplasm, which were produced by natural or artificial mutagenesis (Pavan *et al*., 2011, Pavan *et al*., 2013, Sun *et al*., 2016, Ma *et al*., 2017, Sun *et al*., 2019). The first allele *er1*-1 produced by spontaneous mutation, reported by Harland (1948) has been almost exclusively used in breeding as it imparts strong and reliable resistance in all regions of the world.

Mechanism of powdery mildew resistance in pea

Conidia of *Erysiphe pisi* in susceptible pea genotypes are spread during cool nights and dry winter. Conidia germinates forming a germ tube with a lobed primary appressorium. A penetration peg emerges from the appressorium and penetrates the epidermal host cells through the cuticle and cell wall leading to formation of a biotrophic haustorium within the epidermal cell. Nutrient extracts from the plant cell through the haustorium supports growth of secondary hyphae that radiate across the host epidermis forming hyphal appressoria from which secondary haustoria are formed. Finally, aerial conidiophores emerge from surface hyphae producing conidia capable of initiating a new cycle of infection (Falloon *et al*., 1989). In resistant pea genotypes harbouring *er1* gene, the vast majority of *Erysiphe pisi* conidia germinates and form appressoria. However, no secondary hyphae are formed (Fondevilla *et al*., 2006). Resistance of *er1* was due to avoidance of epidermal cell penetration while *er2*-mediated resistance is mainly based on reduction in penetration success complemented by post-penetration cell death. It is due to the occurrence of hypersensitive response in established colonies (Fondevilla *et al*., 2006). A proteomic study showed that resistant genotypes has higher amount of proteins involved in defence than susceptible genotypes (Curto *et al*., 2006). These proteins are proteins encoded by NBS-LRR resistance genes, PR1 and PR5, Kunitz–trypsin inhibitor that inhibit extracellular fungal proteinases, proteins associated with cell wall reinforcement, proteins involved in tolerance to oxidative stress caused by reactive oxygen species and

proteins implicated in the synthesis of alkaloids compounds (Fondevilla and Rubiales, 2012). In resistant genotypes conferred by *Er3* gene, most conidia are able to penetrate the epidermal pea cells and form secondary hyphae but a strong hypersensitive response stopped the growth of these established colonies. In the case of other uncharacterized sources of incomplete resistance, different mechanisms can contribute to the reduction of disease severity. Complete resistance can be obtained when hypersensitive response occurs fast and in a high proportion of colonies, as in lines harbouring *Er3* gene, or slower and lower proportion of established colonies may result in incomplete resistance (Fondevilla *et al.,* 2007a, b).

Molecular markers for disease resistant gene

Development of cultivars with improved resistance to biotic stresses is a primary goal of plant breeding programs throughout the world. Disease resistant cultivars can reduce both the risk of yield loss and the dependence on pesticides, enabling a more stable crop production (Miklas *et al*., 2006). Advance in genome research and molecular biology has led to the use of DNA markers in plant breeding and crop improvement. Molecular markers linked to resistance genes can obviate the need for field testing of genotypes to identify resistant individuals in early generations of breeding populations and also reducing the number of progeny maintained. It helps to understand complex traits, dissect into single Mendelian components and establish their chromosomal locations using linkage maps and/or cytogenetic stocks (Torres *et al*., 2010). Molecular markers are specific fragments of DNA that can be identified within the whole genome found at specific locations of the genome. They are used to 'flag' the position of a particular gene or the inheritance of a particular character. These are considered valuable tools for crop improvement. The potential usefulness of genetic markers as screening tools in plant breeding was recognized by Sax (1923). Molecular markers command several advantages over morphological markers because of their availability in large numbers, no penetrance and expressivity problem, phenotypically neutral in nature, environmentally neutral, tissue and stage independent expression, rapid screening and applicability across the species (Chopra, 1996). Among the markers, the PCR-based markers are more desirable as it requires small amount of template DNA and can be applied efficiently to large populations. Development of markers from the gene itself is desirable as crossovers between markers and gene of interest may occur if the marker is not tightly linked to gene (Huang and Roder, 2004). RFLPs (Restriction Fragment Length Polymorphisms) are reliable and yield co-dominant data, but are time-consuming and expensive, requiring relatively large amount of highly purified DNA and they do not lend themselves to automation (Gupta *et al*., 2001). RAPD (Random-Amplified Polymorphic DNAs) markers are unreliable with poor replication success among laboratories (Penner *et al.* 1993, Hallde *et al.* 1996). SCAR (Sequence Characterized Amplified Regions) markers are more reliable, but are developed from RAPD markers which limit their utility (Paran and Michelmore, 1993). The dominant nature of AFLP (Amplified Fragment Length Polymorphisms), RAPD and STS (Sequence-Tagged Sites) markers limits to identify heterozygous individuals from segregating populations. SSR (Simple Sequence Repeats) markers, on the other hand, combine reliability and genomic abundance with high levels of polymorphism and allow detection of heterozygotes (Mohan *et al.,* 1997). They do not require sophisticated DNA extraction methods and are ideally suited for high throughput automated scoring and multiplexing (Tang *et al.,* 2002). Genotypic screening of a single gene trait in maize using SSR markers was economically profitable compared to conventional phenotypic screening methods (Dreher *et al.,* 2003, Yu *et al.,* 2000). To ensure optimal cost-effectiveness, molecular markers used for marker assisted selection (MAS) should both permit efficient screening of large populations and show a high degree of reproducibility across laboratories (Mohan *et al*., 1997). The main drawback of SSRs is the initial identification of primer sites to amplify SSR loci, a procedure which is time and resource demanding. Despite this, SSR markers had pervaded the molecular genetics and plant breeding studies until recently whose hegemony was eventually broken by SNP (Single nucleotide polymorphism) markers. SNPs were proved to be universal and most abundant forms of genetic variation among individuals of the same variation providing a dense coverage of the genome for high-resolution mapping of disease resistance (Sun *et al*., 2015b, Jha *et al*., 2016, Sun *et al*., 2019). Development of Next Generation Sequencing (NGS) technologies over the last decade allows rapid inexpensive SNP discovery within genes and avoids highly repetitive regions of a genome (Morozova and Marra, 2008). Two main types of SNP-based markers are CAPS (Cleaved Amplified Polymorphic Sequences) and dCAPS (derived Cleaved Amplified Polymorphic Sequences) (Parsons *et al*., 1997, Neff *et al*., 1998). CAPS are PCR-RFLP markers combined with specific primers, after the digestion of restriction enzymes to detect polymorphism that cannot be directly detected by PCR amplification (Konieczny and Ausubel, 1993, An *et al*., 2021). There are several widely used SNP genotyping platforms among which Kompetitive Allele Specific PCR (KASP) is one that has evolved to be a global benchmark technology (Semagn *et al*., 2014). Genotyping-by sequencing (GBS) has also been widely used for genotyping mapping population for GWAS and QTL analysis (Elshire *et al.* 2011). Other types of markers based on next generation sequencing and array hybridization such as DArT (Diversity Arrays Technology) combined with NGS (DArTseq™) were also used for high throughput genotyping that can generate a greater number of markers at relatively low cost (Kilian *et al.,* 2012).

MAS is viewed as a promising approach to resistance breeding ever since the advent of the first DNA markers. Today, the most successful applications of MAS in plant breeding have been for major disease resistant genes.

Application of MAS requires selection of markers in the vicinity of the resistance genes, investigation of markers in breeding programs and their linkage with resistance genes. Another important factor in MAS program is the cost associated in screening. To ensure optimal cost-effectiveness, molecular markers used for MAS should both permit efficient screening of large populations and show a high degree of reproducibility across laboratories (Mohan *et al.,* 1997). Using the marker maps, putative genes affecting traits of interest have been detected by testing for statistical associations between marker variants and traits (Paterson *et al.,* 1991). Following their identification, useful genes or QTLs can be introgressed into desirable genetic backgrounds using markers which are physically located close to or even within genes of interest. The application of MAS for introgression of genes from one donor to another recurrent genotype through a backcross breeding illustrates the great advantages of the use of molecular markers for indirect selection of traits which would otherwise be difficult to select by standard procedures.

Molecular markers for powdery mildew in pea

The DNA markers linked to resistance genes provide an alternative to disease screening of powdery mildew resistance genes. DNA markers can be used to confirm the presence of multiple resistance genes in the backcross since they are not affected by epistatic interactions. As the pathogen, *Erysiphi pisi* is an obligate parasite, its culture and maintenance are difficult. Molecular marker technology has reduced our dependence on conventional breeding (Tanksley, 1983). Markers tightly-linked to disease resistance genes have been developed for many crops and used successfully in breeding programmes. Some examples of molecular markers linked to powdery mildew resistance gene in various crops other than pea are listed in Table 1.

In pea, RFLP (Dirlewanger *et al.*, 1994), RAPD and SCAR (Timmerman *et al*., 1994, Tiwari *et al*., 1998, Fondevilla *et al.,* 2008, Pereira *et al*., 2010, Srivastava *et al*., 2012) as well as SSR (Loridon *et al.,* 2005*,* Katoch *et al.,* 2010*,* Reddy *et al.,* 2015*,* Cobos *et al.,* 2018) markers have been linked to powdery mildew resistance and reported for the *er1*, *er2* and *Er3* genes. Functional markers corresponding to the *er1* alleles were developed to aid marker assisted selection (Pavan *et al*., 2011, Pavan *et al*., 2013, Sun *et al*., 2019). This provide a powerful tool for breeders, overcoming limitations of previously reported er*1* linked markers due to occurrence of the recombination with resistance locus and/or the lack of polymorphism between parental genotypes. Genetic markers showing linkage to *er1*, *er2* and *Er3* genes as well as functional markers of *er1* alleles in *Pisum sativum* is presented in Table 2.

Sarala (1993) and Timmerman *et al.* (1994) located the powdery mildew resistance gene *er1* to pea linkage group VI using morphological and molecular markers, respectively. Dirlewanger *et al.* (1994) located the *er1* gene at 9.8 cM distance from p236, a RFLP marker. Timmerman *et al.* (1994) reported a RAPD marker, $OPD10_{650}$, at 2.1 cM distance from the *er1* gene, which is a more closely linked marker. The RAPD marker was converted to a SCAR marker which Janila and Sharma (2004) mapped at a distance of 3.4 cM from the *er1* gene. Tiwari *et al.* (1998) identified a RAPD/SCAR marker, $Sc\text{-}OPO\text{-}18_{1200}$, which showed complete linkage to *er1* gene (*i.e.* distance of the marker from *er1* gene was 0.0 cM). Ek *et al.* (2005) developed five

Table 1: Molecular markers linked to powdery mildew resistance gene in various crops.

Source	Causal organism	Gene/allele	Marker	References
Common bean (*Phaseolus vulgaris*)	*Erysiphe*	Two major genes	RAPD, AFLP	Rezende *et al.,* 1999, Johnson *et al.* 1995
Mung bean (*Vigna radiata*)	*Erysiphe polygoni* DC	Two genes (*PMR1*, *PMR2*)	RAPD, RFLP, AFLP, ISSR and ISSR-RGA	Humphry *et al.,* 2003, Chattieng *et al.*, 2002, Miyagi *et al.*, 2004, Tantasawat *et al.*, 2021
Soybean (*Glycine max*)	*Erysiphe*	Single gene (*Rmd*)	RFLP, RAPD, SSR	Lohnes and Bernard 1992, Polzin *et al.,* 1994, Kang and Mian 2010
Cow pea (*Vigna unguiculata* L.)	*Erysiphe polygoni* DC	*Vu-Pm1*	SSR	Wu *et al*., 2014
Common Wheat (*Triticum aestivum*)	*Erysiphe* DC	Multiple loci (more than 30 loci)	RAPD, SSR,SCAR, AFLP,RFLP, CAPS	Huang *et al.,* 2000a, Chantret *et al.,* 2001; Lambreghts *et al*., 2009
Oat (*Avena sativa*)	*Blumeria graminis*	Multiple loci	SCAR, RAPD, Silico DArT	Okoń and Kowalczyk., 2012, Ociepa *et al.,* 2020
Wild tomato (*Lycopersicon parviflorum*)	*Oidium lycopersici*	Two	AFLP, RFLP, CAPS	Bai *et al.,* 2003, Huang *et al.,* 2000b
Apple (*Malus pumila* Mill.)	*Podosphaera Leucotricha*	Single gene (*Pl-w*)	Isozymes, SCAR, SSR, AFLP, RAPD	Evans and James 2003, Liebhard *et al.,* 2002, Hemmat *et al.,* 1994, Batlle and Alston, 1996
Grapes (*Vitis vinifera*)	*Erysiphe necator*	Multiple locus	STS, RAPD, AFLP, CAPS	Dalbó *et al.,* 2001

Table 2: Genetic markers showing linkage to the *er1*, *er2* and *Er3* genes inpea.

Marker	Gene	Marker type	Sequence information	References
AD60	*er1*	SSR	F-CTGAAGCACTTTTGACAACTAC R-CTCATTCAATGATGATAATCCTA	Ek *et al.*, 2005
AA374	*er1*	SSR	F-GTCAATATCTCCAATGGTAACG R-GCATTTGTGTAGTTGTAATTTCAT	Ek *et al,* 2005
A5	*er1*	SSR	F-GTAAAGCATAAGGGGATTCTCAT R-CAGCTTTTAACTCATCTGACACA	Ek *et al.,* 2005
AA369	*er1*	SSR	F-CCCTTCGCACACCATTCTA R-AGTCGTTTTGGAGATCTGTTCA	Ek *et al.*, 2005
AD51	*er1*	SSR	F-ATGAAGTAGGCATAGCGAAGAT R-GATTAAATAAAGTTCGATGGCG	Ek *et al.,* 2005
Sc-OPO-18$_{1200}$	*er1*	SCAR	F-CCCTCTCGCTATCCAATCC R-CCTCTCGCTATCCGGTGTG	Tiwari *et al.,* 1998
ScOPD-10$_{650}$	*er1*	SCAR	F-GGTCTACACCTAAACAGTGTCCGT R-GGTCTACACCTCATATCTTGATGA	Janila and Sharma, 2004
ScOPE16$_{1600}$	*er1*	SCAR	F-GGTGACTGTGGAATGACAAA R-GGTGACTGTGACAATTCCAG	Tiwari *et al.*, 1998
ScAH1R	*er1*	SCAR	F-GATGGACCCCATCAAGTAC R-GCCCCAACTTCATGTCTTG	Pereira *et al.*, 2010
ScOPO06$_{1100}$	*er1*	SCAR	F-CCCCATGTTAGAACCTTGCA R-ACGGGAAGGTCTGACAGTAT	Pereira *et al.*, 2010
ScOPL13$_{990}$	*er1*	SCAR	F-ACCGCCTGCTCTGATGTG R-GCGCTGCTTAATCTCAGG	Pereira *et al.*, 2010
ScAGG.CAA$_{125}$	*er1*	SCAR	F-GAATTCAGGAACATAGCTTC R-CAAGCTAAAAGTCAGAAGAT	Pereira *et al.*, 2010
ScOPT16$_{480}$	*er1*	SCAR	F-GGGCAGAATCAGCTGAGCTC R-GAACAAGGAGAAGAAGAGG	Pereira *et al.*, 2010
ScOPX 04$_{880}$	*er1*	SCAR	F-CCGCTACCGATGTTATGTTTG R-CCGCTACCGAACTGGTTGGA	Srivastava *et al.,* 2011
ScX17$_{1400}$	*er2*	SCAR	F-GGACCAAGCTCGGATCTTTC R-GACACG GACCCAATGACATC	Katoch *et al.*, 2010
AD141	*er2*	SSR	F-AATTTGAAAGAGGCGGATGTG R-ACTTCTCTCCAACATCCAACGA	Katoch *et al.*, 2010
AA278	*er2*	SSR	F-CCAAGAAAGGCTTATCAACAGG R-TGCTTGTGTCAAGTGATCAGTG	Katoch *et al.*, 2010
AC30	*er2*	SSR	F-GCAGCAAGAGTGACGAAGTTATC R-GCCTGACTACCACTTCTGCTG	Katoch *et al.*, 2010
ScAB1$_{874}$	*Er3*	SCAR	F-CCGTCGGTAGTAAAAAAAACTA R-CCGTCGGTAGCCACACCA	Fondevilla *et al.*, 2008
ScW4$_{637}$	*Er3*	SCAR	F-CAGAAGCGGATGAGGCGGA R-CAGAAGCGGATACAGTACTAAC	Fondevilla *et al.*, 2008
AD61	*Er3*	SSR	F-CTCATTCAATGATGATAATCCTA R-ATGAGGTACTTGTGTGAGATAAA	Cobos *et al.*, 2018
AA349	*Er3*	SSR	F-ACCATGAATCCCATATAGAGAG R-GTTTGATCCCAATATCTTACCA	Cobos *et al.*, 2018
er1-1/AsuHPI-B	*er1*-1	CAPS	AGGTTTGCAAGGGACACAAC TGAAGAAGCTAACCTGATTCAACC	Pavan *et al.,* 2013
er1-2/MGB	*er1*-2	STS	CCAAAGGAGGGAAAGGAAAC GGAGCAGGTGACAGGAGAC	Pavan *et al.,* 2013

Table 2: Continue...

Table 2: Continue...

er1-3/XbaI	*er1*-3	dCAPS	CAATTGAAGAGGATTTTAAAGTTGTTCTAG GCCAGATAGTTGGACTGCAAG	Pavan *et al.,* 2013
er1-4/AgsI	*er1*-4	CAPS	GCTGTTGCAGTTGTGTGTCTT GAACAAGGATGCCAAGTTGA	Pavan *et al.,* 2013
er1-5/HRM54	*er1*-5	HRM	GATGAGGAAGTGGAAGACTT AATTGATATTCAACTGTTCTTGTC	Pavan *et al.,* 2013
GIM-300/SmlI	*er1*-5	CAPS	F-TCTGCATATGGAATTCACCAA R-AATTGATATTCAACTGTTCTTGTC	Pavan *et al.*, 2011
SNP1121	*er1*-6	SNP	F-CTGGAGATCACCTTTTCTGGTT R-CATGTACAAACACACATACACACG	Sun *et al.*, 2016
InDel111–120	*er1-7*	InDel	F-GGAGTTAAGGAACGAACTTTGG R-CCATGTCTGCGTCTGTATCTTT	Sun *et al.*, 2016
KASPar-*er1*-1	*er1*-1	KASPar marker	F-C:CCCTTACAATCCATAACAAAATAGGTG F-G: CCCTTACAATCCATAACAAAATAGGTC Common R: TTTGCAAGGGACACAACATTTGGAAGAA	Ma *et al.*, 2017
KASPar-*er1*-3	*er1*-3	KASPar marker	F-G: GTATATTTAATCTTAAGTCACACCTTATTCC F-N/A: AGTATATTTAATCTTAAGTCACACCTTATTCT Common R: AGATCAATTGAAGAGGATTTTAAAGTTGTT	Ma *et al.*, 2017
KASPar-*er1*-4	*er1*-4	KASPar marker	F-A: GTGTCTTGTGTTGCTAGCTGTTTCAA F-N/A: GTGTCTTGTGTTGCTAGCTGTTTCAT Common R: TAGAACGAACCATGCTTAGCTTACCTTT	Ma *et al.*, 2017
KASPar-*er1*-5	*er1*-5	KASPar marker	F-G: ATTCAACTGTTCTTGTCTCATCTTCC F-A: GATATTCAACTGTTCTTGTCTCATCTTCT Common R: TTTCTTCAGATGAGGAAGTGGAAGACTT	Ma *et al.*, 2017
KASPar-*er1*-6	*er1*-6	KASPar marker	F-T: TTGAAGTTACCTGAAAGAGAACAA F-C: CTTTGAAGTTACCTGAAAGAGAACAG Common R: GTCCTCACCTTCTTCTCTTCACGAT	Ma *et al.*, 2017
KASPar-*er1*-7	*er1-7*	KASPar marker	F-TCATGTTATT:AGCTGTTTCAATCTTAATTGAACATATTATT F-N/A: AGCTGTTTCAATCTTAATTGAACATATTATG Common R: ATAGAACGAACCATGCTTAGCTTACCTTT	Ma *et al.*, 2017
KASPar-*er1*-10	*er1*-10	KASPar marker	F-G: TACAATTAGTGGAAGAAATGGAAGC F-A: GCTTACAATTAGTGGAAGAAATGGAAGT Common R: GTTATATGGGCAGGGTGGTATTCTTATTA	Ma *et al.*, 2017
KASPar-*er1*-11	*er1*-11	KASPar marker	F-N/A: ATGCAAATCTCATGCGCGTGTGTA F-GA: GCAAATCTCATGCGCGTGTGTG Common R: TCAGGATTCAAGATGAGATTCATGTACAAA	Ma *et al.*, 2017
InDel-*er1*-8	*er1*-8	InDel	GTTTTGACTGATATGACAGATGGGA GTTTGTAGACTGTCGCTGTTTCC	Sun *et al.*, 2019
KASPar-*er1*-8	*er1*-8	KASPar marker	F-TGG: TGGCAACAGCGCTTAAGAACTGG F: GAGCAACAGCGCTTAAGAACTGG Common R:TGGTTGGTTTCATGGTTGATCCCATC	Sun *et al.*, 2019
KASPar-*er1*-9	*er1*-9	KASPar marker	F-T: TTTTGTTATATGGGCAGGGTGGTATT F: TGTTATATGGGCAGGGTGGTATC Common R:CAAAATGTAGATTATGCTTACAATTAGTGGA	Sun *et al.*, 2019

Table 3: Some sources of powdery mildew resistance of each gene in pea.

Sources	Gene	Origin	Reference
LE 25, ATC 823, Arka Ajit	*er1*	India	Liu *et al.*, 2003, Reddy *et al.,* 2015
JI2480	*er2*	UK	Katoch *et al.,* 2010
Pisum fulvum	*Er3*	Wild relative of pea	Fondevilla *et al.*, 2007

SSR markers linked to *er1* gene covering the regions extended over 49.9 cM. The distance between the *er1* gene and the most closely linked marker (PSMPSAD60) was 10.4 cM. A linkage map of the *er* gene region is found to be located on chromosome VI. Other closely linked SSR markers were AD60, AA374 and A5 at 10.4, 11.6 and 14.9 cM distance, respectively. However, these distances are most likely too large to use in MAS since differentiation of pea germplasm with respect to powdery mildew response would require very large screening populations (Ribaut *et al.,* 2002). When single markers are too distant to the gene of interest, two flanking markers can be used in combination (Werner *et al.,* 2000). *er1* gene was flanked by the markers, AD60 and A5 and use of both markers for selection of resistant plants is estimated to result in 1.6% error in selection (Ek *et al.,* 2005). Tonguc and Weeden (2010) reported the *er1* locus in a position between two markers, BC210 and BA9, at a distance of 8.2 cM from the marker BC210.

Five markers generated the linkage map of *er2* region that covered 51.9 cM and the gene was assigned to LG III, a position different from that reported for *er1* (Katoch *et al.,* 2010). The gene *er*2 was bracketed between the RAPD markers OPX17_1400 and OPY17_550 located at a distance of 2.6 and 21.3 cM, respectively while SSR markers AD141, AA278 and AC30 were reported to be located at a distance of 9.3, 17.9 and 30.6 cM, respectively. Molecular markers linked to *Er*3 gene were located in pea LG IV. SCAR marker $SCW4_{637}$ co-segregate with the resistant gene allowing detection of all the resistant individuals and $SCAB1_{874}$, in repulsion phase with *Er*3 was located at 2.8 cM from the gene. The combined use of $SCW4_{637}$ and $SCAB1_{874}$ allowed the accurate identification of 100% of the resistant plants and was highly efficient in discriminating homozygous and heterozygous resistant plants (Fondevilla *et al.,* 2008). SSR markers AA349 and AD61 were linked to *Er3Er*3 gene and the gene was located in pea LG IV at 0.39 cM downstream of marker AD61 (Cobos *et al.,* 2018). The gene *Er*3 is needed to characterize for utilization to develop pea cultivars resistant to powdery mildew to broaden the genetic horizon of pea as the gene has been reported to be successfully introduced into cultivated pea (Fondevilla *et al.,* 2011, Fondevilla *et al*., 2007, Bobkov and Selikhova, 2021). Moreover, *er1* might not be effective against *Erysiphe baeumleri* and *Erysiphe trifolii* (Fondevilla and Rubiales, 2011).

Functional markers developed for selection of *er1* alleles (*er1*-1 to *er1*-11) includes sequence-tagged site (STS), high- resolution melting (HRM), CAPS and dCAPS. Pavan *et al*. (2011) developed CAPS marker GIM-300/SmlI on the mutation site for *er1*-5 which is associated with a loss-of-function G-A substitution in the PsMLO1 coding sequence. Further, Pavan *et al*., 2013 developed CAPS marker *er1*-1/ AsuHPI-B for *er1*-1 allele, STS marker *er1*-2/ MGB for *er1*-2, derived CAPS (dCAPS) marker *er1*-3/XbaI for *er1*-3 and high resolution melting (HRM) marker *er1*-5/ HRM54 for *er1*-5 for using in developing resistant cultivar. HRM is a technique developed for scanning mutations, SNP detection and genotyping to determine the dissociation behaviour of PCR amplicons (Erali *et al*., 2008).

SNP marker SNP1121 was developed using High Resolution Melting (HRM) technique whose forward and reverse primers were located at 11th exon and 11th intron of the PsMLO1 gene, respectively. SNP1121 successfully distinguished resistant pea landraces carrying the *er1*-6 allele from other *er1* alleles as well as susceptible genotypes (Sun *et al*., 2015a). Sun *et al*., 2016 obtained another functional marker, InDel111-120 located in exon 1 and intron 1 of PsMLO1, specific for *er1*-7 allele which has a 10-bp deletion in position 111-120.

KASPar (Kompetitive allele-specific PCR) assay which is a SNP genotyping system based on fluorescence was deployed by Ma *et al.,* 2017 and proved as an efficient and robust tool for pea breeding. He developed eight KASPar markers *viz*., 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 for *er1* alleles namely, *er1*-1, *er1*-3, *er1*-4, *er1*-5, *er1*-6, *er1*-7, *er1*-10 and *er1*-11 and validated as markers which are breeder-friendly one. The co-dominant functional markers specific to *er1*-8 (InDel-*er1*-8 and KASPar-*er1*-8) and *er1*-9 (KASPar-*er1*-9) were developed by Sun *et al*., 2019. All these functional markers allows rapid identification and characterization of *Erysiphi pisi* resistant alleles at the *er1* locus in pea germplasm. Therefore, such markers aid in marker assisted selection in pea breeding for developing powdery mildew resistance cultivars. Some important sources for each gene are listed in Table 3.

CONCLUSION

The gene *er1* has been widely used in breeding programs but harboring the same resistance source could likely enhance the occurrence of new races of the same pathogen that ultimately will lead to a breakdown of the resistance. A durable resistance can be obtained by combining several major genes from different sources into a variety. If *er1* gene fails to limit the colony establishment, the hypersensitive response of *er2* or *Er3* gene causes death of the established colonies providing a double barrier to disease development. This strategy would provide a complete resistance of pea to powdery mildew disease. This breeding strategy could be aided by the use of the available and more precise molecular markers linked to the powdery mildew resistant genes. Screening for resistance using marker assisted selection needs to be coupled with artificial disease inoculation techniques such as leaf disk assay, detached leaf assay to save time and resources for cultivar development. Moreover, advancement in the use of high throughput next generation sequencing (NGS) and genotyping technologies, the cost of development of molecular markers has been reduced considerably leading to Marker Assisted Breeding more broad, useful, efficient as well as cost-effective in future.

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