DEVELOPMENT AND VALIDATION OF CO-DOMINANT MARKERS FOR PEA POWDERY MILDEW RESISTANCE GENE *er2*

THESIS

By

UJWAL SOOD

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Submitted to

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MASTER OF SCIENCE IN AGRICULTURE

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Dedicated to my Parents

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CERTIFICATE – I

This is to certify that the thesis entitled **"Development and validation of co-dominant markers for pea powdery mildew resistance gene *er2*"** submitted in fulfilment of the requirements for the award of the degree of **Master of Science (Agriculture)** in the discipline of **Agricultural Biotechnology** of Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Ujwal Sood (Admission No. A-2021-30-067)** daughter of **Sh. Bharat Raj Sood** and **Smt. Rajni Sood** under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

Place: Palampur
Dated:

(**Dr. Rajeev Rathour)**
Major Advisor

CERTIFICATE- II

This is to certify that the thesis entitled **"Development and validation of co-dominant markers for pea powdery mildew resistance gene *er2*"** submitted by **Ms. Ujwal Sood (Admission No. A-2021-30-067)** daughter of **Sh. Bharat Raj Sood** to the Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur in partial fulfillment of the requirements for the degree of **Master of Science (Agriculture)** in the discipline of **Agricultural Biotechnology** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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Place: Palampur
Date: June, 2024 ***(Ujwal Sood)***

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LIST OF ABBREVIATIONS USED

Sr. No.	Abbreviation	Meaning
1.	@	At the rate of
2.	%	Percent
3.	µl	Microlitre
4.	µM	Micromolar
5.	BLAST	Basic Local Alignment Search Tool
6.	CAPS	Cleaved Amplified Polymorphic Sequence
7.	cM	Centi Morgan
8.	CTAB	Cetyl Trimethyl Ammonium Bromide
9.	dai	Days after inoculation
10.	DNA	Deoxyribonucleic acid
11.	dNTP	Deoxyribonucleoside Triphosphate
12.	EDTA	Ethylene Diamine Tetra Acetic Acid
13.	et al.	And others
14.	gm	Grams
15.	GB	Giga base pairs
16.	h	Hours
17.	HCl	Hydrochloric acid
18.	i.e.	Id Est.
19.	Kb	Kilo base
20.	KCl	Potassium Chloride
21.	L	Litre
22.	LG	Linkage group
23.	M	Molar
24.	MAS	Marker-assisted selection
25.	Mg	Milligram
26.	MgCl2	Magnesium Choride
27.	Min	Minutes
28.	ml	Milliliter
29.	mm	Millimeter
30.	mM	Millimolar
31.	N	Normal
32.	NaCl	Sodium Chloride
33.	NaOH	Sodium Hydroxide
34.	ng	Nanogram
35.	ºC	Degree Celsius

36.	PCR	Polymerase Chain Reaction
37.	pH	Puissance de hydrogen (Ion Conc.)
38.	PVP	Polyvinylpyrrolidone
39.	QTL	Quantitative Trait Locus
40.	RAPD	Random Amplified Polymorphic DNA
41.	RNase A	Ribonuclease A
42.	SCAR	Sequence Characterized Amplified Region
43.	SNP	Single nucleotide polymorphism
44.	SSR	Simple Sequence Repeats
45.	STS	Sequence Tagged Site Markers
46.	TAE	Tris-acetate EDTA buffer
47.	Taq	*Thermus aquaticus* DNA polymerase
48.	Tris	Tris (Hydroxy Methyl) amino methane
49.	UV	Ultra Violet
50.	Viz.	Namely

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Title of the research project:	:	Development and validation of co-dominant markers for pea powdery mildew resistance gene *er2*
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ABSTRACT

In present study attempts were made to develop codominant markers for a recessive powdery mildew resistance gene *er2*. For this purpose, pea genome sequences around a previously known *er2* linked SCAR marker ScX17_1400 were chosen for developing a set of ten STS markers. All the ten STS markers developed from a 1038 kb sequence around the region of ScX17_1400 marker were monomorphic in pea genotypes Azad P-1 and JI2480 and therefore were not immediately useful as markers. Of the ten STS markers five namely, PsLG3STS-1, PsLG3STS-2, PsLG3STS-3, PsLG3STS-4 and PsLG3STS-6 could be successfully converted into Cleaved Amplified Polymorphic Sequence (CAPS) markers after digestion with restriction enzymes HpaII, ScaI and HaeII, DraI, HaeIII and TaqI, and TaqI, respectively. The polymorphic CAPS markers developed during the present study and two pea LG3 specific SSR markers, AA278 and AA5, exhibited close linkage to *er2* when tested on 110 homozygous recessive powdery mildew resistant BC_7F_2 plants of cross Azad P-1 x JI2480. The linkage map of *er2* locus constructed with these six markers spanned 5.9 cM. The SSR marker AA278 was most closely linked to the *er2* gene at 1.8 cM while the CAPS-ScX17 was the next closely linked marker situated at a genetic distance of 2.7 cM from the gene. The three cosegregating markers PsLG3_CAPS-1, PsLG3_CAPS-3 and PsLG3_CAPS-4 were located at a distance of 3.2 cM from the gene. The codominant markers exhibited polymorphism between *er2* donor line JI2480 and 6 to 22 commercial pea varieties tested. The SSR AA278 was the most polymorphic marker differentiating 22 pea varieties from JI2480. The CAPS markers PsLG3_CAPS-1, PsLG3_CAPS-3 and PsLG3_CAPS-4 showed polymorphism in 13, 15 and 16 commercial pea varieties, respectively. The codominant markers developed in present study collectively differentiated 26 commercial pea varieties from JI2480 thus suggesting their wide applicability in marker-assisted selection of *er2* gene.

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1. INTRODUCTION

Pea (*Pisum sativum* L.; 2n = 2x = 14) is one of the most important cool season legumes consumed both as a vegetable and as a pulse. There are two types of cultivated pea, i.e., garden pea and field pea. Garden peas are harvested in an immature condition and cooked as fresh or canned for subsequent uses. Peas are low in fat but high in fiber, protein, vitamin C, ß-carotene, thiamine, riboflavin and iron content, thereby making it a healthy food capable of meeting the global dietary needs of over 900 million undernourished people (Devi et al. 2022). Garden pea is an important nitrogen-fixing vegetable crop that contributes significantly to sustainable cropping systems and helps to preserve soil health and productivity (Rana et al. 2023).

Globally, the garden pea is grown over an area of 2.53 million hectares (mha) with a production of 19.86 million tonnes (mt) (FAO 2020). India ranks second next to China both in terms of area and production with total area and production of 0.56 million ha and 5.70 million tonnes, respectively **(**FAO 2020**)**. In north-western Himalayan region of India, particularly Himachal Pradesh, pea is the most widely grown vegetable with a production of 3,28,804 metric tonnes (Anonymous, 2020). A great variety of agro-climatic conditions enable Himachal Pradesh to produce pea crop through-out the year and thus brings handsome income to the growers of the State.

Pea productivity is constrained by numerous fungal pathogens of which powdery mildew (PM) caused by *Erysiphe* species (order Erysiphales, family Erysiphaceae) is the major one. Until recently, *E. pisi* was thought to be the only causal agent of powdery mildew in pea. Recently, a new powdery mildew species, *E. trifolli*, infecting pea under greenhouse and fields has been reported from the USA, Spain and India (Attanayake et al. 2010; Fondevilla et al. 2013). The disease first appears as small, round, whitish or grey spots on the top of mature leaves. All aerial parts of the plant may subsequently become covered with white powdery film resulting in withering of whole plant. The disease can cause 25-50% yield losses, reducing total yield biomass, number of pods per plant, number of seeds per pod, plant height and number of nodes (Ghafoor et al. 2012). In Himachal the disease occurs every year in the month of March and April in mid-hills and August to September in higher hills causing considerable reduction in yield and quality of crop. This disease also occurs in serious proportions in other States of India and reductions in pea yield to the tune

of 26-47 per cent of pod yield have been attributed to this disease (Munjal *et al.*, 1963; Nene, 1988).

Although a range of protective and systematic fungicides to control the powdery mildew is available in the market (Singh and Shekhar 2020), the chemical control remains the least preferred option due to associated economic and health concerns. The breeding of powdery mildew resistant cultivars remains the most effective means of controlling the disease. Several sources of powdery mildew resistance have been identified among pea germplasm screened by various workers (Heringa et al. 1969; Tiwari et al. 1997; Vaid and Tyagi, 1997) and till date two recessive powdery mildew resistance genes, *er1* and *er2* have been identified from pea (Harland 1948; Heringa et al. 1969). Recently, one dominant gene *Er3* has been identified in *Pisum fulvum* and has been successfully transferred into pea by cross breeding (Fondevilla et al. 2007). The *er1* gene is reported to confer complete resistance to powdery mildew infection by offering resistance to pathogen penetration, while the resistance imparted by *er2* present in pea accession 'JI2480' results in reduction in penetration success complemented by post-penetration cell death. Although the *er1* resistance has been widely used in pea breeding for past six decades, recent reports suggest that the gene is not effective against *E. trifolli* (Fondevilla et al. 2013), while *er2* resistance is effective against both the species of the pathogen. The susceptibility of *er1* harbouring lines that are resistant to powdery mildew isolates from dry-temperate zone has also been recently noted under Palampur conditions, while the *er2* harbouring lines were resistant to powdery isolates from both the regions (R. Rathour, personal communication). These results suggest that *er2* gene can be effectively used in pea breeding to provide broader protection against powdery mildew. The gene can be also effectively combined with other powdery resistance genes to provide broad-spectrum and durable resistance against the powdery mildews in pea.

The conventional breeding for recessively inherited resistance genes like *er2* is more difficult and time consuming because the resistance gene cannot be phenotypically selected in heterozygous progenies which thus need to be self-pollinated to select resistant plants. The situation is further complicated when both *er1* and *er2* genes are to be simultaneously selected in the progenies; the high level of penetration resistance mediated by *er1* will preclude the detection of *er2* in the breeding material.

The codominant DNA makers linked to resistance genes offer solution to these difficulties by allowing the direct selection of heterozygous of carriers of the recessive resistance genes among backcross progenies without resorting to progeny tests. Since the DNA markers are not affected by epistatic interactions, they can also be used to confirm the presence of multiple resistance genes in the backcross progenies without disease screening, thereby increasing efficiency of selection and reducing time span for the introgression of resistance genes. While a number of closely linked codominant SSR and functional gene-based based markers have been developed for *er1* gene (Ek et al. 2005; Pavan et al. 2013), the *er2* has been mapped with a tightly linked SCAR marker 'ScX17_1400' that shows dominant inheritance (Katoch et al. 2010). Since the dominant marker 'ScX17_1400' cannot differentiate between heterozygous and homozygous carriers of the *er2* gene, there is an urgent need to develop closely linked codominant markers for the gene for its speedy and precise introgression into the powdery mildew susceptible pea cultivars through marker- assisted breeding.

The recent sequencing efforts in pea by Pea Genome Consortium have led to the development of a high-quality chromosomal-level genome assembly of the reference genotype 'Caméor' that spanned 3.92 Gb of the genome (https://urgi.versailles.inra.fr/Species/Pisum/Pea-Genome-project). This genome assembly can be utilized to develop a range of sequence based markers by targeting the genomic regions around the region of ScX17_1400 marker for generating codominant markers like sequence tagged site (STS) and cleaved amplified polymorphic sequence (CAPS) markers. In view of the above, the present study has been planned with following objectives:

Objective(s):

1. Development of sequence-based PCR markers from pea LGIII using the pea genome sequence information available under public domain.
2. Validation of developed markers for polymorphism in powdery mildew resistant and susceptible genotypes.
3. Confirmation of linkage of parental polymorphic markers to *er2* gene using appropriate mapping population.

2. REVIEW OF LITERATURE

Pea is a significant nitrogen-fixing vegetable crop forming a significant component of a sustainable cropping system, helping to preserve soil health and output. The crop is considered a prime protein-supplying vegetable crop with 7.2 g of digestible protein (Sirwaiya et al. 2018). The crop is affected by multiple fungal, viral, bacterial, and nematode diseases. Under appropriate conditions, these diseases decrease the quality and quantity (Grunwald et al. 2004). In temperate pea-growing areas of the world, powdery mildew caused by *Erysiphe pisi* D.C. poses a serious threat to pea cultivation (Katoch et al. 2010). The disease affects both crop quality and yield; in epidemic years, yield reduction of up to 26-27% have been ascribed to the disease (Munjal et al. 1963; Warkentin et al. 1996). The identification of resistant sources and their incorporation into modern cultivars is the most efficient way to control the disease. To date, two recessive powdery mildew-resistance genes, *er1* and *er2* and one dominant gene *Er3* have been identified from peas (Harland 1948; Heringa et al. 1969; Fondevilla et al. 2007). The identification of molecular markers linked to these powdery mildew resistance genes and their utilization in selection of resistance has been one of the most important aims of pea breeding research. The recent release of draft genome assembly of pea genotype by International Pea Genome Consortium has offered enormous possibilities for the development of sequence based markers for genetic mapping of genes of economic importance in pea and their utilization in speedy generation of new varieties of peas by genomics-assisted breeding.

In the following sections, the available literature pertaining to the powdery mildew resistance in peas, mapping of powdery mildew resistant genes, validation of powdery mildew linked markers in breeding material and germplasm, and genome sequencing in pea has been summarized under the following heads:

2.1. Powdery mildew resistance in peas

2.2. Mapping of powdery mildew resistance genes and validation of markers

2.3. Genome sequencing in pea

2.1. Powdery mildew resistance in pea

Several sources of powdery mildew resistance have been identified among pea germplasm screened by various workers (Heringa et al. 1969; Tiwari et al. 1997; Vaid and

Tyagi, 1997) and till date two recessive powdery mildew resistance genes, *er1* and *er2* have been identified from pea (Harland 1948; Heringa et al. 1969). Recently, one dominant gene *Er3* has been identified in *Pisum fulvum* and has been successfully transferred into pea by cross breeding (Fondevilla et al. 2007).

Of the three known powdery mildew resistance genes, *er1* gene has been widely deployed in pea breeding for imparting resistance to powdery mildew and has maintained its resistance under most of the environments. However in recent year several cases of breakdown *er1* resistance have been reported (Ondrej et al. 2005; Lahoz et al. 2013). Ondrej et al. (2005) reported susceptibility to powdery mildew in 16 of the 19 pea genotypes carrying *er1* genes tested under field and glasshouse conditions. The powdery mildew species attacking the *er1* genotypes was identified to be as *E. baeumleri* on the basis of characteristics of its conidia and ascocarps. Later, Lahoz et al. (2013) reported susceptibility to powdery mildew in three pea resistance cultivars carrying *er1* gene under field and glasshouse conditions in Italy. Morphological and molecular analyses of the fungus that induced disease on *er1* genotypes suggested that the species belonged to *E. pisi* producing conidia and cleistothecia characteristic of the species. However, in both of the above studies a few er1 carrying genotypes maintained their resistance to powdery mildew thus suggesting that although *er1* resistance is conferred by loss of function of PsMLO gene different mutations may differ in their resistance spectrum. Attanayake et al. (2010) investigated the diversity of powdery mildew pathogens infecting pea in US Pacific Northwest using molecular and morphological techniques. Phylogenetic analysis of rDNA ITS sequences in combination with assessment of morphological characters defined two groups of powdery mildew infecting pea, one of which is 99% similar to those of *E. pisi* in GeneBank and other similar to *E. trifolii* with 14 nucleotide differences in the ITS region between the two groups. Recently, *E. trifolii* has also been shown to be the causal agent of powdery mildew symptoms in pea lines carrying *er1* in Spain and India (Fondevilla et al. 2013).

Fondevilla et al. (2006) studied macroscopic and histological features of the powdery mildew resistance conferred by *er1* and *er2* genes. The *er1* gene present in genotype JI1559 was shown to confer complete or almost complete resistance to the pathogen which was not associated with macroscopically visible necrosis. In *er1* genotypes epidermal cell penetration was prevented and very few haustoria or colonies were formed. Resistance in line JI2480 (carrying *er2*) was mainly based mainly on post-penetration cell

death, complemented by a reduction of percentage penetration success in mature leaves. The *er2*- mediated resistance was reported to increase with temperature and leaf age, and complete resistance was expressed only at high temperature (25°C) or in mature leaves, whereas *er1* resistance was not affected by temperature or plant growth stage.

Fondevilla et al. (2007) identified a dominant gene *Er3* conferring resistance to powdery mildew in *Pisum fulvum*. The gene has been successfully introduced into adapted pea genotypes by sexual crossing and has been shown to segregate independently from *er1* and *er2* genes (Fondevilla et al. 2007; Fondevilla et al. 2011).

Fondevilla et al. (2013) tested the efficacy of pea accessions carrying powdery mildew resistance genes *er1*, *er2* and *Er3* against *E. trifolii* under controlled conditions at 20 and 25 °C and under field conditions in Spain and in India. Analysis of the ribosomal DNA (rDNA) internal transcribed spacer (ITS) sequences showed that *E. trifolii* was the causal agent of powdery mildew symptoms in lines carrying *er1* in Spain and that this pathogen was also present in India. *E. trifolii* was able to overcome *er1* and also *Er3* resistance under some conditions. In contrast, *er2* provided high level of resistance against *E. trifolii* in all the conditions and locations studied. The pea accession JI2480, containing *er2*, was highly resistant and JI2302 containing *er1* was susceptible to *E. trifolii* at both temperatures, whereas P660-4 containing *Er3* was resistant at 20 °C but susceptible at 25 °C.

In a recent publication, Devi et al. (2022) provided a detailed review of the work done in peas on the genetics of resistance to powdery mildew including allelic variations of the *er* gene, marker linkage analysis in peas, and proposed future strategies to exploit this knowledge for targeted breeding for resistance to powdery mildew in Pisum sativum. Modern tools of genomics, MAS, transcriptomics, and genomic selection, genome editing using CRISPR/Cas9 have been suggested for developing resistant cultivars.

2.2. Mapping of powdery mildew resistance genes and validation of markers

Availability of molecular markers tightly linked to the pea powdery mildew resistance genes is a pre-requisite for pursuing marker-assisted breeding for powdery mildew resistance. Timmerman et al. (1994) did linkage analysis of *er-1* and demonstrated genetic linkage between *er-1* and linkage group 6 markers after analyzing the progeny of 2 crosses, an F_2 population and a set of recombinant inbred lines. A RAPD marker tightly

linked to the *er-1* was identified by bulked segregant analysis and specific PCR primers were designed to convert it into SCAR. Tiwari et al. (1998) identified three RAPD markers viz., $OPO18_{1200}$, $OPE16_{1600}$ and $OPE6_{1900}$, linked to powdery mildew resistance gene *er1*. The RAPD markers $OPO18_{1200}$ and $OPE16_{1600}$ were successfully converted into SCAR markers, Sc-$OPO18_{1200}$ and Sc-$OPE16_{1600}$ that proved effective in identifying *er1* in diverse genetic backgrounds. The marker Sc-$OPE16_{1600}$ was found to be useful in marker assisted selection of heterozygous BC_{nF1} individuals, while Sc-$OPO18_{1200}$ proved useful in identifying homozygous resistant individuals in F_2 and subsequent generations. Tiwari et al. (1999) identified five AFLP markers linked to powdery mildew resistance gene *er2*. Three of these markers (generated with AFLP primer pairs E+ACT/M+CGC, E+ACG/M+CCC and E+AGG/M+CTA) were linked in *trans* to *er2*, while two AFLP markers generated with primer pair E+AGG/M+CTG were linked in *cis* to *er2*.

Rakshit et al. (2001) tested the utility of the marker $OPD10_{650}$ developed by Timmerman et al. (1994) in marker aided selection of powdery mildew resistance gene *er1*. The marker was found to be efficient in differentiating the heterozgous (*Erer*) and homozygous (*ErEr*) susceptible plants among the backcross progeny. Janila and Sharma (2004) identified two RAPD markers, OPO-02_{1400} and OPU-17_{1000} linked to powdery mildew resistance gene *er1*. They also validated the previously identified RAPD and SCAR markers (Timmerman et al. 1994; Tiwari et al. 1998) in indigenous pea germplasm.

Janila and Sharma (2004) identified two RAPD markers, OPO-02_{1400} and OPU-17_{1000} linked to powdery mildew resistance gene *er1*. They also validated the previously identified RAPD and SCAR markers (Timmerman et al. 1994; Tiwari et al. 1998) in indigenous pea germplasm.

Ek et al. (2005) used 315 different SSR markers for testing their linkage to powdery mildew resistance *er1* using an F_2 population derived from the cross of susceptible line 'Majorret' and resistant line '955180'. The powdery mildew resistance gene was mapped on linkage group VI with closely linked markers PSMPSAD60, PSMPSAA374 and PSMPA5 mapping at a distance of 10.4, 11.6 and 14.9 cM, respectively.

Fondevilla et al. (2008) identified four RAPD markers linked in coupling phase (OPW04_637, OPC04_640, OPF14_1103, and OPAH06_539) and two in repulsion phase (OPAB01_874 and OPAG05_1240) to *Er3* gene. Two of these markers, OPW04_637 and OPAB01_874, have been converted into Sequence Characterized Amplified Region

(SCAR) markers. Recently, *Er3* gene has been localized to pea linkage group IV through linkage analysis of susceptible cv. Messire and a resistant near isogenic line of Messire (cv. Eritreo, *Er3Er3*) with position-specific SSR makers (Cobos et al. 2018). The SSR markers AA349 and AD61 located on LGIV were found to be linked to *Er3* and to the RAPD and SCAR markers previously reported to be linked to this gene.

Katoch et al. (2010) identified and mapped a recessive powdery mildew resistance gene '*er2*' from a pea genotype 'JI2480'. The gene '*er2*' was mapped to pea LG III. A RAPD marker OPX-17_{1400} linked in coupling phase (2.6 cM) to *er2* was converted into a Sequence Characterized Amplified Region (SCAR) marker 'Sc-OPX-17_{1400}' for speedy and precise introgression of the *er2* gene into the powdery mildew susceptible pea cultivars.

Pavan et al. (2011) reported that the powdery mildew resistance *er1* in pea is caused by loss-of-function in the *PsMLO1* gene. A single nucleotide polymorphism in the *PsMLO1* gene sequence, causing premature termination of translation and a non-functional protein, was shown to be responsible for the *er1* resistance. A cleaved amplified polymorphic sequence (CAPS) marker developed from the mutation site was shown to be fully cosegregating with resistance in segregating F_2 of the cross between mutant line RO13/02 and susceptible cultivar Progress9.

Srivastva et al. (2012) identified a tightly linked sequence characterized amplified regions (SCAR) marker for *er1* gene using NILs. A total of 620 random amplified polymorphic DNA (RAPD) markers were screened for length polymorphism between seven sets of NILs. The 880 bp polymorphic band of the tightly linked RAPD marker OPX 04_{880} was cloned, sequenced and a SCAR marker ScOPX-04_{880} was developed. The markers was tightly linked to *er1* gene at 0.6 cM in coupling phase and could correctly differentiate homozygous resistant plants from the susceptible accessions with more than 99 % accuracy.

Pavan et al. (2013) developed functional makers for *er1* gene by targeting *PsMLO1* polymorphisms directly responsible for the resistant phenotype of five different *er1* alleles (*er1-1* to *er1-5*) that have been identified in pea till date. Highly informative cleaved amplified polymorphic sequence (CAPS), derived cleaved amplified polymorphic sequence (dCAPS), sequence tagged site (STS) and high resolution melting (HRM) markers were developed which enabled the selection of each of the five *er1* alleles identified from pea genotypes, JI1559, Franklin, JI210, JI1951and RO13/02.

Bheri et al. (2015) tested garden pea germplasm lines and cultivars against *E. pisi* under green house conditions and further characterized the resistant lines using markers linked to powdery mildew resistance genes, *er1*, *er2* and *Er3*. Out of the forty six pea lines tested, three lines were found to be highly resistant, three lines were resistant, eighteen lines were moderately resistant, twelve were moderately susceptible, six were susceptible and four lines were highly susceptible. The previously known SCAR markers for *er1* gene, Sc-OPO-18_{1200}, Sc-OPE-16_{1600}, ScOPX 04_{880} and ScOPD 10_{650} were found to be unsuitable for the detection of *er1* gene either due to lack of expected amplification with these markers or lack of polymorphism between resistant and susceptible controls. The SCAR marker for *er2* gene, ScX17_1400 led to identification of twenty-four lines carrying *er2* gene, while SCAR markers for *Er3* gene, SCW4637 and SCAB1874, revealed four lines as potential sources of *Er3* gene.

Reddy et al. (2015) tested SCAR and SSR markers linked to powdery mildew resistance genes *er1* and *er2* across a panel of nine parental genotypes used for pea breeding in India. Although SCAR markers amplified specific loci, none, including $ScX17_{1400}$, could differentiate the resistant or susceptible genotypes. The SSR marker A5 for *er1* gene clearly distinguished allelic conditions of *er1* in homozygous resistant and susceptible parents and three F_2 progenies of crosses 'Arka Priya' × 'IP-3', 'Arka Pramod' × 'IP-3', and 'Arka Ajit' ×'Azad-Pea'.

Sun et al. (2016) identified a novel allele of the powdery mildew resistance gene *er1* from a resistant genotype 'DDR11'. The novel allele designated*, er1-7*, had a 10-bp deletion in position 111–120 of *PsMLO1* gene that caused a frame-shift mutation, resulting in a premature termination of translation of PsMLO1 protein. A co-dominant functional Indel marker developed by targeting 10-bp deletion was found to cosegregate with *E. pisi* resistance in a mapping population derived from a cross of susceptible genotype, Bawan 6 and DDR-11.

Ma et al. (2017) developed 8 breeder-friendly kompetitive allele-specific PCR(KASPar) markers to overcome the problems of gel-based markers and increase the efficiency of genotypic screening. Both phenotyping and genotyping revealed that one pea accession, PI 142775, carried the allele *er1-1* for resistance to powdery mildew indicating that KASPar markers might be a powerful and valuable tool for use in pea breeding to develop varieties resistant to powdery mildew disease.

Sharda and Makandar (2023) evaluated a set of 46 pea genotypes against *E. pisi* under greenhouse conditions to identify resistant genotypes. Subsequently, the phenotyped genotypes were analysed with gene-specific sequence characterized amplified region (SCAR) markers for powdery mildew resistance genes *er1, er2* and *Er3*. While the tested markers amplified resistance gene specific bands in the control pea genotypes; JI2302 for *er1* gene, JI2480 for *er2* gene and P660-4 for *Er3*, these markers also amplified resistance specific bands in many of the susceptible genotypes. These results suggested that the SCAR developed form the linked regions of the resistance genes may not be 100% predictive of the presence of resistance gene in certain cross combinations involving susceptible and resistant genotypes.

2.3. Genome sequencing in pea

The genome is often described as the information repository of an organism. Complete and accurate reference genomes provide basic resources for functional genomics and molecular genetic research to accelerate crop breeding. The pea genome spans 4.45 Gb organized into seven chromosomes. The new and improved reference genome is required for a better understanding of phenotypic variation and genome evolution and broaden our understanding of the genetics underlying the giant size of the pea genome. With its large and complex genome with preponderance of repetitive sequences, peas posed a real challenge to genome sequencing. Pea genomics has lagged behind that of legumes with smaller genomes due to its large genomic size. A combined use of novel approaches for reducing genome complexity and advent of Next-generation sequencing (NGS) techniques has provided the means to combat the challenge of sequencing the large genomes of plants like pea that have high levels of repeated sequences. First genome-wide sequencing effort in peas was made by Boutet et al. (2016) who identified 419,024 genomic SNPs using HiSeq whole genome sequencing of four pea lines. Among these, 213,030 SNPs appeared robust for genotyping. The study led to development of first WGGBS-derived map in pea containing 64,263 markers including new genomic SNPs added to 910 other markers thus allowing development of a new high-density genetic map. This new WGGBS map showed an average density of 62.6 markers per cM and marker density for different LGs ranged from 52 markers/cM (PsLGI) to 74 markers/cM (PsLGIV)

In 2013, the Pea Genome International Consortium launched a program dedicated to the development of a quality reference draft sequence for French pea variety ‘Cameor’.

The effort has led to the release of the first seven-chromosome draft assembly of the inbred pea cultivar "Caméor" (Kreplak et al. 2019). The sequenced genome spans 3.92 GB or approximately 88% of the estimated 4.45 GB of the pea genome with 82.5% (3.23 GB) of the sequences assigned to the seven pseudomolecules, and 14,266 unassigned scaffolds representing 685Mb. The pea genome sequence is publicly available at https://urgi.versailles.inra.fr/Species/Pisum/Pea-Genome-project. The high quality-annotated pea genome sequence represents a valuable resource for fundamental research and improvement of pea through genomics assisted approaches.

Shirasawa et al. (2021) determined the genome sequence of the pea line with yellow pods to identify the genomic region controlling pod color. Utilizing Nanopore sequencing technology, genome sequence reads were assembled into 117,981 contigs (3.3 Gb) with an N50 value of 51.2 kb, and a total of 531,242 potential protein-coding genes were predicted, of which 519,349 (2.8 Gb) were positioned within repetitive sequences (2.8 Gb). This study has facilitated the identification of the gene-controlling one of the traits studied by Mendel and is expected to accelerate the pan-genome studies in peas. Yang et al. (2022) reported a de novo genome assembly of the Chinese pea cultivar, ZW6 with a contig N50 of 8.98 Mb featuring a 243-fold increase in contig length and evident improvements in the continuity and quality of sequence in complex repeat regions compared to the existing one. A pan-genome of 118 pea accessions including cultivated and wild pea lines was also constructed. The study revealed distinct functional enrichment of pan-genes of *P. abyssinicum* and *P. fulvum* in peas, indicating the potential value of them as pea breeding resources in the future.

3. MATERIALS AND METHODS

The present research work entitled "Development and validation of co-dominant markers for pea powdery mildew resistance gene *er2"* was conducted at the Department of Agricultural Biotechnology, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur during the period 2022-2024. The material and methods used in the present research work are described in the following sections:

3.1. Plant Material

The *er2* donor line "JI2480" procured from the John Innes Centre (JIC), Norwich, UK, and a susceptible pea line "Azad P-1" were used for testing the polymorphism of developed markers. A BC_7F_2 population derived from the cross Azad P-1 × JI2480 was used to test the linkage of polymorphic markers to *er2* gene. A set of 30 genotypes representing 28 commercial pea varieties and lines with known powdery resistance genes *er1* and *er2* were utilized to test the suitability of markers for marker-assisted selection of *er2* gene in diverse pea genetic backgrounds (Table 3.1).

Table 3.1 List of pea genotypes used in the study

Sr. No.	Line no.	Source	Remarks
1	Arka Ajit	ICAR-Indian Institute of Horticulture Research, Bengaluru	-
2	Arka Kartik	ICAR-Indian Institute of Horticulture Research, Bengaluru	-
3	Arka Priya	ICAR-Indian Institute of Horticulture Research, Bengaluru	-
4	Arka Uttam	ICAR-Indian Institute of Horticulture Research, Bengaluru	-
5	Kashi Ageti	Indian Institute of Vegetable Research, Varanasi	-
6	Kashi Mukti	Indian Institute of Vegetable Research, Varanasi	-
7	Kashi Nandani	Indian Institute of Vegetable Research, Varanasi	-
8	Kashi Purvi	Indian Institute of Vegetable Research, Varanasi	-
9	Kashi Samarth	Indian Institute of Vegetable Research, Varanasi	-
10	Kashi Samridhi	Indian Institute of Vegetable Research, Varanasi	-
11	Kashi Shakti	Indian Institute of Vegetable Research, Varanasi	-
12	Kashi Tripti	Indian Institute of Vegetable Research, Varanasi	-
13	Kashi Udai	Indian Institute of Vegetable Research, Varanasi	-
14	NDVP-250	Indian Institute of Vegetable Research, Varanasi	-
15	VRP Sel.-17	Indian Institute of Vegetable Research, Varanasi	-
16	Pant Mattar-2	Indian Institute of Vegetable Research, Varanasi	-
17	PC-531	Indian Institute of Vegetable Research, Varanasi	-
18	Pusa Pragati	Indian Institute of Vegetable Research, Varanasi	-
19	Shijara Local	Indian Institute of Vegetable Research, Varanasi	-
20	VRP-343	Indian Institute of Vegetable Research, Varanasi	-
21	Azad P-3	Department of Vegetable Science, PAU, Ludhiana	-
22	Arkel	Department of Seed Science and Technology, UHF, Nauni, Solan	-
23	Him Palam-1	Department of Seed Science and Technology, CSKHPKV, Palampur	-

24	Him Palam-2	Department of Seed Science and Technology, CSKHPKV, Palampur	Known to harbour PM resistance gene *er2*
25	Palam Priya	Department of Seed Science and Technology, CSKHPKV, Palampur	-
26	Lincoln	Department of Seed Science and Technology, CSKHPKV, Palampur	-
27	PB-89	Department of Vegetable Science, PAU, Ludhiana	-
28	Azad P-1	Department Agricultural Biotechnology, CSKHPKV, Palampur	-
29	JI1559	The John Innes Centre, Norwich, UK	Known source of *er1* gene
30	JI2480	The John Innes Centre, Norwich, UK	Known source of *er2* gene

3.2 Development of STS markers and conversion to cleaved amplified polymorphic sequence (CAPS) markers

A previously known sequence characterized amplified region (SCAR) marker (ScX17_1400) showing *cis* phase linkage (2.6 cM) to *er2* (Katoch et al., 2010) was used as a reference point for the development of co-dominant markers for *er2*. The ScX17_1400 amplicon was amplified from the *er2* carrying genotype JI2480 using the primers and PCR conditions as described in Katoch et al. (2010). The ScX17_1400 amplicon was cloned into pGEM-T Easy Vector (pGEM-T Easy Cloning Kit, Promega) and transformed in *Escherichia coli* strain DH5-α following manufacturer's instructions. The putative positive clones were selected by blue and white selection of transformed *E. coli* cells on LB agar medium containing ampicillin (100 µg/ml). The positive clones were identified by colony PCR using ScX17_1400 specific primers. Plasmid DNA from the two selected clones was isolated by alkali lysis method (Birnboim and Doly 1979) and was got custom sequenced by Sanger sequencing from Eurofins Genomics India Pvt Ltd., Banglore. The deduced DNA sequence of ScX17_1400 was aligned against the reference Pea genome (*Pisum sativum* genome assembly version 1a) available at http: https://urgi.versailles.inra.fr using BLAST tool. Based on BLAST analysis a 1038 kb region spanning from 240.617 to 241.655 Mb of PsLG3 was downloaded and targeted for designing ten STS markers using Primer3 software (Untergasser et al., 2012). The uniqueness of the designed primers was checked using BLAST analysis. The details of developed STS markers is given in Table 3.2.

Table 3.2 List of Sequence Site Tagged markers designed from pea LG3

Markers	Primer sequence(5'---3')	Genomic position (bp)	Annealing Temp. (°C)	Expected size (bp)
PsLG3-STS-1	F: ATGGTCCCAGTCCTTCAGAGTA R: AGGGATGGGGTTGAAGTTTTAT	240617105..240618630	59	1526
PsLG3-STS-2	F: AGGTTGAATCTGTGGCCTTAAA R: TCCTCCAGAGTTAGGATTTGGA	241030785..241032017	59	1233
PsLG3-STS-3	F: GGTTTTCTTATCCCCAATCTCC R: TCTGTTAGGTGAGCTCCAGTGA	241112089..241113436	59	1348
PsLG3-STS-4	F: TGAAAGACGATTATTGCCACAC R: TCCAAACCTATGCAGTGTCATC	241225388..241226587	59	1200
PsLG3-STS-5	F: GTGGAAACATAATGGGTGTGTG R: TGCCATAGTCAACCTTTCCTTT	241241624..241242893	59	1270
PsLG3-STS-6	F: ATCAATTGGCTTTTTGATTGCT R: CAGCACCATTGGAAGATAACAA	241301451..241302778	59	1328
PsLG3-STS-7	F: TGGTGAGATGCCATACTTGAAC R: AAAGCAACAAAGGGCAATAAAA	241310970..241312404	59	1435
PsLG3-STS-8	F: CCATTCAGTACCTCACCCTCTC R: CAACCGAAAAATAAAAGCCAAG	241393222..241394690	59	1469
PsLG3-STS-9	F: TGATTGGCTTGACCTAATGTTG R: CAACAAACGCATAAATTCCAGA	241502003..241503208	59	1206
PsLG3-STS-10	F: CAATCTTGCACTCCATAACCAA R: CACCACAGAGGGATACAGATCA	241653959..241655448	59	1490

[a] The genomic position was deuced by landing the primer sequences on the genome sequence of reference pea cultivar 'Caméor' available at https://urgi.versailles.inra.fr/Species/Pisum/Pea-Genome-project

The designed STS markers were used for the PCR amplification of the DNA of AP-1 and JI2480. PCR amplification with STS markers was carried out in a 12.5 µl reaction volume containing 20 ng template DNA, 0.2 mM of each dNTP, 0.2 µ*M* of each primer, 1.5 mM $MgCl_2$, 1X PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3) and 1 unit *Taq* polymerase (Gotaq® DNA Polymerase, Promega). PCR amplification of different markers was carried out in a thermocycler (Proflex PCR System; Applied Biosystems, Life Technologies, USA) using the PCR cycling parameters consisting of initial denaturation at 94 ºC for 5 min followed by 35 three-step cycles specific for each marker; PsLG3-STS-1 to STS-10: 94 ºC for 1 min, 59 ºC for 1 min, 72 ºC for 1 min 30 seconds; ScX17_1400: 94 ºC for 1 min, 65 ºC for 1 min, 72 ºC for 1 min 30 seconds; The Final extension step for all markers was carried out at 72 ^{0}C for 5 min. The PCR products were resolved on 2.0% agarose gel prepared in 1X Tris borate-EDTA (TBE) buffer (0.05 M Tris, 0.05 *M* boric acid, 1 mM EDTA, pH 8.0) at a constant voltage of 100 volts for 1 h. The PCR products were visualized by staining with ethidium bromide (0.5µg/ml) and a photographed of the gel was stored in Gel-Doc system (BIO-RAD Laboratories Inc., USA).

The STS markers showing monomorphic amplification pattern in parental genotypes i.e. AP-1 and JI2480 were converted to cleaved amplified polymorphic sequence (CAPS) marker. For conversion of STS markers into CAPS marker, *in silico* restriction map of the corresponding amplified region was constructed using Restriction Site Mapper (http://www.arabidopsis.org/tools/) to select potential restriction enzymes for restriction analysis. The amplified products were digested in a 10 µl reaction containing 1X restriction buffer, 10U of fast digest restriction enzyme (Thermo Fisher Scientific, Mumbai, India), and 5 µl PCR product in a PCR tube. The restriction reactions for different enzymes were incubated under specific temperature as provided in the manufacturer's instructions. The restriction-digested products of AP-1 and JI2480 for each STS marker were resolved on 2.0% agarose gel to select the restriction enzymes generating polymorphic restriction patterns in the parental genotypes.

3.3 Testing of the polymorphic markers for linkage to *er2*

The polymorphic CAPS markers developed during the study along with two other pea LG3 specific SSR markers, AA278 and AA5, were tested for their linkage to *er2* using a BC_7F_2 population of cross Azad P-1 x JI2480.

3.3.1 Phenotyping for PM resistance

The parental genotypes and BC_7F_2 plants of cross AP-1 x JI2480 were evaluated for powdery mildew reaction using the detached leaf technique (Vaid & Tyagi, 1997). Briefly, a single colony isolate of *Erysiphe pisi* was collected from naturally infected plants of susceptible pea line Azad P-1. The pathogen culture was maintained and propagated on plants of 'Azad P-1' under spore-proof chambers. Two leaflets from 25-30-day-old plants were floated on 40 ppm benzimidazole solution in 90 mm Petri dishes and inoculated with powdery mildew using camel hair brush. The leaflets were incubated for 10 days at 25±1°C with 16-hour photoperiod. After 10 days of inoculation, the leaflets were assessed macroscopically, and microscopically using a stereoscopic microscope (X 90). The disease reaction was evaluated on a scale ranging from 0 to 4, where 0 indicates macroscopically or microscopically no mycelial growth, 1 indicates microscopically sparse mycelial growth with rare conidiophores, 2 indicates slight growth of mycelium microscopically and little sporulation and individual conidiophores on colony can be counted easily, 3 indicates moderate development of mycelium microscopically with moderate to heavy sporulation, and 4 indicates microscopically abundant development of mycelium with heavy sporulation. The leaflets with reaction types 0, 1, 2 were categorised as resistant while those with reaction types 3 and 4 were deemed to be susceptible. The segregation data of number of resistance and susceptible BC_7F_2 plants was subjected to Chi-square analysis to determine the goodness of fit to the Mendelian ratios expected for a recessive monogenic trait.

3.3.2 DNA isolation

The genomic DNA from parental genotypes and BC_7F_2 plants was isolated using standard Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray & Thompson, 1980). Briefly, about 200 mg fresh leaf tissue from individual plants was ground to a fine powder in the presence of liquid nitrogen using autoclaved pestle mortar. The powder was transferred into a 1.5 ml Eppendorf tube using autoclaved spatula and 800µl of pre-warmed (65 °C) extraction buffer (2% CTAB, 100mM Tris, 20mM ethylene diamine tetra acetic acid (EDTA), 1.4M Sodium chloride (NaCl) and 1% polyvinylpyrrolidone (PVP), pH 8.0) added and incubated with repeated shaking at 65 °C in the water bath for 1 hour. An equal volume of Chloroform: Isoamyl alcohol (24:1) was added to each tube and mixed by gentle inversions. The tubes were centrifuged at 10,000 rpm for 10 minutes at 4 °C and the upper

aqueous phase was carefully transferred to a fresh tube to which 2 µl of RNase A was added and incubated at 37 °C for 1 hour followed by addition of 800 µl of CI and centrifugation at 10,000 rpm for 10 minutes at 4℃. The upper aqueous phase was carefully transferred into a new tube and 600 µl pre-chilled isopropanol was added. All the tubes were incubated at -20 °C for at least 1 hour to aid precipitation followed by DNA sedimentation using centrifugation at 10,000 rpm for 10 minutes. The supernatant was discarded and pellets were washed twice with 200 µl of pre-chilled ethanol (70%). The pellets were allowed to air dry for 3-4 hours at room temperature and later dissolved in 100 µl of TE buffer (10 mM Tris-HCL and 0.1 mM EDTA, pH 8.0). The quality and quantity of DNA was checked using the on 0.8% agarose gel. The isolated DNA stock was appropriately diluted with nuclease free water to make final working stock (20ng/µl).

3.3.3 Linkage analysis

The polymorphic CAPS markers developed during the study along with two other pea LG3 specific SSR markers, AA278 and AA5, were tested for their linkage to *er2* employing recessive class approach (Zhang et al. 1994). The approach entailed the use of 110 homozygous recessive powdery mildew resistant BC_7F_2 plants to ascertain the association of the markers with *er2* gene. The PCR conditions for SSR markers were essentially similar to that described previously for STS markers except that the PCR cycling parameters for these markers consisted of initial denaturation at 94 ^oC for 5 min followed by 35 three-step cycles at 94 ^oC for 45 seconds, 61 ^oC for 45 seconds, 72 ^oC for 45 seconds. The SSR amplicons were resolved on 4% agarose gel prepared in 1X Tris borate-EDTA (TBE) buffer and visualized by ethidium bromide staining as described in preceding section for STS markers. The BC_7F_2 plants inheriting marker allele similar to the susceptible parent were scored as ‘A’ and those inheriting allele of the resistant parent were scored as ‘B’. The heterozygous plants were scored as ‘H’. Linkage analysis of the marker and powdery mildew resistance data of BC_7F_2 individuals performed with Map DistoVersion 2.0 software (Lorieux 2012) and the recombination frequency was converted to map distance expressed in cM (centi Morgan) using the Kosambi function (Kosambi 1944).

Table 3.3. List of markers tested for linkage to powdery mildew resistance gene *er2*

Markers[a]	Primer sequence (5'---3')	Type of marker	Annealing Temp. (° C)	Restriction enzyme	Source
PsLG3_CAPS-1	F: ATGGTCCCAGTCCTTCAGAGTA R: AGGGATGGGGTTGAAGTTTTAT	CAPS	59	HpaII	Present study
PsLG3_CAPS-3	F: GGTTTTCTTATCCCCAATCTCC R: TCTGTTAGGTGAGCTCCAGTGA	CAPS	59	Dra-I	Present study
PsLG3_CAPS-4	F: TGAAAGACGATTATTGCCACAC R: TCCAAACCTATGCAGTGTCATC	CAPS	59	Hae-III, Taq-I	Present study
PsLG3_CAPS-X17	F: GGACCAAGCTCGGATCTTTC R: GACACGGACCCAATGACATC	CAPS	65	Hae-III	Present study
AA5	F: TGCCAATCCTGAGGTATTAACACC R: CATTTTTGCAGTTGCAATTTCGT	SSR	61	-	Loridon et al. (2005)
AA278	F: CCAAGAAAGGCTTATCAACAGG R: TGCTTGTGTCAAGTGATCAGTG	SSR	61	-	Loridon et al. (2005)

3.4 Validation of markers in commercial pea varieties

The *er2* linked polymorphic CAPS markers developed herein and two previously known *er2* linked markers ScX17_1400 and AA278 were tested on a panel 28 commercial pea genotypes to evaluate their applicability for marker assisted selection of *er2* in crosses involving these genotypes as recipient or recurrent parents. The seeds of commercial pea varieties and genotypes carrying known powdery mildew resistance genes were sown in plastic pots (30 cm diameter) filled with potting mixture (soil:sand :: 3:1) and kept under naturally ventilated net house for plant growth. The genomic DNA from the young leaflets of these genotypes was isolated by CTAB method. The isolated DNA was diluted to working stocks (20ng/µl) with nuclease free water and was analyzed with different *er2* linked markers as previously described in section 3.2.

4. RESULTS AND DISCUSSION

The present study aimed at the development of codominant markers for powdery mildew resistance gene *er2* by utilizing the pea genomic resources available under public domain. A novel set of codominant Cleaved Amplified Polymorphic Sequence (CAPS) markers were developed and validated for their linkage to *er2* gene using a BC_7F_2 population segregating for *er2* resistance. The tightly linked marker developed during the study were also tested across a panel of commercial pea varieties of the country to ascertain their applicability for marker- assisted introgression of *er2* in these varieties. The results pertaining to various aspects of the study are discussed hereunder different heads.

4.1 Development of co-dominant markers for *er2* gene

4.1.1 Deducing the genomic location of ScX17_1400 on pea genome.

To deduce the genomic location of ScX17_1400 on pea genome, the PCR fragment amplified with this marker from the *er2* donor line JI2480 was sequenced and aligned on the reference pea genome. The deduced DNA sequence of ScX17_1400 marker from JI2480 measured 1457 bp in length and is deposited in Genbank under accession no. PP856163. Consistent with the reported location of *er2* on LG3 (Katoch et al. 2010), the DNA sequence of ScX17_1400 marker physically mapped to pea LG3 pseudomolecule showing 99.51% homology with position 241616882 to 24618336 bp of the pseudomolecule (Table 4.1).

Table 4.1. Pea genome sequence producing significant alignment with sequence of SCAR marker ScX17_1400 amplified from pea genotype JI2480.

Description	Alignment score	Identity	E value	Max. identity	Start	End
Chrm5LG3 *Pisum sativum* genome assembly version 1a	2592	1448/1455	0.0	99.51%	241616882	24618336

4.1.2 Designing of STS markers from *er2* region and conversion into CAPS markers

A 1038 kb sequence around the region of ScX17_1400 spanning from position 240.617 to 241.655 Mb of PsLG3 was targeted for designing ten sequence tagged site (STS)

markers with expected amplicon length ranging from 1200 to 1526 bp. All the ten STS markers amplified expected sized monomorphic PCR amplicons with the DNA of Azad P-1 and JI2480 (Fig 4.1), thus were not *immediately* useful as markers for genetic studies. In order to convert STS markers into polymorphic Cleaved Amplified Polymorphic Sequence (CAPS) markers, the parental PCR amplicons obtained with these markers were digested with a panel of 7 to 15 restriction enzymes (Table 4.2). Of the ten STS markers targeted for conversion into CAPS, polymorphic restriction patterns were generated for five STS markers, PsLG3STS-1, PsLG3STS-2, PsLG3STS-3, PsLG3STS-4 and PsLG3STS-6 when the parental amplicons obtained with these markers were digested with restriction enzymes HpaII, ScaI and HaeII, DraI, HaeIII and TaqI, and TaqI, respectively (Table 4.2; Fig 4.2). These results suggested that single-nucleotide polymorphisms (SNPs) existed in the genomic regions defined by these STS markers which created or disrupted the recognition sites for certain restriction enzymes resulting in CAPS polymorphisms in the parental genotypes. The CAPS markers developed herein from PsLG3STS-1, PsLG3STS-2, PsLG3STS-3, PsLG3STS-4 and PsLG3STS-6 were designated as PsLG3_CAPS-1, PsLG3_CAPS-2, PsLG3_CAPS-3, PsLG3_CAPS-4 and PsLG3_CAPS-6, respectively. Furthermore, the previously known *er2* linked SCAR marker ScX17_1400 which amplified monomorphic amplicons in pea genotypes Azad P-1 and JI2480 could also be converted into a CAPS marker by digesting the PCR products with enzyme HaeIII (Fig 4.2). The SCAR derived from ScX17_1400 was designated as CAPS-ScX17. Previous studies have reported SNP polymorphisms in pea genome with a frequency of 1.49 to 20.2 SNPs/Kb in different regions of pea genome (Sindhu et al. 2014; Boutet et al. 2016). Nearly 30-40% of SNPs polymorphisms are expected to alter restriction enzymes recognition sites (Wicks et al. 2001), thus have potential to be converted into CAPS markers as has been demonstrated in present study. Previously, Pavan et al. (2013) have reported the development of CAPS markers for the identification of different alleles of pea powdery mildew resistance gene *er1* by targeting the allele specific SNPs in the gene. Due to their ease of obtainment and low-cost, the CAPS markers are ideally suited for routine use in pea breeding programmes which cannot afford *prohibitively* expensive and time consuming sequencing-and array-based techniques for the genotyping of SNPs linked to economically important traits.

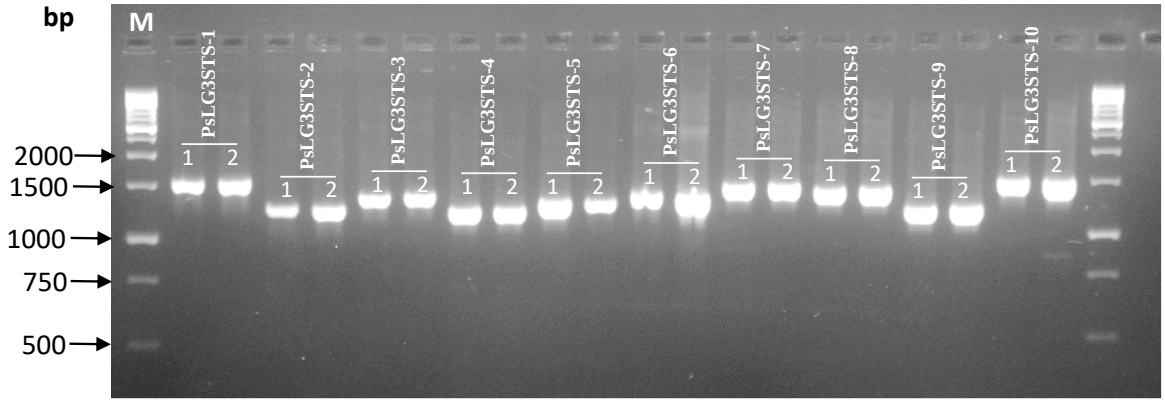

Fig 4.1. Amplification profiles of pea genotypes Azad P-1 and JI2480 with sequence tagged site markers developed from Pea LG3.

1= Azad P-1; 2= JI2480. The PCR products were resolved on 1.5% agarose gel and visualized by ethidium bromide staining. M: 1Kb molecular weight ladder.

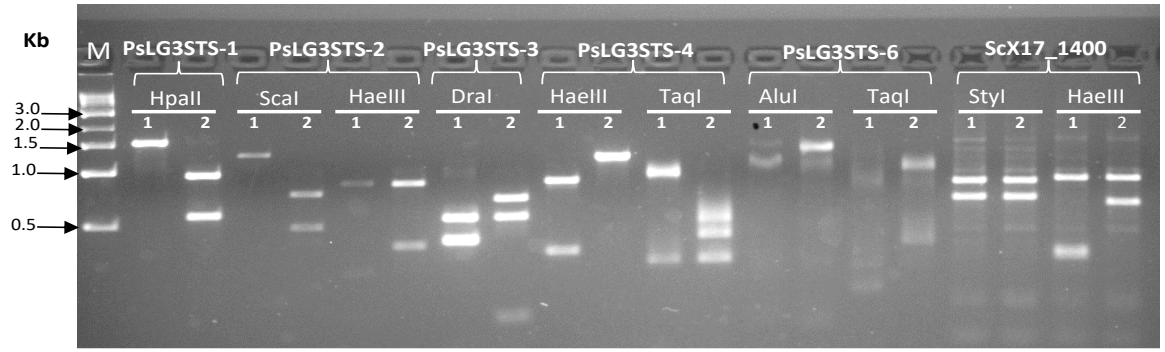

Fig 4.2. Restriction digestion of STS amplicons of the parental genotypes with different enzymes generating polymorphic patterns. 1= Azad P-1; 2= JI2480. M= 1 kb DNA ladder.

Table 4.2. List of restriction enzymes used for CAPS analysis of parental genotypes.

Markers	Restriction enzymes used	Enzyme(s) generating polymorphic restriction pattern
PsLG3-STS-1	AluI, DpnI, HaeII, HinfI, HpaII, NdeI, RsaI, StyI, TaqI, TruI, KpnI	HpaII
PsLG3-STS-2	AluI, BamHI, DpnI, DraI, HaeIII, HindIII, HinfI, KpnI, RsaI, ScaI, TaqI, TruI, HphI	Sca-I, Hae-III
PsLG3-STS-3	AluI, DpnI, DraI, HindIII, HinfI, HphI, SacI, StI, TruII	Dra-I
PsLG3-STS-4	DpnI, DraI, HaeIII, HhaI, HinfI, HpaI, HphI, RsaI, ScaI, TaqI, TruII	Hae-III, Taq-I
PsLG3-STS-5	AluI, BamHI, DpnI, EcoRI, HaeIII, HhaI, HindIII, HinfI, HphI, NdeI, RsaI, StyI, TaqI, TruII, XhoI	-
PsLG3-STS-6	AluI, DpnI, DraI, EcoRI, HhaI, HinfI, NdeI, SalI, TaqI, HphI	TaqI
PsLG3-STS-7	AluI, DpnI, DraI, EcoRI, HaeIII, HhaI, HindIII, HinfI, HpaII, HphI, NdeI, RsaI, SacI, TaqI	-
PsLG3-STS-8	AluI, DpnI, DraI, HhaI, HinfI, HpaII, HphI, RsaI, ScaI, TaqI	-
PsLG3-STS-9	AluI, DpnI, HinfI, HphI, RsaI, StyI, TaqI	-
PsLG3-STS-10	AluI, DpnI, EcoRI, HhaI, HinfI, HpaI, HphI, MvaI, NdeI, RsaI, SacI, StyI, TaqI	-
ScX17_1400	AluI, DpnI, EcoRI, HaeIII, HhaI, HpaI, HpaII, HphI, RsaI, StyI, TaqI	HaeIII

4.2. Confirmation of linkage of markers to *er2* gene

4.2.1 Genetics of powdery mildew resistance

In order to validate the linkage of polymorphic markers generated in present study to powdery mildew resistance gene *er2,* a BC_7F_2 population derived from cross Azad P-1 x JI2480 and parental genotypes were phenotyped for powdery mildew resistance using detached leaf assay. The parental genotype Azad P-1 exhibited a highly susceptible reaction (reaction type 4) to powdery mildew, while JI2480 exhibited resistance reaction (reaction type 1) characterized by sparse mycelia growth and hypersensitive flecking of the infected leaf tissue, a characteristic feature of the *er2* mediated resistance. Of the 439 BC_7F_2 plants tested for reaction to powdery mildew 325 were susceptible and remaining 114 exhibited resistance reaction (Table 4.3). The segregation of resistance and susceptible plants in BC_7F_2 showed a good fit to 3: 1 (susceptible: resistance) ratio ($0.50 < P < 0.75$) suggesting

monogenic recessive inheritance of powdery resistance in pea genotype JI2480. These results are in agreement with previous reports on the presence a single recessive gene *er2* for resistance to powdery mildew in pea genotype JI2480 (Tiwari et al. 1997; Katoch et al. 2010).

Table 4.3. Segregation of resistance in BC_7F_2 progenies of a cross Azad P-1 x JI2480

Generation	No. of progenies		Expected ratio (S: R)	χ^2	P value
	S	R [a]			
F_1	5	0	all : 0	0.0	1.0
F_2	325	114	3 : 1	0.22	0.50< P < 0.75

[a] R= Resistant; S= susceptible. χ^2 = the actual value of Chi-square test for susceptible / resistant ratio.

4.2.2 Linkage analysis of markers

The four polymorphic CAPS markers developed during the present study along with two pea LG3 specific SSR markers, AA278 and AA5, were tested for their association with *er2* employing recessive class approach (Zhang et al.1994). A total of 110 homozygous recessive powdery mildew resistant BC_7F_2 plants were genotyped with different markers to deduce the intensity of their linkage with *er2* gene (Fig 4.3 to 4.7). Among different markers the least number of recombinants (4) were detected with AA278, while maximum recombination events (9) were recorded with SSR marker AA-5. The CAPS markers PsLG3_CAPS-1, PsLG3_CAPS-3 and PsLG3_CAPS-4 identified seven identical recombinants in the population of resistant plants, while six recombinants were detected with CAPS-ScX17. Of the ten recombination events detected during the linkage analysis of 110 resistant plants, four (# 47, 49, 53 and 61) were detected with all the markers suggesting that these markers are located on the same side of the *er2* gene. The linkage map of *er2* locus constructed with genotypic data of six markers spanned 5.9 cM (Fig 4.8). The SSR marker AA278 was most closely linked to the *er2* gene at 1.8 cM while the CAPS-ScX17 was the next closely linked marker situated at a genetic distance of 2.7 cM from the gene. The three cosegrating markers PsLG3_CAPS-1, PsLG3_CAPS-3 and PsLG3_CAPS-4 were located at a distance of 3.2 cM from the gene.

4.3 Validation of markers in commercial pea genotypes

The five codominant markers developed during the present study and a previously known *er2* linked SCAR marker ScX17_1400 were tested on a panel of 28 commercial pea varieties and powdery mildew resistant genotypes JI1559 (*er1*) and JI2480 (*er2*) to assess their applicability for marker assisted introgression of *er2* gene in diverse genetic backgrounds. The amplification or restriction patterns obtained with different markers in various genotypes are shown in Fig 4.9 and the information on polymorphisms detected with these markers in different genotypes is graphically summarized in Fig 4.10. The different markers showed polymorphic banding patterns in comparison to *er2* donor JI2480 in 6 to 22 varieties tested. The SSR AA278 was the most polymorphic marker which differentiated 22 pea varieties from JI2480. The CAPS markers PsLG3_CAPS-1, PsLG3_CAPS-3 and PsLG3_CAPS-4 showed polymorphism in 13, 15 and 16 commercial pea varieties, respectively. The SCAR marker ScX17_1400 generated dominant polymorphism in seven genotypes *viz*., Lincoln, Arkel, PB89, Him Palam-1, Pant Mattar-2, VRP-343 and Arka Ajit indicating limited applicability of this marker for marker assisted selection of *er2*. However, the CAPS marker CAPS-X17 derived from the same marker detected polymorphism in additional six genotypes *viz*., JI1559, AP-1, Palam Priya, Kashi Samarth, Kashi Samridhi and *Shijara Local*. Similarly, the different CAPS markers generated polymorphic banding patterns in pea varieties that could not be differentiated with AA278 e.g. PsLG3_CAPS-3 and PsLG3_CAPS-4 generated polymorphic banding pattern in pea genotypes PB89, Kashi Samridhi, Pant Mattar-2 and Pusa Pragati which could not be differentiated from JI2480 with marker AA278. All the markers generated monomorphic banding pattern in pea varieties Him Palam Mattar-2, AP-3 and Kashi Tripti. While the results are on expected line for variety Him Palam Mattar -2, which harbours *er2* gene introgressed from JI2480, the monomorphism in other two varieties suggested the need for testing additional restriction enzymes for generating CAPS polymorphisms between these varieties and JI2480. The CAPS markers and AA278 collectively differentiated 26 commercial pea varieties from JI2480 thus suggesting wider applicability of these markers in marker-assisted selection of *er2* gene. Most importantly, all the codominant markers developed during the present study have generated polymorphic banding pattern in *er1* donor genotype JI1559. Thus these markers can be effectively utilized to combine *er2* with *er1* gene in diverse genetic backgrounds for achieving broad spectrum and durable resistance against different powdery mildew species infecting peas.

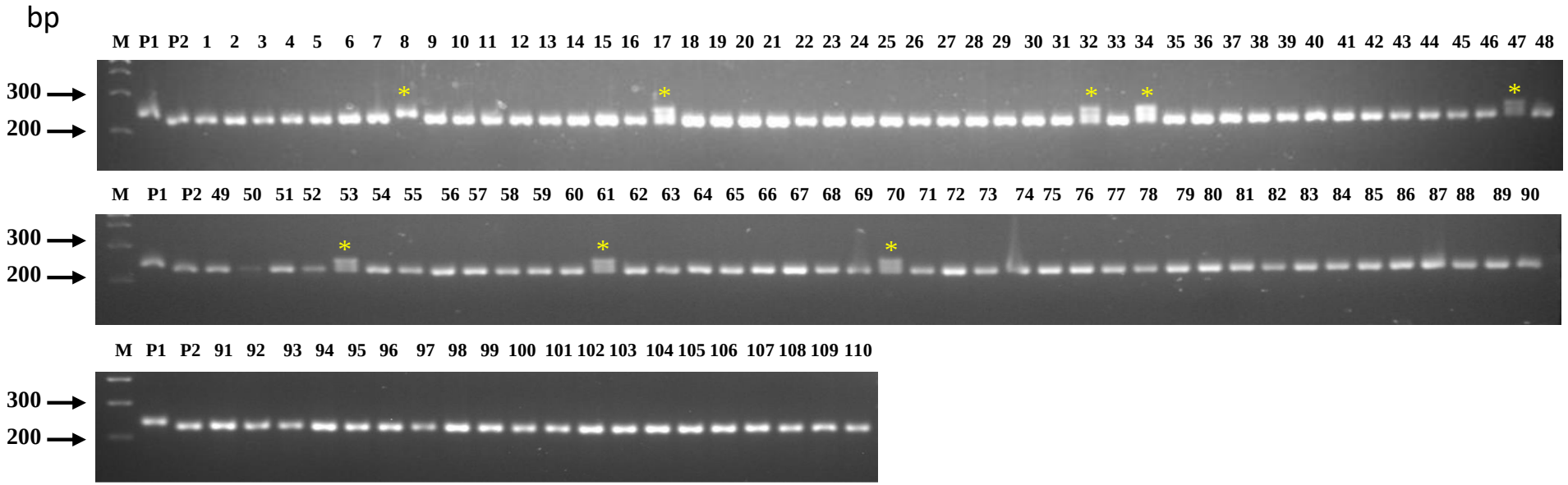

Fig 4.3. Genotyping of 110 powdery mildew resistant BC_7F_2 plants of cross Azad P-1 x JI2480 with SSR marker AA5. P1= Azad P-1; P2= JI2480. M:100 bp DNA ladder. The PCR products were resolved on 4 % agarose gel and visualized by ethidium bromide staining. ***Plants marked with asterisk are the recombinants.***

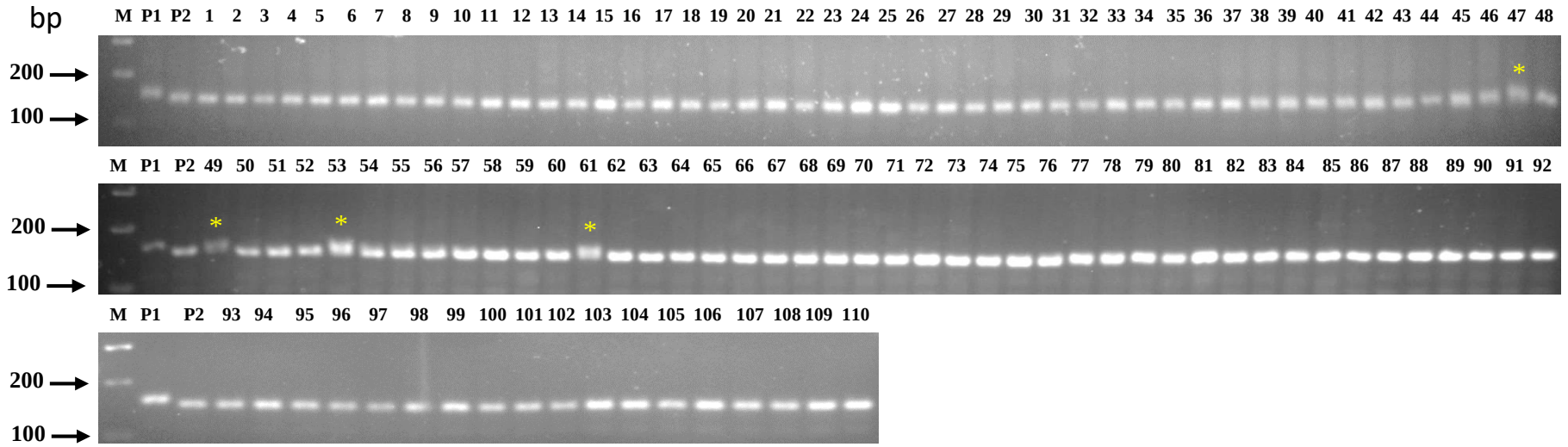

Fig 4.4. Genotyping of 110 powdery mildew resistant BC_7F_2 plants of cross Azad P-1 x JI2480 with SSR marker AA278. P1= Azad P-1; P2= JI2480. M:100 bp DNA ladder. The PCR products were resolved on 4 % super fine resolution agarose gel and visualized by ethidium bromide staining. ***Plants marked with asterisk are the recombinants.***

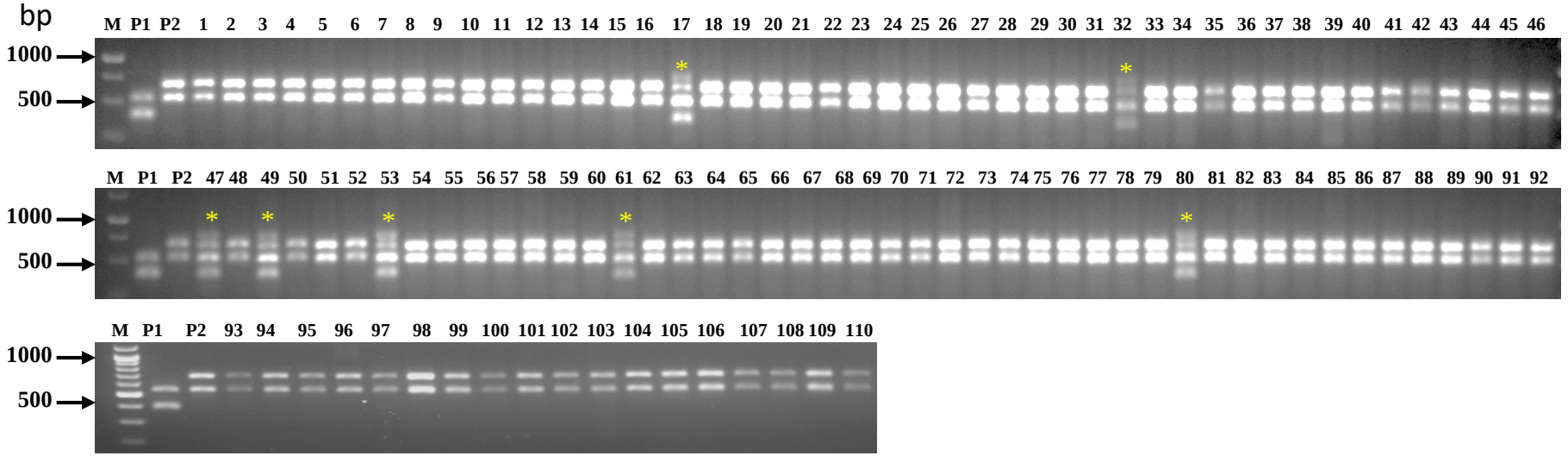

Fig 4.5. Genotyping of 110 powdery mildew resistant BC_7F_2 plants of cross Azad P-1 x JI2480 with Cleaved Amplified Polymorphic Sequence (CAPS) marker PsLG3_CAPS-3. P1= Azad P-1; P2= JI2480. M: 100 bp DNA ladder. The PCR products digested with restriction enzyme *DraI* were resolved on 2% agarose gel and visualized by ethidium bromide staining. *Plants marked with asterisk are the recombinants.*

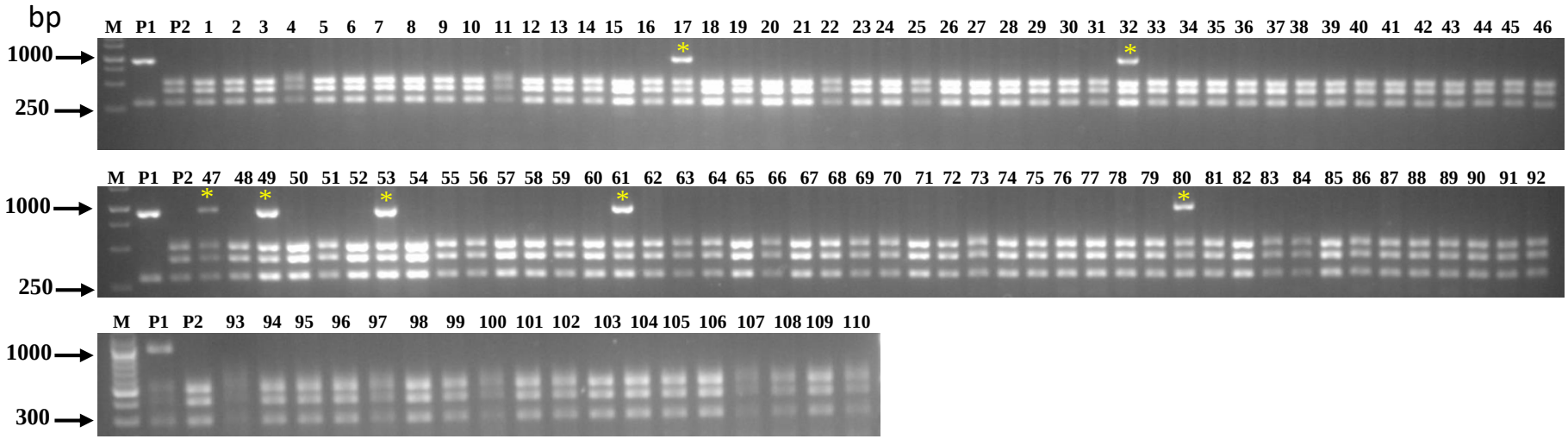

Fig 4.6. Genotyping of 110 powdery mildew resistant BC_7F_2 plants of cross Azad P-1 x JI2480 with Cleaved Amplified Polymorphic Sequence (CAPS) marker PsLG3_CAPS-4. P1= Azad P-1; P2= JI2480. M:100 bp DNA ladder. The PCR products digested with restriction enzyme *TaqI* were resolved on 2 % agarose gel and visualized by ethidium bromide staining. *Plants marked with asterisk are the recombinants.*

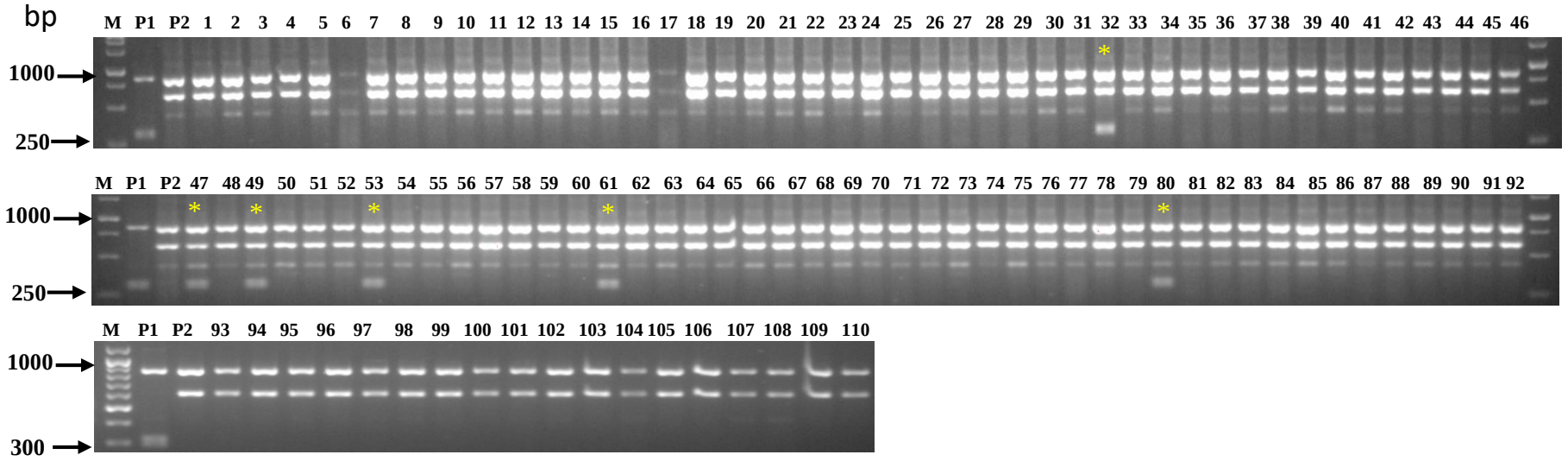

Fig 4.7. Genotyping of 110 powdery mildew resistant BC_7F_2 plants of cross Azad P-1 x JI2480 with Cleaved Amplified Polymorphic Sequence (CAPS) marker CAPS-ScX17. P1= Azad P-1; P2= JI2480. M:100 bp DNA ladder. The PCR products digested with restriction enzyme *HaeIII* were resolved on 2 % agarose gel and visualized by ethidium bromide staining. *Plants marked with asterisk are the recombinants.*

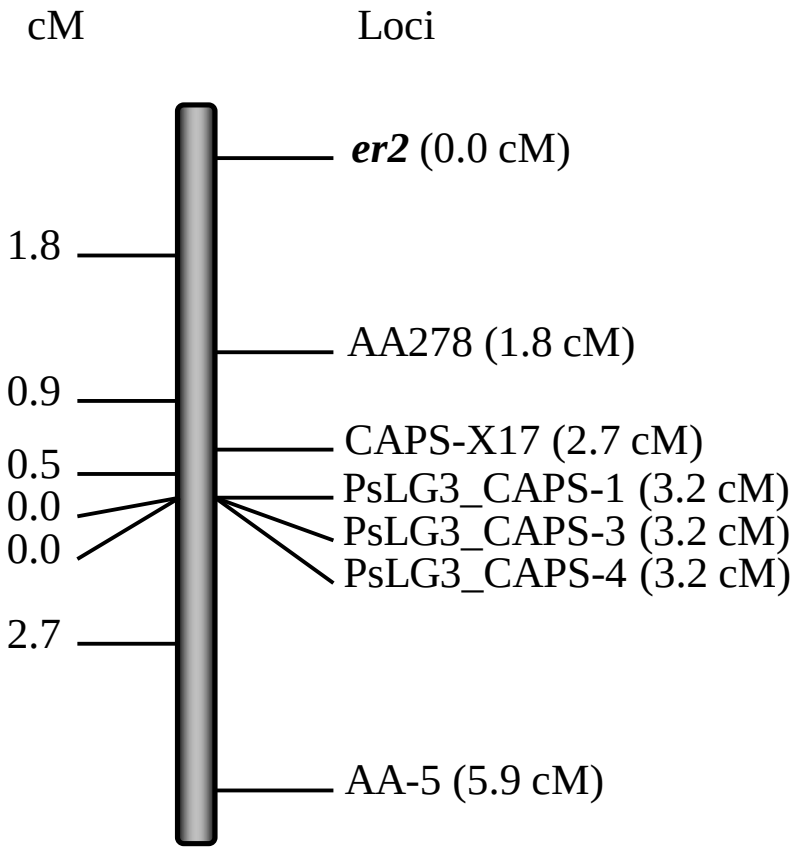

Fig 4.8. Linkage map of powdery mildew resistance gene *er2* on pea linkage group 3. Linkage map was constructed from the segregation analysis of 110 powdery mildew resistant BC_7F_2 progenies of cross Azad P-1/JI2480 with LG3 specific SSR and sequence tagged site makers. The maximum likelihood map order was determined with a LOD score threshhold of 5.0 and map distances (cM) are reported in Kosambi units.

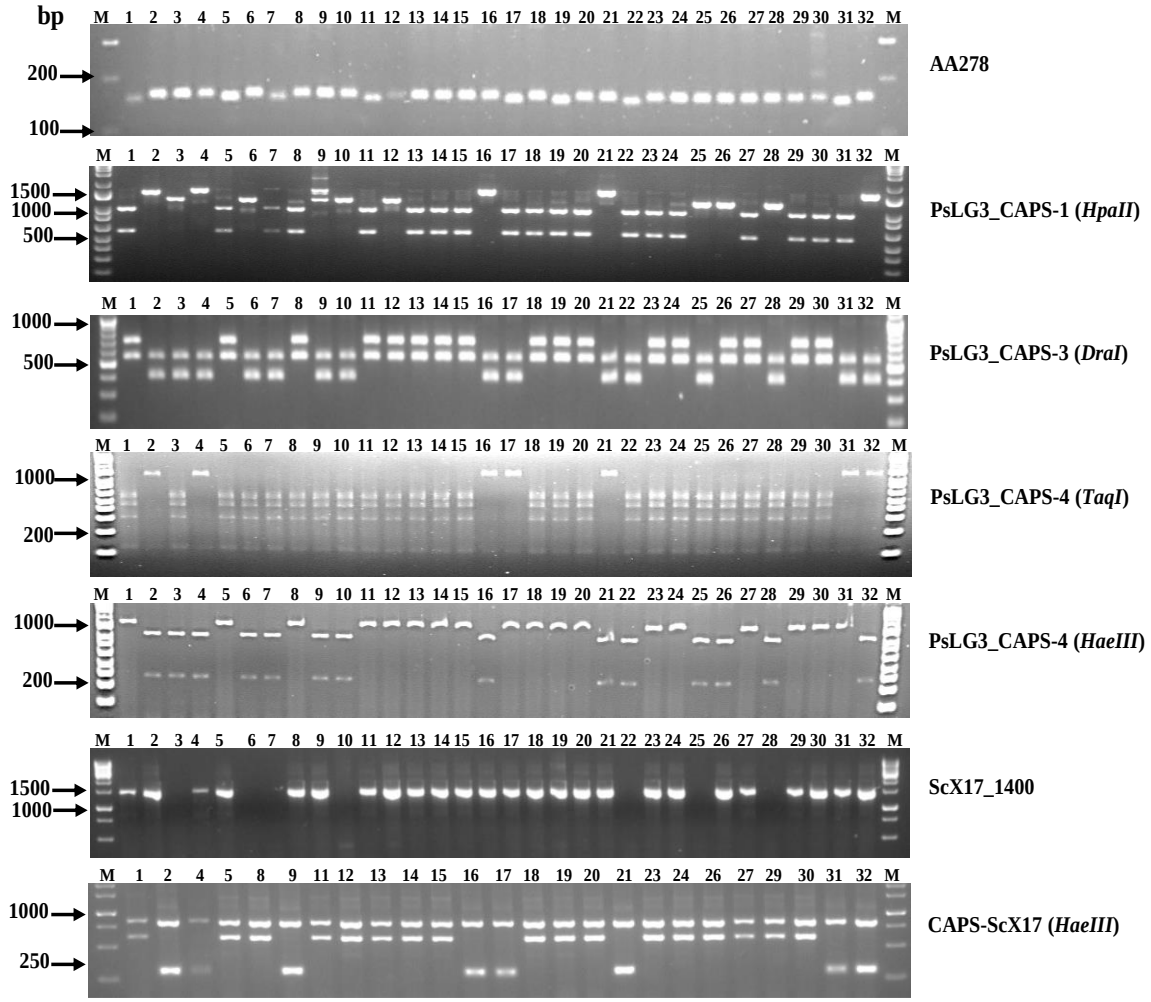

Fig 4.9. Validation of *er2* linked markers in commercial pea genotypes.

1= JI2480; 2= JI1559; 3= Lincoln; 4= Azad P-1 ; 5= Azad P-3 ; 6= Arkel ; 7= PB89 ; 8= PC-531 ; 9= Palam Priya ; 10= Him Palam Mattar-1; 11= Him Palam Mattar-2; 12= Kashi Ageti; 13= Kashi Mukti ;14= Kashi Nandini ;15= Kashi Purvi ;16= Kashi Samarth ; 17= Kashi Samridhi ; 18 Kashi Shakti ; 19= Kashi Tripti ; 20= Kashi Udai; 21= *Shijara Local* ; 22= Pant Mattar-2 ; 23= Kashi Nandini ; 24= Kashi Udai; 25= VRP343; 26= NDVP-250; 27= VRP Sel.-17; 28= Arka Ajit; 29= Arka Kartik; 30= Arka Priya; 31= Arka Uttam; 32= Pusa Pragati**.** M= Molecular weight ladder. The PCR products for AA278 were resolved on 4.0 % agarose gel while for others markers 2.0% agarose gel was used. *Note the genotypes Kashi Nandini (14, 23) and Kashi Udai (20, 24) were analyzed in duplicate to serve as a check for the reproducibility of each marker.*

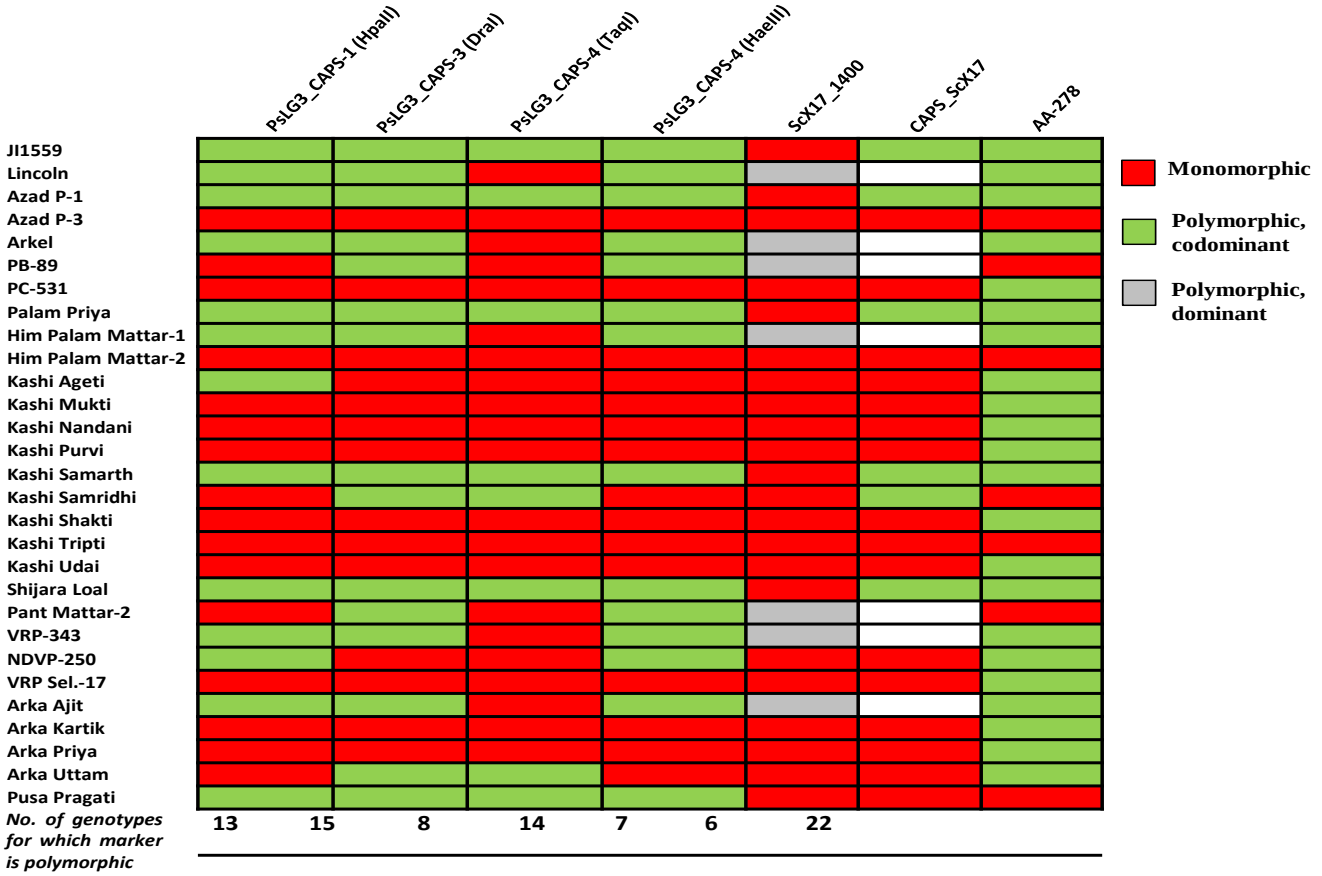

Fig 4.10. Graphical representation of polymorphisms between pea line JI2480 and commercial pea varieties for *er2* linked markers.

5. SUMMARY AND CONCLUSIONS

The present study was carried out to develop codominant markers for a recessive powdery mildew resistance gene *er2*. For this purpose, pea genome sequences around a previously known *er2* linked SCAR marker were chosen for developing a new set of STS markers, which were subsequently converted into polymorphic CAPS markers. The CAPS markers were analysed for their linkage to *er2* gene using a segregating mapping population. Finally, the markers showing tight linkage to *er2* gene were validated across a panel of 28 popular pea varieties that are cultivated in different parts of the country. The significant findings and implications of the study are summarized below:

1. The DNA sequence of ScX17_1400 marker from *er2* donor line JI2480 measured 1457 bp in length. Consistent with the reported location of *er2* on LG3, the DNA sequence of ScX17_1400 marker physically mapped to the genomic sequence of PsLG3 pseudomolecule released by Pea Genome International Pea genome Consortium (https://urgi.versailles.inra.fr), showing 99.51% homology with position 241616882 to 24618336 bp of the pseudomolecule.

2. All the ten STS markers developed from a 1038 kb sequence around the region of ScX17_1400 amplified monomorphic PCR patterns with the DNA of Azad P-1 and JI2480 and therefore were not *immediately* useful as markers.

3. When the STS markers were targeted for conversion into CAPS, polymorphic restriction patterns were generated for five STS markers, PsLG3STS-1, PsLG3STS-2, PsLG3STS-3, PsLG3STS-4 and PsLG3STS-6 when the amplicons obtained with these markers were digested with restriction enzymes HpaII, ScaI and HaeII, DraI, HaeIII and TaqI, and TaqI, respectively.

4. The previously known *er2* linked SCAR marker ScX17_1400 which amplified monomorphic amplicons in pea genotypes Azad P-1 and JI2480 could also be converted into a CAPS marker by digesting the PCR products with enzyme HaeIII. The CAPS marker derived from ScX17_1400 was designated as CAPS-ScX17.

5. The segregation of resistance and susceptibility to powdery mildew in BC_7F_2 population of cross Azad P-1 x JI2480 showed a good fit to 3: 1 (susceptible: resistance) ratio ($0.50 < P < 0.75$) suggesting the presence a single recessive gene *er2* for resistance to powdery mildew in pea genotype JI2480.

6. The four polymorphic CAPS markers developed during the present study and two pea LG3 specific SSR markers, AA278 and AA5, exhibited close linkage to *er2* when tested on 110 homozygous recessive powdery mildew resistant BC_7F_2 plants of cross Azad P-1 x JI2480. The linkage map of *er2* locus constructed with these six markers spanned 5.9 cM. The SSR marker AA278 was most closely linked to the *er2* gene at 1.8 cM while the CAPS-ScX17 was the next closely linked marker situated at a genetic distance of 2.7 cM from the gene. The three cosegrating markers PsLG3_CAPS-1, PsLG3_CAPS-3 and PsLG3_CAPS-4 were located at a distance of 3.2 cM from the gene.
7. The five codominant markers developed during the study exhibited polymorphism between *er2* donor line JI2480 and 6 to 22 commercial pea varieties tested. The SSR AA278 was the most polymorphic marker differentiating 22 pea varieties from JI2480. The CAPS markers PsLG3_CAPS-1, PsLG3_CAPS-3 and PsLG3_CAPS-4 showed polymorphism in 13, 15 and 16 commercial pea varieties, respectively.
8. The previously known marker ScX17_1400 for *er2* gene generated dominant polymorphism in seven genotypes *viz*., Lincoln, Arkel, PB89, Him Palam-1, Pant Mattar-2, VRP-343 and Arka Ajit indicating limited applicability of this marker for marker assisted selection of *er2*. However, the CAPS marker CAPS-X17 derived from the same marker detected polymorphism in additional six genotypes *viz*., JI1559, AP-1, Palam Priya, Kashi Samarth, Kashi Samridhi and *Shijara Local*.
9. The CAPS markers and AA278 collectively differentiated 26 commercial pea varieties from JI2480 thus suggesting wider applicability of these markers in marker-assisted selection of *er2* gene. These markers were also polymorphic in *er1* donor genotype JI1559. Thus these markers can be effectively utilized to combine *er2* with *er1* gene in diverse genetic backgrounds for achieving broad spectrum and long lasting resistance against powdery mildew disease in peas.

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Appendix-I

DNA Sequence of ScX17_1400 fragment amplified from pea genotype JI2480 (*5′*-to-*3′*)

GACACGGACCCAATGACATCTTCCATGTCTTCGTCATGACCCGCACCACTAGCT
TGACCACTACCTCCTGCACACCCCATGAATGTTGGTCTGCCCTCATGCCAATTA
GAATAAGATACGTACTCCGCCTAAGTCGGAAAAGTGCGGGCTTCGGGTGCTAA
GAATGTGTTCTGGAACTGCTGCTGCATGACATCAAATAAGAATGATTGAGACCT
CCTAGAAGCCTCAAAGTTTTCACAGCAGTAAGTGGCAAACGCCATCTGATCAA
AAACTGGTGGACAGCGGGCACGAGACCTAGATGAAGAAGTGCCTGCTCCCTC
AGCTCTATCCATCTGACTTTGGCAGAACCTGTTCAAAAACACATCATTAATCAC
ACTGGTTATAGAAAGGTGCACCTCATCCGGAATAGGCACATTTGCCCTTCGGCA
AAGCCCCATAATCAGCCCCGGATACAAGAGCACACCGACTCTCCCTCTGAACT
TTGTCCCGCTAGTTGCCACTTTTCTCATCTCCTCCGCGATGATAGACGCCACATC
CACCGATTTCCCAATCATAATGTAATACAAAAGGCAACCGACATCGAGTGGAAT
TGTGGTACTATGGCTCCGCGGCCGAATGTTGTTCAACAAAAGAACCAAATAAG
CCCGGGCTTCCAAATTAAGGTTTTCCCTCTTCCAAGATTTTGCAAATCCTTGATC
GTTCAAGTCATACGTCTTTCCCTCAAGGCAAAGGTGAGCACTAACATCAGTAAT
ATCCCAATCATTTGCGGTCTCTTTAGCACGGTATTCACACCGCATGTTACCCGCC
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TGGATTAGCTTTGTTCCCTAACATTGACGGTTTTGTTGATGTTAACGCCATTAGA
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GCATCTAAGGAATTCTAAGGGCGGTGGGGATATTGTCTGTTGCATTCCTGTTTTG
TACAGGTGGTTTATTTTGCACTTGCCTCAGACGCCTGCTTTTGTGGAGAACAAA
CAATGTCTATGATGGTCTCAGAGACTTATGTCTCTCACTAATGATGATATAGTTTG
GTATGATCCTTCTTTGAGCAGTTTGGAGATTATTGATTGTTGTGGTGAATTCTCT
AATGTGTCTCTCATTGGTACACAAGGAGGAATTAACTACAACCCTGCTTTGGCT
CGTCGTCAACTTGGGTTCCCCTTGAGAGACAAACCTAATAACATGTTGTTAGAA
GGTATTTTCTATCAAGAGGGTATAGATCCCCAACATTTGAAGTAGAAGATTGTGC
ATGCTTGGCATAATGTGCATAGGAAAGAAAGATCCGAGCTTGGTCC

Appendix-II

DNA sequences of STS markers retrieved from Pea Genome Consortium database (https://urgi.versailles.inra.fr/Species/Pisum/Pea-Genome-project).

>Chr5LG3: 240617105..240618630 (PsLG3STS-1)

ATGGTCCCAGTCCTTCAGAGTATCATGGGCGTTCATACATACTAAGGCACTGGT
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TTTCTAAGAGCAGGTTGATGGTTCCTTCAAACTCAACTAGGTTCAGATGGTGAA
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CACTTTGAACTTGTTGTATTATCTGCATCATAAATTCTTGTTGTTGTTGTTGTTGC
ATCTACAGTTGAAATTGTTGCATATACAAATGATGTTGTTGTTGCTGGTGCATTA
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CATGACCTTGCACCTGAAATATTGAGAATGGGGTGAAATACAACTATCTTATTG
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GTAACTCAGGGATGATTAGGATAATGAACGATCATAATAATCTACTCATCCTTTC
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GTCCACCAAATATGACTTCTATGGACTATGGTAGTACCTATGACACCCTAAACCC
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AACAAATTCTCATACTTGAGTAACATGTTCATGATTCAAGATAAAACATTGGTTC
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> Chr5LG3: 241030785..241032017 (PsLG3STS-2)

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AACTCTGAGTCAGAAGATACAAAAGCATTATTTTCTCACCTCTCAAAATCTAAT
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> Chr5LG3:241112089..241113436 (PsLG3STS-3)

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TTCTTCCAGTCTTGCAATGGTTGAACCAAGCTTCTCCTTTTCTAGGAGTAAAAC
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CATGTCTCTATAATTTTCAGCAAGATCTTCTGATGACATCTCTTCATCAGTAGAC
TCACTACAAGAGTCATATTTCCTAGTAAAAGCCATTACTTTATTAGTTATTTCAGC
ATTAGACTCAGACCGAGCGATTGATAATTCCTTTTTCTATTTCTTTAGAAAAGTG
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ATCATTCAAGGTCATCTCAAAGTTTTATAGGGAACCAATGAGTTCATCAACTTTT
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GGAAATGATTTGAGAATTTTCCTGGAAATATTTTTTTCAAACATATCTTCACCTA
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> Chr5LG3:241225388..241226587 (PsLG3STS-4)

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ATTCATCAGGCCAGAGTGAAGAGACTTGTCTCCCTCCTTCCTTACACTACATTC
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> Chr5LG3:241241624..241242893 (PsLG3STS-5)

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> Chr5LG3:241301451..241302778 (PsLG3STS-6)

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> Chr5LG3:241310970..241312404 (PsLG3STS-7)

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> Chr5LG3:241393222..241394690 (PsLG3STS-8)

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> Chr5LG3:241502003..241503208 (PsLG3STS-9)

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> Chr5LG3:241653959..241655448 (PsLG3STS-10)

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CCAATTTTGGGTCTGTGTCCATGGATACGGCAACTCTGACACGGTGCTTAGCAG
AAACTGAACTATCCACAGTTACATCATCTGATCTTTGGAGCGCTTCACTGCATG
ATCTGTATCCCTCTGTGGTG

BRIEF BIODATA OF STUDENT

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Permanent Address	:	Shakuntla Complex, VPO Rajpur, Tehsil Palampur, Distt. Kangra, Himachal Pradesh. Pin-176061

Academic Qualifications

Qualification	Year	Marks/ %/ CGPA	Subject	Institution / University
10th	2014	60%	English, Hindi, History, Civics and Geography, Mathematics, Science, Computer	Mount Carmel Sr. Sec. School, Palampur
12th	2016	74.50%	English, Mathematics, Physics, Biology, Chemistry	St Paul's Sr. Sec. School, Palampur
B.Sc. (Hons.) Agriculture	2021	68.50%	All courses offered in B.Sc. (Hons.) Agriculture	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur
M.Sc. (Agricultural Biotechnology)	2024	69.30%	Agricultural Biotechnology	CSK Himachal Pradesh Krishi Vishvavidyalaya

Title of M.Sc. Thesis: "Development and validation of co-dominant markers for pea powdery mildew resistance gene *er2*"