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# Gene-Specific Marker and Trait-Based Evaluation of Powdery Mildew Resistance in Garden Pea (*Pisum sativum var Hortense* L.)

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**Abstract**

Powdery mildew (PM), caused by *Erysiphe pisi* DC ex. Saint-Amans, poses a major constraint in pea cultivation, underscoring the identification of resistant genotypes for effective disease management. The current study employed *in-vitro, in-vivo*, and molecular screening methods to assess the PM reaction behaviour of 11 pea genotypes aiming to identify reliable resistance source. Field assessments during two crop growth phases were carried out at two locations namely Hawalbagh (Almora) and Mukteshwar (Nainital). Among the genotypes tested VP-2020-101 and VP- 2024-55 were categorised as resistant and showed the lowest disease severity at both locations, with significantly limited Disease Incidence (DI) and Percent Disease Index (PDI). These results were corroborated by detached leaf method assay conducted under polyhouse (spore proof chamber) and incubator conditions, where VP-2020-101 and VP- 2024-55 consistently showed minimal sporulation macroscopically and sparse mycelial development microscopically. Molecular validation with gene-specific SCAR markers revealed the presence of resistance genes viz. *er1*, *er2*, and *Er3* in VP-2020-101, while VP- 2024-55 carried only *er1*. Marker ScOPE-$16_{1600}$ relaibly tracked *er1*, while ScOPX-$17_{1400}$ and $ScW4_{637}$ confirmed the presence of *er2* and *Er3*, respectively. Integrating phen-c and geno-typic data strongly supports VP-2020-101 as a promising donor genotype for pyramiding powdery mildew resistance in the popular garden pea cultivars/genotypes. These findings demonstrate the importance of integrated disease screening approaches to precisely identify robust sources of durable resistance facilitating their effective deployment in future pea breeding programs.

**Keywords:** Disease screening, Garden pea, Powdery Mildew, Gene specific markers.

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## Declarations

### Ethics approval and consent to participate

This study does not involve any human or animal participation

### Consent for publication

All authors have given their consent for publication

### Availability of data and materials

The original contributions presented in this study are included in the article.

### Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Author contributions

**NKH**: Conceptualization, reviewing and editing, **SS**: Conceptualization, Investigation, Data Curation, Formal analysis, Writing - Original Draft; **KKM:** Pathological investigation **AS**: Investigation and data curation, writing original draft, **AT**: Data curation and writing review, **RD**: reviewing and editing, **SK**: Supervised molecular work, reviewing and editing **LK**: Resources, Supervision and reviewing.

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## 1. Introduction

Garden pea (*Pisum sativum var Hortense* L, 2n = 2x = 14) is a cool season legume vegetable crop grown for its tender green pods in temperate and tropical highlands of the world (Gupta et al. 2024; Azmat et al. 2010). It is one of the oldest model plants in plant genetics and is considered among the most extensively studied genetic systems in crops next to maize. The green and dry foliage are used as cattle feed and green pods being highly nutritious are preferred for culinary purpose (Devi et al., 2018). Globally it is consumed as green tender and dried forms and also processed into canned, dehydrated, and frozen forms. Pea has numerous nutritional benefits due to presence of functional compounds like essential amino acids (threonine, lysine, methionine, cysteine, and tryptophan), minerals, fatty acids (oleic, linolenic, and linoleic acid), and carbohydrates (Villalobos Solis et al. 2013). It is also rich in proteins, minerals, vitamins and fibres. Protein content in pea ranges from 26-33% and 23-31 % in wrinkle and smooth seeded varieties of pea with fibre content between 4 to7% (Cousin 1997; Janani et al. 2024). India ranks second in area and production of peas as cultivated on 0.563 million hectares' land, yielding 5.703 million metric tonnes, with a productivity of about 10.13 t/ha (NHB 2020). Having its own importance there are several stress constrains hindering in achieving potential production of garden peas, one among them is powdery mildew (PM). PM is one of the major threat in peas cultivation leading up to 50% yield and quality loss (Warkentin et al. 1996; Katoch et al. 2010).

PM in peas is caused by *Erysiphe pisi* DC ex. Saint-Amans, an obligate biotrophic fungal phytopathogen that depends on photosynthetic activity of the host and cannot survive on photosynthetically inactive tissues (Carver and Jones 1988). Temperature plays an important role in PM development and epidemics in peas with an ideal range of 20-24□ favouring conidial germination and proliferation of the pathogen (Smith 1970; Pheirim et al. 2021). The disease shows its prominence in warm dry days and cool nights (Sillero et al. 2006). PM lesions appear as white talcum like growth in the aerial parts of the plants. In cases of severe infestation, the fungus can penetrate up to seeds causing seed discoloration and leading to pre-mature drying of whole plant (Singh et al. 1995; Pheirim et al. 2021). This disease can be controlled by cultural practises and use of fungicides but their efficacy is limited and also lead to environmental pollution. Identification and development of cultivars having inherent resistance is effective method to manage this disease in farmer field. But the developing resistant cultivars requires reliable sources and effective screening method also. In *Pisum* species, two monogenic recessive resistant genes namely *er1* (Harland 1948) and *er2* (Heringa et al. 1969) as well as one monogenic dominant gene *Er3* (Fonddevilla et al. 2007) have been identified as confering inherent resistance to PM. Molecular markers linked to these resistance genes can be effectively employed in early screening of genotypes harbouring resistance to PM. Among various molecular markers, PCR based markers are more desirable as it requires template DNA in small quantity and can be employed in large populations. Sequence Characterized Amplified Region (SCAR) and Simple Sequence Repeats (SSR) markers reported to be linked with powdery mildew resistance in peas (Katoch et al. 2010; Reddy et al. 2015; Cobos et al. 2018; Pheirim et al. 2021; Gupta et al. 2024). These markers can be effectively employed for molecular screening of genotypes and also valuable for pyramiding of PM resistance genes into a single genetic background. In present investigation in-vitro, in-vivo characterization of institute developed garden pea varieties and lines for PM resistance have been made and genes responsible for powdery mildew resistance in these lines were validated using gene specific SCAR markers.

## 2. Materials and method

### 2.1 Experimental location and materials

The present experiment was carried out at ICAR-Vivekananda Parvathiya Krishi Anusandhan Sansthan (VPKAS), Almora, experimental farm Hawalbagh (Coordinates 29.61□N, 79.67□E) and High-Altitude Testing site (HATS), ICAR-VPKAS, Mukteswar, Nainital (Coordinates 29.4722°□N, 79.6482°□E) located in mid-hills of Himalayas in Indian state of Uttarakhand. The plant material used for experiment comprised of 11 genotypes of garden pea maintained in the institute Table 1. The screening of germplasm was carried in both in-vivo and in-vitro condition in *rabi* (a standard Indian term for winter season) 2024-2025.

**Table 1: Experimental material used in the study**

| Genotypes | Remarks | Release date |
|---|---|---|
| Vivek Matar -11 | Released varieties of vegetable type pea from ICAR-VPKAS, Almora | 12-8-2010 |
| Vivek Matar -12 | | 17-8-2015 |
| VL Sabji Matar-15 | | 05-02-2019 |
| Arkel | Released variety, seeds maintained at ICAR-VPKAS, Almora | Introduced variety in 1970 |

| | |
|---|---|
| VP-2317<br>VP-2321<br>VP-1920<br>VP-2206<br>VP-2307<br>VP-2020-101<br>VP- 2024-55 | Advance lines of vegetable type garden pea from ICAR-VPKAS, Almora |

### 2.2 *In-vivo* screening

*In-vivo* screening was carried under naturally ventilated Poly-house condition at both the above mentioned locations. The experiment was laid out in a Randomized Block Design (RBD) with three replications. Each genotype was sown in ten rows, eachof 3 m length, maintaining standard spacing between rows and plants. Disease development and scoring was recorded at two key growth stages viz. pod development and first picking. At Hawalbagh site, sowing took place on Dec 12, 2024 with disease scoring at pod development on February 22, 2025 (70 days after sowing) and at first picking on March 3, 2025 82 days after sowing). At Mukteshwar, sowing was done on Dec 18, 2024 with disease scoring at pod development on April 2, 2025 (105 days after sowing) and at first picking on April 18, 2025 (111 days after sowing).

The individual plants were categorized into different classes as per disease severity scale (Table 2) following the 0–5 scale (Gawande and Patil, 2003). Percent disease index (PDI) was calculated by following formula given by Pandey et al. (2003) and Rasool et al. (2021) while disease incidence was calculated as per Awan et al. (2018). The host plant reaction was categorised as highly resistant (HR) (0–5 percent), resistant (R) (5.1–12.0 percent), moderately resistant (MR) (12.1–25.0 percent), moderately susceptible (MS) (25.1–50.0 percent), susceptible (S) (50.1–75.0 percent), and highly susceptible (HS) (>75 percent) based on the mean PDI.

$$\text{Percent disease index (\%)} = \frac{\text{Sum of all rating}}{\text{Total number of observation} \times \text{Maximum rating grade}} \times 100$$

$$\text{Disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

To eliminate chances of disease escape, in vitro multiplied conidial inoculums of the disease maintained in susceptible cv. Arkel in greenhouse of ICAR-VPKAS was collected and dusted on the plants twice with camel brush hair (55-days old plant and 75-day old plant) for uniform development of disease infestation to facilitate effective screening of lines for resistance under polyhouse condition. Also, the susceptible genotype Arkel (Sharada and Makandar 2023) was used as spreader or infector line after every ten rows. Arkel was also used as susceptible check in the experiment.

**Table 2: 0-5 Scale used for powdery mildew disease scoring in field under poly-house condition**

| Scale Used | Leaf area affected | Disease Reaction |
|---|---|---|
| 0 | 0% | Immune (I) |
| 1 | 0.1-10.0% | Resistant (R) |
| 2 | 10.1-25.0% | Moderately resistant (MR) |
| 3 | 25.1-50% | Moderately susceptible (MS) |
| 4 | 50.1-75% | Susceptible (S) |
| 5 | 75.1-100.0% | Highly susceptible (HS) |

### 2.3 *In-vitro* screening

The detached leaf method, as described by Banyal (1994) and Vaid and Tyagi (1997), was used for in-vitro screening and evaluating powdery mildew reaction on the genotypes. In brief, four to five leaflets detached from 30-40 days old seedlings of each genotype were floated in 90mm Petri dishes containing 25 ml of 50ppm solution of benzimidazole to enhance leaf longevity. The leaflets were dusted with PM inoculum collected from the PM infected pea fields at Hawalbagh site. One set of PM inoculated Petri dishes, along with uninoculated controls were sealed using parafilm and incubated at 25 ± 1□ under 16 h photoperiod while second set was kept in a spore proof chamber in polyhouse condition at room temperature. After 10 days of inoculation, the disease reaction was assessed microscopically through compound microscope (Olympus CX21, Tokyo, Japan) using a 0–4 scale (Vaid and Tyagi 1997). The leaflets rated 0, 1 and 2 were classified as resistant (Table 3) and those rated 3 and 4 declared susceptible (Banyal 1994; Vaid and Tyagi 1997).

**Table 3: 0-4 scale used for scoring disease reaction in detached leaf method**

| Scale | Description | Rate |
|---|---|---|
| 0 | macroscopically or microscopically no mycelial growth is evident | Resistant |
| 1 | microscopically sparse mycelial growth with rare conidiophores is seen | Resistant |
| 2 | microscopically slight growth of mycelium with a little sporulation is seen and individual conidiophores on a colony can be easily counted | Resistant |
| 3 | microscopically moderate development of mycelium with moderate to heavy sporulation is seen | Susceptible |
| 4 | microscopically abundant development of mycelium with heavy to very heavy sporulation is visible | Susceptible |

### 2.4 Molecular screening

Molecular screening was carried using a set of 6 SCAR markers reported to be linked with PM resistant genes namely *er1, er2* and Er3 (Table 4). For SCAR based molecular screening genomic DNA was extracted using young leaves of the genotype using CTAB method as suggested by Murray and Thompson (1980) with slight modification. The integrity of DNA was assessed using agarose gel electrophoresis and quantified with a Nano-drop spectrophotometer (Thermo scientific Nano-drop 2000) (Ausubel et al. 1995). DNA was suspended in Milli-Q water to a concentration of 50 ng/µl and stored at −20°C until further use.

Polymerase chain reaction (PCR) was carried out that each reaction comprises of 1× reaction buffer, 0.76 U Taq DNA polymerase, 20µM dNTPs, 20ng each of forward and reverse primers and 50 ng of genomic DNA. The PCR amplifications were carried out in a thermocycler (ABI, Thermo Scientific, USA). The thermal profiling for PCR was as follows: an initial denaturation at 94 °C for 5 min followed by 35 cycles of 94 °C for 1.0 min, the annealing temperature for 2 min, extension at 72 °C for 2 min, with a final extension at 72 °C for 10 min followed by storage at 4°C. To evaluate the DNA amplification,10 µl of each PCR product was mixed with 3 µl of 6X gel loading dye and electrophoresed on 2.5% agarose gel.. Agarose gels were visualized and documented using the gel documentation system (Protein simple, Alpha Imager EC, USA). Amplicons of the expected size corresponding to markers linked with powdery mildew resistance genes were successfully detected.

**Table 4: List of SCAR markers used in the study**

| Gene | Marker | Forward primer | Reverse primer | Band size (bp) | References |
|---|---|---|---|---|---|
| *er1* | ScOPX $04_{880}$ | CCGCTACCGATGTT ATGTTTG | CCGCTACCGAACTGGTT GGA | 880 | Srivastava *et al*., 2011 |
| *er1* | Sc-OPO-$18_{1200}$ | CCCTCTCGCTATCC AATCC | CCTCTCGCTATCCGGTGT G | 1200 | Tiwari *et al*., 1998 |
| *er1* | ScOPE-$16_{1600}$ | GGTGACTGTGGAAT GACAAA | GGTGACTGTGACAATTC CAG | 1600 | |
| *er2* | ScX$17_{1400}$ | CAGAAGCGGATGAG GCGGA | GACACG GACCCAATGACATC | 1400 | Katoch *et al*., 2010 |
| Er3 | SCAB$1_{874}$ | CCGTCGGTAGTAAA AAAAACTA | CCGTCGGTAGCCACACC A | 874 | Fondevilla *et al*., 2008 |
| Er3 | SCW$4_{637}$ | CAGAAGCGGATGAG GCGGA | CAGAAGCGGATACAGTA CTAAC | 637 | |

## 3. Results

### 3.1 *In-vivo* screening

*In-vivo* screening of 11 pea genotypes for PM at both locations—Hawalbagh and Mukteshwar—across two growth stages: pod development and first picking revealed variable disease progression among genotypes and between locations. The weather parameters including minimum and maximum temperature and relative humidity of both the locations during the experimental growth period is given in the Supplementary table. At Hawalbagh, no disease symptoms were observed at the pod development stage across all genotypes (Table 5). However, as the crop matured, a gradual increase in disease severity was recorded. By the first picking stage, the highest disease score (scale: 3) was recorded in VP-2206 as well in Arkel a susceptible check, showed moderately susceptible disease reaction. Except for Vivek Matar-11 which showed moderately resistant (2.0) disease reaction, all other genotypes (Vivek Matar-12, VLSM-15, VP-2317, VP-2321, VP-2307, VP-2020-101 and VP- 2024-55) exhibited resistant (scale: 1.0) disease reaction. At Mukteshwar, disease onset of PM occurred at an earlier growth stage due to favourable temperature and humidity conditions for PM development, with several genotypes showing disease symptoms as early as the pod development stage (Table 5). At the first picking stage, overall disease severity was higher, with Arkel showing highly susceptible disease reaction (scale: 5.0) followed by three susceptible genotypes (Vivek Matar-11, Vivek Matar-12 and VP-2321) and two moderately susceptible genotypes (VLSM-15 and VP-2317). Moderately resistant disease reaction was displayed by VP-1920 and VP-2206, whereas, VP-2020-101 and VP- 2024-55 exhibited the least disease progression by the final stage and were classified as resistant genotypes (scale 1.0).

Disease scoring at Mukteshwar was employed to calculate Disease Incidence (DI) and Percent Disease Index (PDI). Except for VP-2020-101 and VP- 2024-55, all the genotypes showed 100% disease incidence and were classified as Susceptible (S), with PDI values ranging from 60.23% (VL Sabji Matar-15) to 70.74% (Arkel). The second highest PDI was observed in VP-2321 (67.36%) followed by VP-2206 (67.28%), and Vivek Matar-11 (66.67%). In contrast, VP-2020-101 and VP- 2024-55 recorded disease incidences of 86.667% and 83.333%,

193 respectively, along with significantly lower PDI values of 11.11% and 11.52%. Accordingly, these two genotypes
194 were therefore classified as Resistant (R) to powdery mildew under Mukteshwar conditions (Table 5, Figure 1).

195 Briefly, VP-2020-101 and VP- 2024-55 hold potential, as sources of resistance to powdery mildew, while the
196 remaining ten genotypes are susceptible and showed progressive disease development throughout the crop growth
197 stages at both locations.

198

Table 5: **Powdery mildew resistance Scoring of 11 garden pea genotypes across two growth stages and location at 0–5 scale; DI and PDI under Mukteshwar field condition**

| Genotypes | Disease score | | | | | | | | Disease incidence (%) | Percent Disease Index (%) | Disease reaction[$] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hawalbagh | | | | Mukteshwar | | | | Location: Mukteshwar | | |
| | Pod development stage | | First picking | | Pod development stage | | First picking | | | | |
| | Rating | Reaction | Rating | Reaction | Rating | Reaction | Rating | Reaction | | | |
| Vivek Matar -11 | 0 | I | 2 | MR | 3 | MS | 4 | S | 100 | 66.67 | S |
| Vivek Matar -12 | 0 | I | 1 | R | 1 | R | 4 | S | 100 | 63.13 | S |
| VL Sabji Matar-15 | 0 | I | 1 | R | 2 | MR | 3 | MS | 100 | 60.23 | S |
| Arkel | 0 | I | 3 | MS | 3 | MS | 5 | HS | 100 | 70.74 | S |
| VP-2317 | 0 | I | 1 | R | 2 | MR | 3 | MS | 100 | 63.4 | S |
| VP-2321 | 0 | I | 1 | R | 2 | MR | 4 | S | 100 | 67.36 | S |
| VP-1920 | 0 | I | 0 | I | 1 | R | 2 | MR | 100 | 65.81 | S |
| VP-2206 | 0 | I | 3 | MS | 1 | R | 2 | MR | 100 | 67.28 | S |
| VP-2307 | 0 | I | 1 | R | 1 | R | 3 | MS | 100 | 64.81 | S |
| VP-2020-101 | 0 | I | 1 | R | 1 | R | 1 | R | 86.667 | 11.11 | R |
| VP- 2024-55 | 0 | I | 1 | R | 1 | R | 1 | R | 83.333 | 11.52 | R |

[$] *S: Susceptible; MS: Moderately susceptible; MR: Moderately Resistant; R: Resistant*


**Figure 1: Powdery mildew incidence in field condition at Mukteshwar in resistant (a: VP-2020-101 and b: VP- 2024-55) and susceptible (c: Arkel) genotypes.**

### 3.2 *In-vitro* screening

Detached leaf assay was conducted to assess the resistance response of 11 pea genotypes to powdery mildew under three experimental conditions: control (uninoculated, incubated), incubator (inoculated and incubated) and polyhouse (inoculated in spore proof chamber in polyhouse). Under the control condition, no disease symptoms were observed in any genotype throughout the experimental period. This confirms the absence of natural inoculum and rules out contamination during the assay.

Under polyhouse conditions, seven genotypes namely Vivek Matar-11, VP-2321, VP-1920, VP-2020-101, and VP- 2024-55—exhibited a resistant (R) reaction, with a disease severity score of 2 on a 0–4 scale. In contrast, six genotypes viz. Vivek Matar-12, VL Sabji Matar-15, Arkel, VP-2317, VP-2206, and VP-2307—were classified as susceptible (S) as displayed higher disease scores of 3 or 4. Under incubator conditions, only five genotypes—VL Sabji Matar-15, VP-2317, VP-2321, VP-1920, VP-2020-101 and VP- 2024-55—maintained resistant reactions with low disease scores (1–2). Whereas the remaining genotypes, including Vivek Matar-11, Vivek Matar-12, Arkel, VP-2206, and VP-2307, were categorized as susceptible due to higher disease scores (≥3) (Table 6).

Notably, genotype VP-2020-101 consistently exhibited resistance across all conditions. It recorded no symptoms (Scale: 0) in the control condition, minimal infection (scale: 1) in the incubator and a slightly higher (scale: 2) but still resistant response in the polyhouse. These outcomes indicated that VP-2020-101 possesses a strong and stable resistance to powdery mildew. Similarly, VP- 2024-55 also demonstrated a high degree of resistance mechanism effective under both controlled (incubator) and semi-natural (polyhouse) conditions. In both of these genotypes, the resistance was characterised by the presence of sparse mycelial growth and minimal sporulation of the pathogen as observed microscopically and macroscopically (Figure 2). Arkel, VP-2206, and VP-2307 showed susceptibility under polyhouse and incubator conditions evidenced by presence of abundant mycelial growth and

profuse sporulation of the pathogen depicting. The detached leaf assay effectively distinguished varying levels of resistance among genotypes. The results were largely consistent with field observations, further validating the reliability of the assay in screening for powdery mildew resistance under in-vitro condition.

**Table 6: Scoring of garden pea genotypes for powdery mildew resistance under in-vitro condition (Detached leaf method)**

| Genotypes | Control | | Polyhouse | | Incubator | |
|---|---|---|---|---|---|---|
| | Scale | Rate | Scale | Rate | Scale | Rate |
| Vivek Matar -11 | 0 | R | 2 | R | 3 | S |
| Vivek Matar -12 | 0 | R | 4 | S | 3 | S |
| VL Sabji Matar-15 | 0 | R | 3 | S | 2 | R |
| Arkel | 0 | R | 4 | S | 3 | S |
| VP-2317 | 0 | R | 3 | S | 2 | R |
| VP-2321 | 0 | R | 2 | R | 2 | R |
| VP-1920 | 0 | R | 2 | R | 2 | R |
| VP-2206 | 0 | R | 3 | S | 3 | S |
| VP-2307 | 0 | R | 3 | S | 3 | S |
| VP-2020-101 | 0 | R | 2 | R | 1 | R |
| VP- 2024-55 | 0 | R | 2 | R | 2 | R |



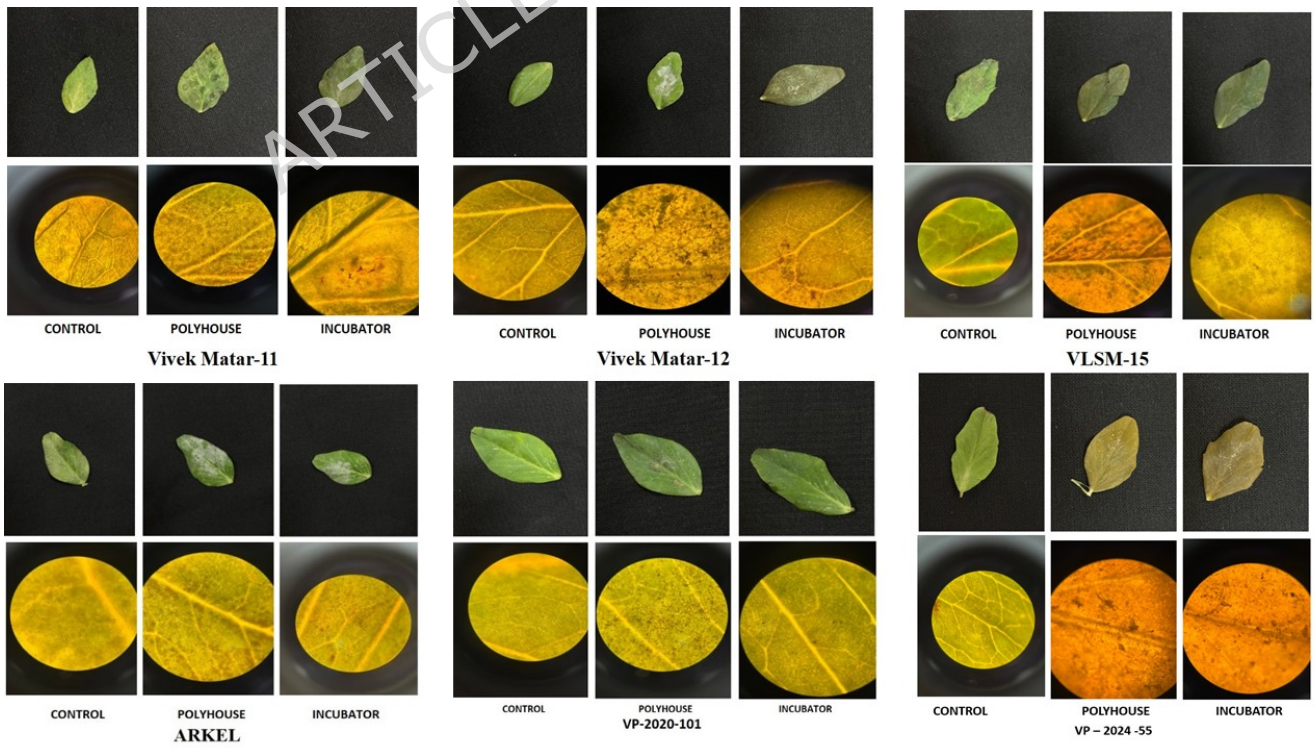

**Figure 2: Microscopic and macroscopic views of garden pea genotypes (VM-11, VM-12, VLSM-15, Arkel, VP-2020-101 and VP- 2024-55) showing resistant phenotype in both the inoculated condition (polyhouse and incubator) and control check.**

### 3.3 Molecular screening

For validation of powdery mildew resistance, six markers namely, ScOPX $04_{880,}$ Sc-OPO-$18_{1200}$ and ScOPE-$16_{1600}$ linked to *er1*, ScOPX-$17_{1400}$ linked to *er2* and SCAB$1_{874}$ and SCW$4_{637}$ linked to *Er3* were used. The resistant check included, *er1* donor er000202, *er2* donor er000203 and *Er3* donor P660-4 were used, whereas, Arkel was used as a susceptible check. The amplification profiles for *er1, er2* and *Er3* linked markers are presented in the Figure 3. Among the *er1* linked marker, ScOPE-$16_{1600}$ was found to be polymorphic between the resistant check, susceptible check and the genotypes showing resistance in the in-vivo condition. Amplicon of size 1600bp was detected in donor er000202 and genotypes VP- 2024-55 and VP-2020-101, but it was absent in the susceptible check i.e. Arkel. These results highlighted the potential of ScOPE-$16_{1600}$ for effectively tracking the presence of the *er1* gene among the garden pea genotypes. For *er2* linked marker ScOPX-$17_{1400}$ amplified a 1400bp amplicon product in resistant check er000203 and genotype VP-2020-101, indicating the presence of *er2* gene. Similarly, ScW$4_{637}$ marker linked to the *Er3* gene generated a 637bp amplicon in resistant check P660-4 and genotype VP-2020-101, confirming the presence of *Er3* gene.

Genotype VP-2020-101 was found to carry all three resistance genes (***er1*, *er2,*** and *Er3*), as evidenced by the presence of respective markers. Additionally, genotype VP- 2024-55 was positive for the *er1* gene. These findings suggest that VP-2020-101, with its pyramided resistance genes, holds significant potential as a genetic resource in breeding programs aimed at developing durable powdery mildew resistance in pea. Moreover, its resistance validates the results observed under both in vitro and in vivo conditions.

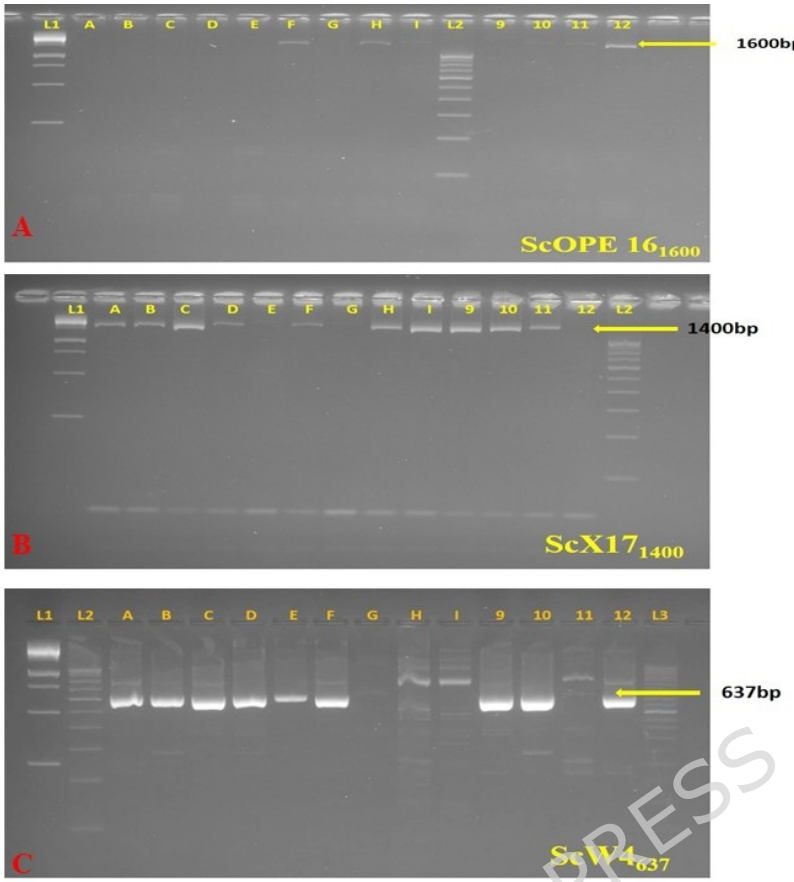


**Figure 3: Agarose gel images depicting polymorphism of amplification products linked to powdery mildew resistance genes (*er1, er2* and Er3) in pea genotypes. 3A shows a 1600□bp product amplified by the *er1*-linked marker ScOPE-16$_{1600}$, 3B shows a 1400□bp product with the *er2*-linked marker ScX-17$_{1400}$, and 3C shows a 637□bp product amplified by the *Er3*-linked marker ScW4$_{637}$. Lane details for all gels include: C. Arkel (susceptible check), G. P660-4 (resistant check for Er3), H. er000203 (resistant check for er2), I. er000202 (resistant check for er1), 10. VP-2024-55, 11. VP-2020-101, L1: 1□kb ladder (Puregene), L2: 100□bp ladder (GeNei), and L3: 50□bp ladder (GeNei). These gel images without labelling is also presented as supplementary information.**

## 4. Discussion

Powdery mildew caused by *Erysiphe pisi* is regarded as one of the major constrain in achieving potential production causing economical losses by having significant impact on the quantity and quality of pea crop. One conventional way to control the PM disease is the use of Sulphur containing chemical fungicide (Warkentin et al. 1996). However, their unsustainability and the high cost of repeated applications preclude their extensive use in many countries (Fondevilla et al. 2012). Thus, the development of genetic resistance is more favoured to attain a stable resistance in the cultivated genotypes of garden pea. Historically, genotype resistant to powdery mildew was first described by Hammarlund (1925). Numerous resistant lines have been identified in earlier studies for powdery mildew resistance through both natural epiphytotic conditions and controlled artificial inoculation techniques (Rana et al. 2023). So far, three genes, two recessive and a dominant, have been identified that confers resistance to powdery mildew in pea. Harland (1948) first reported resistance as a monogenic recessive trait and

designated the gene as *er1*. In contrary, Heringa et al. (1969) identified the resistance gene *er2* in Pervuian material that was confined to leaves of pea providing complete resistance above 25˚C. Later, Fondevilla et al. (2007) reported a third dominant gene (*Er3*) in *P. fulvum* that segregates independently from both *er1* and *er2* genes.

The expression and presence of above genes can be evaluated through conventional (field screening and detached leaf assay) as well as molecular assay techniques. Though, the conventional methods give proper result especially field-based screening but the screening become complicated during experiment associated with obligate fungus like *Erysiphe* spp. and resistance governed by recessive genes (Raj et al. 2024). Therefore, the current study evaluated powdery mildew resistance across two different agro-climatic areas using a multifaceted strategy by integrating in-vitro assay, molecular marker analysis, and in-vivo testing to identify the resistance sources suitable for incorporation in the gene pyramiding and breeding programs. Strong insights into the resistance behaviour of these genotypes have been obtained by the integration of field, controlled environment, and molecular data. Earlier, Rana et al. (2013) also screened the pea germplasm in different agro-climatic regions under natural epiphytotic conditions. The multi-location trials help to avoid the ambiguities in disease reaction due to environmental factors.

Field screening at Hawalbagh and Mukteshwar highlighted significant variability in powdery mildew incidence and progression across both locations and developmental stages. The result is in agreement with Fondevilla et al. (2007b). At Hawalbagh, disease symptoms were absent at the pod development stage but increased steadily with crop maturity, whereas at Mukteshwar, symptoms appeared earlier and intensified due to conducive environmental conditions, particularly favourable temperature and humidity levels at Mukteshwar. During the disease assessment stage (February–March) at Hawalbagh, the average maximum and minimum temperatures were around 23 °C and 1 °C, respectively, with mean relative humidity below 45%. Such dry conditions are generally unfavorable for the development and spread of foliar fungal pathogens like powdery mildew. In contrast, at Mukteshwar, during May, the mean temperature remained around 23 °C with minimum temperatures above 8 °C and relative humidity averaging 70%. These moderate temperature combined with higher humidity created a congenial microclimate that favoured pathogen establishment and sporulation, resulting in greater disease incidence and severity. Theseobservations were corroborated by Pheirim et al. 2021. Genotype-specific differences were evident, with VP-2020-101 and VP- 2024-55 consistently exhibiting the lowest disease severity at both sites, suggesting inherent resistance. Importantly, disease incidence reached 100% in all genotypes except VP-2020-101 and VP- 2024-55 at Mukteshwar, reaffirming the high disease pressure at this site. The varied degree of disease reaction in pea germplasm has been reported earlier by Chaudhary and Banyal (2017). The differential response of genotypes across the two locations necessitated screening the germplasm under controlled conditions using the detached leaf assay.

Detached leaf assays under polyhouse and incubator conditions provided a controlled setting to validate field resistance (Özer et al. 2018). Disease development patterns were largely consistent with in-vivo data, supporting the effectiveness of detached leaf assays as a reliable screening method. The result aligns well with whole-plant assay outcomes and are consistent with findings from previous studies utilizing detached leaf assays (Miller-Butler et al. 2018). Previous studies of Sharma et al (1992) and Thakur et al (1996) reported (in)complete resistance due to presence of multiple resistance genes and interaction of *er-2* with environment. This may be the

reason for detection of different number of resistance/susceptible plants in polyhouse (whole plant and detach leaf assay) and incubator. Notably, VP-2020-101 and VP- 2024-55 maintained resistance across all artificial environments, characterized by low disease scores and sparse mycelial growth. Conversely, some genotypes, such as Vivek Matar-12 and VL Sabji Matar-13, which showed susceptible responses in vitro despite field-level variability, indicate that environmental stress can reveal cryptic susceptibility, potentially due to inducible or unstable resistance mechanisms. The breakdown of resistance in certain genotypes under induced conditions also underscores the importance of integrating both field and controlled environment evaluations to capture the full spectrum of disease responses.

Molecular markers enable cost-effective, reliable selection of germplasm and breeding lines through DNA assays, minimizing the need for extensive phenotypic evaluation when closely linked to target traits to reduce recombination (Javid et al. 2017). In current study, molecular characterization using gene-specific markers linked to *er1* (major resistance gene), *er2* express under specific conditions**),** and *Er3* (isolated from *P. fulvum*) resistance genes provided genetic validation of phenotypic observations. VP-2020-101 was found to harbour all three resistance genes as its amplicon profiling perfectly matched with respective donor (*er-1*: er000202; *er-2*: er000203 and *Er-3*: P660-4), indicating a pyramided resistance genotype. This likely contributes to its robust and consistent resistance across all test conditions/locations, making it an ideal parent in resistance breeding programs. The genotypes habouring *er 1* and *er 2* have been reported to be resistant and validated through these markers previously (Sharma et al., 2025). Given the breakdown of resistance under varying environmental conditions, the most viable strategy is to incorporate multiple resistance genes into cultivated genotypes through gene pyramiding (Devi et al. 2022). This is a significant finding, as pyramiding resistance genes is a proven strategy for achieving durable and broad-spectrum resistance. In contrast, VP- 2024-55 was positive only for *er1*, suggesting that while it exhibits high resistance, it may be more vulnerable to virulent pathogen races that overcome single-gene resistance. Resistance due to *er1* gene is said to be complete and durable as it avoids the epidermal cell penetration of the peg that emerges from the appressorium formed after the conidia germination and penetrates the epidermal host cells through the cuticle and cell wall. Earlier Tiwari et al (2017) also reported that mere *er-1* is sufficient to mitigate the PM in pea. The results of this study is in agreement with Tiwari et al (2017) as *er-1* containing both genotypes exhibited resistance.

## 5. Conclusion

This study underscores the critical importance of multi-environment screening and the integration of molecular tools for robust validation of resistance against powdery mildew. The genotypes VP-2020-101 and VP-2024-55 consistently exhibited resistance across diverse environments, highlighting their potential as valuable donor parents in resistance breeding programs. These genotypes can be effectively utilized in the development of mapping populations, thereby facilitating QTL mapping and further genetic dissection of resistance traits. Future research should focus on detailed patho-typing of powdery mildew isolates to understand genotype-pathogen interactions better and on mapping quantitative trait loci (QTLs) associated with resistance expression under varying environmental conditions.

## Abbreviations

PM - Powdery mildew

QTL- Quantitative trait loci

PDI – Percent Disease Incidence

S: Susceptible

MS: Moderately susceptible

MR: Moderately Resistant

R: Resistant

PCR: Polymerase chain reaction

CTAB: Cetyltrimethylammonium bromide

ICAR-VPKAS: ICAR-Vivekananda Parvathiya Krishi Anusandhan Sansthan

HATS: High-Altitude Testing Site

SCAR: Sequence Characterized Amplified Region

SSR: Simple Sequence Repeats



**Reference**

1. Ausubel, F. M. *et al.* Short protocols in molecular biology. Wiley, New York (1995).

2. Awan, Z. A., Shoaib, A. & Khan, K. A. Variations in total phenolics and antioxidant enzymes cause phenotypic variability and differential resistant response in tomato genotypes against early blight disease. *Sci. Hortic.* 239, 216–223 (2018).

3. Azmat, M. A. *et al.* Single recessive gene controls powdery mildew resistance in pea. *Int. J. Veg. Sci.* 16, 278–286 (2010).

4. Banyal, D. K. & Chand, J. C. Evaluation of pea genotypes for resistance against powdery mildew caused by *Erysiphe pisi*. *Indian Phytopathol.* 70, 69–74 (2017).

5. Banyal, D. K. Studies on powdery mildew of pea caused by *Erysiphe pisi* DC. Ph.D. Thesis, Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India, 80 pp. (1994).

6. Carver, T. L. W. & Jones, S. W. Colony development by *Erysiphe graminis* f. sp. *hordei* on isolated epidermis of barley coleoptile incubated under continuous light or short day conditions. *Trans. Br. Mycol. Soc.* 90, 114–116 (1988).

7. Cousin, R. Peas (*Pisum sativum* L.). *Field Crops Res.* 53, 111–130 (1997).

8. Devi, J. *et al.* Gene based resistance to *Erysiphe* species causing powdery mildew disease in peas (*Pisum sativum* L.). *Genes* 13, 316 (2022).

**9**. Devi, M., Bilashini, M., Devi, M. T., Jha, A. K., Yumnam, A., Balusamy, A., Verma, V. K., Talang, H. D., Deshmukh, N. A., Rymbai, H. & Assumi, S. R. Yield and yield attributes of garden pea (Pisum sativum var. hortense L.) as influenced by nutrient management practices under agroclimatic conditions of Meghalaya. *Int. J. Curr. Microbiol. Appl. Sci.* **7**, 3447–3454 (2018)

10. Fondevilla, S., Carver, T. L. W., Moreno, M. T. & Rubiales, D. Identification and characterization of sources of resistance to *Erysiphe pisi* Syd. in *Pisum* spp. *Plant Breed.* 126, 113–119 (2007).

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

12. Fondevilla, S., Rubiales, D., Moreno, M. T. & Torres, A. M. Identification and validation of RAPD and SCAR markers linked to the gene *Er3* conferring resistance to *Erysiphe pisi* DC in pea. *Mol. Breed.* 22, 193–200 (2008).

13. Fondevilla, S., Torres, A. M., Moreno, M. T. & Rubiales, D. Identification of a new gene for resistance to powdery mildew in *Pisum fulvum*, a wild relative of pea. *Breed. Sci.* 57, 181–184 (2007).

14. Gawande, V. L. & Patil, J. V. Genetics of powdery mildew (*Erysiphe polygoni* DC) resistance in mungbean (*Vigna radiata* L.). *Crop Prot.* 22, 567–571 (2003). https://doi.org/10.1016/S0261-2194(02)00202-8

15. Gupta, A. *et al.* Identification of SSR markers linked to powdery mildew resistance in table pea (*Pisum sativum var. hortense* L.). *Legume Res.* 47, 1032–1038 (2024).

16. Harland, S. C. Inheritance of immunity to mildew in Peruvian forms of *Pisum sativum*. *Heredity* 2, 263–269 (1948).

17. Heringa, K. J., Vannorel, A. & Tazelaar, M. F. Resistance to powdery mildew (*Erysiphe polygoni* D.C.) in peas (*Pisum sativum* L.). *Euphytica* 18, 163–169 (1969).

18. Janani, R. *et al.* Physiological and biochemical responses of garden pea genotypes under reproductive stage heat stress. *Genet. Resour. Crop Evol.* 71, 1177–1200 (2024).

19. Javid, M. *et al.* Validation of molecular markers associated with boron tolerance, powdery mildew resistance, and salinity tolerance in field peas. *Front. Plant Sci.* 6, 917 (2015).

20. Tiwari, K. R., Penner, G. A. & Warkentin, T. D. Inheritance of powdery mildew resistance in pea. *Can. J. Plant Sci.* 77, 307–310 (1997).

21. Katoch, V. *et al.* Molecular mapping of pea powdery mildew resistance gene *er2* to pea linkage group III. *Mol. Breed.* 25, 229–237 (2009).

22. Miller Butler, M. A., Smith, B. J. & Babiker, E. M. Comparison of whole plant and detached leaf screening techniques for identifying anthracnose resistance in strawberry plants. *Plant Dis.* 102, 2112–2119 (2018).

23. Murray, M. G. & Thompson, W. F. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* 8, 4321–4326 (1980).

24. Özer, N., Kün, A. & İlbi, H. Detached leaf test for evaluation of resistance to powdery mildew in pepper. *Agric. Res. Technol. Open Access J.* 14, Article 555923 (2018).

25. Pandey, K. K., Pandey, P. K., Kalloo, G. & Banerjee, M. K. Resistance to early blight of tomato with respect to various parameters of disease epidemics. *J. Gen. Plant Pathol.* 69, 364–371 (2003).

26. Pheirim, R., Konjengbam, N. S. & Mahanta, M. Molecular markers for powdery mildew in pea (*Pisum sativum* L.): A review. *Legume Res.* 45, 399–409 (2022).

27. Raj, P. *et al.* Evaluation of powdery mildew resistant pyramid vegetable pea genotypes introgressed with *er1* and *er2* genes for yield and related traits in mid hills of Himachal Pradesh. *Veg. Sci.* 51, 335–341 (2024).

28. Rana, C. *et al.* In vivo and in vitro validation of powdery mildew resistance in garden pea genotypes. *Sci. Rep.* 13, 2243 (2023).

29. Rasool, M., Akhter, A., Soja, G. & Haider, M. S. Role of biochar, compost and plant growth promoting rhizobacteria in the management of tomato early blight disease. *Sci. Rep.* 11, 6092 (2021).

30. Sharada, P. & Makandar, R. Assessing garden pea germplasm for powdery mildew resistance through disease phenotyping and genotyping using molecular markers. *Plant Gene* 35, 100425 (2023).

31. Sharma, N. Evaluation of varietal susceptibility in pea to *Erysiphe polygoni*. *Ann. Appl. Biol.* 120, 110–111 (1992).

32. Sillero, J. C. *et al.* Screening techniques and sources of resistance to rusts and mildews in grain legumes. *Euphytica* 147, 255–272 (2006).

33. Singh, U. P., Prithviraj, B., Wagner, K. G. & Schumacher, K. G. Effect of ajoene, a constituent of garlic (*Allium sativum*), on powdery mildew (*Erysiphe pisi*) of pea (*Pisum sativum*). *J. Plant Dis. Prot.* 102, 399–406 (1995).

34. Smith, C. G. Production of powdery mildew cleistocarp in a controlled environment. *Trans. Br. Mycol. Soc.* 55, 355–365 (1970).

35. Srivastava, R., Mishra, S., Singh, A. & Mohapatra, T. Development of a coupling phase SCAR marker linked to the powdery mildew resistance gene *er1* in pea (*Pisum sativum* L.). *Euphytica* 186, 855–866 (2012).

36. Thakur, B. R., Kapoor, A. S. & Jamwal, R. S. Varietal resistance of pea to powdery mildew in dry temperature zone of Himachal Pradesh. *Indian Phytopathol.* 49, 92–93 (1996).

37. Tiwari, K. R. & Penner, G. A. Identification of coupling and repulsion phase RAPD markers for powdery mildew resistance gene *er1* in pea. *Can. J. Plant Sci.* 78, 361–367 (1998).

39. Vaid, A. & Tyagi, P. D. Genetics of powdery mildew resistance in pea. *Euphytica* 96, 203–206 (1997).

40. Villalobos Solis, M. I., Patel, A., Orsat, V. *et al.* Fatty acid profiling of the seed oils of some varieties of field peas (*Pisum sativum*) by RP LC/ESI MS/MS: towards the development of an oilseed pea. *Food Chem.* 139, 986–993 (2013).

41. Warkentin, T. D., Rashid, K. Y. & Xue, A. G. Fungicidal control of powdery mildew in field pea. *Can. J. Plant Sci.* 76, 933–935 (1996).

42. Sharma, A., Banyal, D. K., Dhole, V. J., Bansuli, Rana, R. S., Kumar, R., ... & Sharma, A. (2025). Development of new powdery mildew resistant lines in garden pea (Pisum sativum L.) using induced mutagenesis and validation of resistance for the er1 and er2 gene through molecular markers. *Frontiers in Plant Science*, *15*, 1501661.

**Table legends**

**Table 1:** Experimental material used in the study

**Table 2:** 0-5 Scale used for powdery mildew disease scoring in field under poly-house condition

**Table 3:** 0-4 scale used for scoring disease reaction in detached leaf method

**Table 4:** List of SCAR markers used in the study

**Table 5:** Powdery mildew resistance Scoring of 11 garden pea genotypes across two growth stages and location at 0–5 scale; DI and PDI under Mukteshwar field condition

**Table 6:** Scoring of garden pea genotypes for powdery mildew resistance under in-vitro condition (Detached leaf method)

**Figure legends**

**Figure 1:** Powdery mildew incidence in field condition at Mukteshwar in resistant (a: VP-2020-101 and b: VP-2024-55) and susceptible (c: Arkel) genotypes.

**Figure 2:** Microscopic and macroscopic views of garden pea genotypes (VM-11, VM-12, VLSM-15, Arkel, VP-2020-101 and VP- 2024-55) showing resistant phenotype in both the inoculated condition (polyhouse and incubator) and control check.

**Figure 3:** Agarose gel images depicting polymorphism of amplification products linked to powdery mildew resistance genes (*er1, er2* and Er3) in pea genotypes. 3A shows a 1600□bp product amplified by the *er1*-linked marker ScOPE-$16_{1600}$, 3B shows a 1400□bp product with the *er2*-linked marker ScX-$17_{1400}$, and 3C shows a 637□bp product amplified by the *Er3*-linked marker ScW$4_{637}$. Lane details for all gels include: C. Arkel (susceptible check), G. P660-4 (resistant check for Er3), H. er000203 (resistant check for er2), I. er000202 (resistant check for er1), 10. VP-2024-55, 11. VP-2020-101, L1: 1□kb ladder (Puregene), L2: 100□bp ladder (GeNei), and L3: 50□bp ladder (GeNei).