16 November 2024: Received
02 February 2025: Revised
28 February 2025: Accepted
04 March 2025: Available Online

https://aatcc.peerjournals.net/

Original Research Article

Open Access

Genetic analysis and identification of molecular markers for Fusarium wilt resistance gene in Garden pea (*Pisum sativum* (L.) var. hortense)

Kumari Shubha[1,4*], Shri Dhar[1], Amol K. U Solnake[2], Harswardhan Choudhary[2], S. C. Dubey[3], Anirban Mukherjee[4]

[1] *ICAR- Indian Agricultural Research Institute, New Delhi, India*
[2] *ICAR-National Research Centre on Plant Biotechnology, New Delhi, India*
[3] *ICAR-National Bureau of Plant Genetic Resources, New Delhi, India*
[4] *ICAR Research Complex for Eastern Region, Patna, Bihar, India*

ABSTRACT

Fusarium wilt, caused by Fusarium oxysporum f. sp. pisi, is a severe disease that significantly impacts garden pea (Pisum sativum (L.) var. hortense) yields worldwide. This study delves into the genetic basis of resistance to Fusarium wilt in garden peas, focusing on identifying molecular markers associated with resistance genes. Genetic resistance is a more cost-effective and environmentally friendly alternative to fungicide applications. Through detailed genetic analysis, the study confirmed that resistance to Fusarium wilt is monogenic and dominant. This was demonstrated by the segregation patterns observed in different generations: F_1 (100% resistant), F_2 (124 resistant: 36 susceptible), and backcross generations ($BC_1F_1P_1$: 19 resistant: 16 susceptible; $BC_1F_1P_2$: 35 resistant: 0 susceptible), using 'GP-6' as the resistant parent and 'Arkel' as the susceptible parent. The resistance gene, derived from the resistant parent 'GP-6', was characterized through phenotypic screening and validated using molecular markers. The SCAR marker Y15_999Fw identified the 999 bp band associated with resistance in 131 out of 160 F_2 plants. Additionally, the polymorphic ISSR marker UBC-812 was found to be closely linked to the Fusarium wilt resistance locus, exhibiting a 3:1 Mendelian segregation ratio in the F_2 population. Marker linkage analysis placed UBC-812 at a distance of 5.01 cM from the resistance locus. These findings offer crucial insights into the genetic mechanisms behind Fusarium wilt resistance in garden peas, supporting the development of marker-assisted breeding strategies to cultivate wilt-resistant pea varieties.

Keywords: *Fusarium wilt, garden pea, genetic analysis, Mendelian segregation ratio, Linkage analysis*

Introduction

Rapidly changing surroundings, including climate change and population growth, pose significant challenges to our food systems [1]. In the quest for a more sustainable agricultural model, legumes like Garden pea (*Pisum sativum* (L.) var. hortense) play a crucial role. Nitrogen-fixing bacteria, which reside as nodules in the roots and aid in the growth and development of garden pea, offer several benefits. These include reducing the need for fertilizers, thereby decreasing chemical usage, preventing soil pollution, and promoting more economical agricultural practices [2,3]. Moreover, peas are abundant sources of carbohydrates, minerals, fiber, vitamins, and particularly, proteins [4]. Hence, as an ecologically responsive alternative, peas serve as a superb substitute for more expensive animal-based proteins [5,6].

Worldwide, around 21.77 million tons of peas are produced each year. India is the world's second-largest green pea producer, contributing around 4.8 million tons, following China's leading production of 12.2 million tons [7]. Uttar Pradesh, Madhya Pradesh, Punjab, and Himachal Pradesh are the major pea-cultivating areas within India [8].

*Corresponding Author: **Kumari Shubha***

DOI: https://doi.org/10.21276/AATCCReview.2025.13.01.534

Garden peas are utilized in various cuisines and are highly suitable for human consumption, offering a diverse nutrient profile that includes vitamins, minerals, and lysine, which is often lacking in cereals [9,10]. Per 100 grams, garden pea seeds contain significant amounts of carbohydrates (17-22g), starch (20-50g), dietary fiber (14-26g), protein (6.2-6.5g), fat (0.4g), and ash (1.0g). They are also rich in essential minerals such as calcium (9-10mg), sodium (3-5mg), and potassium (97-99mg), and vitamins including riboflavin (0.7mg), thiamine (5-6mg), and folate (0.54mg) per kilogram [11,12]. Consuming garden peas can help in managing type-2 diabetes, reduce the risk of colon cancer, coronary disease, and lower LDL-cholesterol levels [12]. Additionally, fresh pea contains a considerable amount of folic acid, ß-sitosterol, and vitamins K and C [14].

The main challenge to production of garden pea is biotic stress, particularly soil-borne diseases, which pose the most significant threat to pea cultivation worldwide. Among these, Fusarium wilt (FW), caused by *Fusarium oxysporum* f. sp. *pisi* (Fop), stands out as a major biotic stressor, resulting in continuous and severe yield losses. Despite early sowing in late September offering higher profits, the prevailing high temperatures (28-30°C) and humidity create ideal conditions for wilt disease development, leading to significant yield and quality losses [14-17]. *Fusarium oxysporum* f. sp. pisi, a fungal pathogen from the family Tubercullariaceae and the order Hyphomycetales within the Deuteromycotina, is responsible for causing severe damage to peas [10].

In India, Fusarium wilt was first reported in Bombay by Sukapure et al. [18].

The disease, caused by *Fusarium oxysporum* f. sp. pisi (Fop), was initially recognized in Minnesota in 1918 by Chupps and Sherf. Jones [19] distinguished pea wilt disease from root rot and named it an undescribed wilt disease. By 1928, Linford identified the causative agent as Fothoceras App and Wr var. *pisi*, later renamed race 1 of *Fusarium oxysporum* Schl f. sp. pisi. Fusarium belongs to the class Fungi *imperfecti*, as reported by Snyder and Hansen [20]. Kraft [21] classified isolates of Fop, noting that its impact is determined by the genetic makeup of both the host and pathogen. The fungus invades root tissues, blocking xylem vessels, which restricts water and nutrient flow, causing yellowing and wilting. Early-stage infections can kill the plant, while later-stage infections result in yield loss and kernel shrinkage. The fungus can enter through wounds or directly penetrate the epidermis [22]. Infections via wounds escalate symptom severity and wilting [23]. Both *Fusarium solani* f. sp. pisi and *Fusarium oxysporum* f. sp. pisi infect roots and seeds, causing systemic disease [24]. Lin [25] reported that wilt during flowering leads to leaf yellowing and discoloration. Six races of Fusarium have been identified, with races 1 and 5 posing the greatest threat, predominantly affecting garden pea crops in India [26]. Race 1 manifests early in the season, localized within specific field areas, while Race 2 prevails later and spreads more broadly. This soil-borne fungus persists for years, causing significant crop losses, with reported losses ranging from 13.9% to 95% [15,16,24]. Utilizing disease-resistant cultivars is the most effective and economical method for disease prevention [16]. The *Pisum* germplasm collection at Washington State University has been evaluated for resistance to Fusarium wilt and Aphanomyces root rot [27]. While wild peas offer disease resistance potential, their use is limited due to undesirable traits and reproductive barriers [28]. Partial resistance to various pathogens like *F. solani* has been observed in certain wild pea accessions [29]. Some accessions, such as PI 125673 and Banner, exhibit resistance to *F. solani* [30]. Genotypes with pigmented flowers generally show lower disease scores, except for PI 180693 [31].

Previous studies have identified different resistant lines, with resistance to Fusarium wilt reported as dominant polygenic or monogenic [32-37]. Marker-assisted breeding holds promise for accelerating the development of wilt-resistant varieties, with various molecular markers linked to resistance genes reported in previous studies [38-41]. However, these markers have seen limited use in breeding programs, especially in the Indian context.

This study aims to elucidate the genetic mechanisms underlying Fusarium wilt resistance in garden pea and identify molecular markers linked to resistance genes. By unravelling the genetic basis of resistance, the research seeks to facilitate the development of marker-assisted breeding strategies for selecting and breeding wilt-resistant varieties. Ultimately, the goal is to enhance the sustainability and productivity of pea cultivation by providing farmers with effective tools to manage Fusarium wilt and mitigate its detrimental impact on crop yield and quality.

1. Materials and Methods

1.1 Parental lines and development of population

The research was conducted at the ICAR-Indian Agricultural Research Institute's (IARI) Division of Vegetable Science's Research Farm in New Delhi, India. New Delhi experiences a subtropical climate with scorching summers exceeding 40°C and chilly winters dipping below 4°C. The temperature during sowing and germination was 28-30°C which is most favourable temperature for Fusarium wilt infestation. Based on this preliminary evaluation (Figure 1A-F), the highly commercialized but susceptible variety 'Arkel' and the resistant line 'GP-6' were selected. 'GP-6' is an advanced breeding line characterized by its dwarf stature, leafless trait, and high resistance to Fusarium wilt; however, it has a low yield and poor pod quality. In contrast, 'Arkel' is a high-yielding variety with early maturation and attractive pods, but it is susceptible to Fusarium wilt used to develop the population. The parental lines were sown in the first fortnight of October in wilt-free soil in pots. Crosses were attempted to develop F_1 plants. Next year F_1s were backcrossed to both the parents to get 35 $BC_1F_1P_1$ (F_1 backcrossed to susceptible parent), and 35 BC_2 (F_1 backcrossed to resistant parent) generations and the self-pollination of F_1 plants generated 160 F_2plants.

1.2 Preparation of inoculums and Screening

We obtained a virulent strain of the fungal pathogen *Fusarium oxysporum* f. *sp. pisi* from pea plants exhibiting wilt symptoms. The Division of Plant Pathology at IARI, New Delhi, confirmed the identification of the fungus. To assess its host range (host specificity), the fungus was first grown and maintained on a standard culture medium called potato dextrose agar (PDA). Additionally, sorghum seeds were disinfected by soaking them in tap water overnight. Subsequently, 250 grams of these pre-soaked seeds were added to conical flasks for further experimentation. To eliminate any existing microorganisms, the flasks were sterilized using an autoclave at a pressure of 1.1 kg/cm^2 for a duration of 20 minutes. This autoclaving process was repeated on two consecutive days. After the flasks cooled down, a sterile cork borer was used to cut 4mm plugs from a 7-day-old, actively growing culture of *Fusarium oxysporum* f. sp. pisi. These agar plugs were then introduced into the sterilized flasks to inoculate them with the fungus. The flasks were subsequently incubated at a constant temperature of 25°C for 10 days. Separately, plastic pots with a diameter of 15 cm were surface-disinfected using a solution of 0.1% mercuric chloride. These pots were then filled with 2.0 kg of sterilized soil that had been previously treated with 1.0% formalin over a period of 15 days to ensure the elimination of any potential soil-borne pathogens. The soil was inoculated with a 15-day-old culture of the pathogen, which had been propagated on sorghum grains (10 g/kg soil) seven days prior to sowing, following the method outlined by Dubey et al. (2010). Control pots without inoculated soil were also maintained for comparison. The field and artificial screening were conducted at a wilt sick plot established at the Vegetable Farm and the Centre for Protection Cultivation Technology (CPCT), both located at ICAR-IARI, New Delhi. Both susceptible and resistant pea cultivars were included in the experiment. Seeds were planted in the pots, with 10 seeds placed in each pot (Figure 2A-B). The plants were allowed to grow for the entire growing season, and the percentage of plants showing wilt symptoms (wilt incidence) was documented every 15 days until harvest.

1.3 Phenotyping of parents, F1, F2 and backcross (BC) population

Seeds of parental lines, F_1, F_2, $BC_1F_1P_1$ and $BC_1F_1P_2$ were grown in sick pots. The observations of disease symptoms were recorded in 0-5 scale where 0 indicated no disease symptom; 1 indicated plants with pale yellowish green curling leaf margin at downward and stipules; 2 indicated plants with yellow leaves

from base to apex; 3 indicated plants with stunting and wilting symptom; 4 indicated plants with dried lower leaves, discoloration of the vascular system in the upper taproot spreading up the stem; 5 indicated plants with completely wilted or died. Finally, the following formula was used to calculate disease incidence (DI),

$$\text{Percent disease incidence} = \frac{\text{Number of wilted plants}}{\text{Total number of plants}} \times 10$$

The disease incidence (DI) of 0-10% was considered as highly resistant, between 11-20% as resistant, between 21-30% as moderately resistant, between 31-50% as susceptible and above 50% as highly susceptible [42].

1.4 DNA isolations and marker study

The young leaves from 16 garden pea lines including parents and 160 F_2 plants were collected and DNA was isolated as per method based on Doyle and Doyle [43] with certain modifications. The DNA was quantified in 0.8% agarose gel and the final concentration was made to 25–50 ng/µl for PCR analysis.

1.5 Marker Analysis Using PCR Amplification

SCAR and ISSR marker analyses were performed using PCR amplification on a Mastercycler Gradient Thermal Cycler (Eppendorf). For SCAR analysis, the PCR profile procedure included 30 cycles, each comprising 30 seconds at 94°C, 30 seconds at 60°C, and 1 minute at 72°C. The resulting amplification products were then separated on 2% agarose gels under a constant voltage of 120V for 1.5 hours. In the ISSR analysis, PCR reactions were carried out in a total volume of 25 µl, including 18.9 µl sterile water, 1.0 µl PCR buffer, 0.25 µl dNTP, 1 µl primer (0.5 µM), 5U Taq DNA polymerase, and 1 µl template DNA (50ng). The PCR protocol began with an initial denaturation step at 94°C for 4 minutes. This was followed by 45 cycles, each consisting of 1 minute at 94°C, 1 minute at 50°C, and 1 minute at 72°C. A final extension step was conducted at 72°C for 10 minutes. The amplified products were separated on 2% agarose gels at a constant voltage of 120V for 2.5 hours. Fragment sizes at each locus were determined using a 1 Kb DNA plus ladder. The amplicons were visualized by staining and observed with a Gel Documentation System under fluorescence.

1.6 Validation of linked SCAR markers in garden pea lines

For the marker validation study, previously identified SCAR markers *Y15_999Fw* [39] linked to the *Fw* gene were used to validate in set of 16 pea lines *viz*. GP-6, GP-47, GP-48, GP-55, Arkel, GP-17, Pusa Pragati, GP-941, GP-942, VRP-6, EC-677211, EC-677213, EC-677212, EC-677214, EC-677216, EC-677215. The PCR protocol as described was followed as mentioned section 2.5.

1.7 Polymorphic marker identification and bulk segregant analysis (BSA)

Parental polymorphism was surveyed using 60 ISSR and 62 SSR [44-46] markers. The polymorphic markers between the susceptible and resistant parents were used for BSA to identify putatively linked molecular markers to the Fusarium wilt resistance locus [47]. The DNA of 8 resistant F_2 plants was pooled to make a resistant bulk (R-bulk). Similarly, DNA of 8 susceptible F_2 plants was pooled to make susceptible bulk (S-bulk). The identified polymorphic marker was then used for genotyping each F_2plant along with parents.

1.8 Data analysis and inheritance study

To test the goodness of fit, Chi Square (χ^2) test was carried out and the ratio of resistant and susceptible plants in F_2 and backcross generations ($BC_1F_1P_1$ and $BC_1F_1P_2$) were tested for significance [47]. The association of markers and the resistant gene was carried out using JoinMap 3.0 [48] to estimate genetic intervals in cM with the Kosambi mapping function with the threshold for goodness of fit (< = 5.0 with LOD scores >1.0 and a recombination frequency <0.4).

2. Results

2.1 Genetic analysis of Fusarium wilt resistance

In this study, Fusarium wilt incidence was observed early in the season, primarily confined to specific areas in the field, contrasting with Race 2, which emerged later and scattered throughout the field. Microscopic examination of fungal spores confirmed the presence of race 1, highlighting its devastating nature. The disease manifested shortly after germination, with the commercial but susceptible variety 'Arkel' displaying high susceptibility, while 'GP-6' exhibited strong resistance to Fusarium wilt. Screening facilitated clear categorization of susceptible and resistant plants (Figure 1A-F). Table 1 illustrates the reactions of the parents, F_1s, and F_2 generation. All F_1 plants demonstrated resistance to Fusarium wilt. Among the 160 F_2 plants, 124 were resistant, and 36 were susceptible (Figure 2A-B). Chi-square (χ^2) analysis indicated that the segregation of resistant and susceptible plants followed a 3:1 ratio (χ^2=0.533), with a confidence level of p=0.465. In the $BC_1F_1P_1$ population, the ratio of resistant to susceptible plants was 19:16 (1:1) with confidence (p=0.356), and in $BC_1F_1P_2$, it was 35:0 (1:0).

2.2 Validation of SCAR marker Y15_999Fw in F2 plants

Initially, sixteen garden pea lines, comprising susceptible and resistant parents, underwent rigorous phenotyping. Subsequently, genotyping was performed on the susceptible and resistant parents using the SCAR marker Y15_999Fw to detect the presence of a 999 bp band. Among these lines, the marker revealed the 999 bp band in 10 resistant lines, including the resistant parent GP 6, while it was absent in six susceptible lines, including the susceptible parent Arkel. Subsequently, the SCAR marker was employed to genotype 160 F_2 plants, revealing the presence of the 999 bp band in 131 plants and its absence in 29 plants. Notably, 22 phenotypically susceptible F_2 plants exhibited the 999 bp band, while 17 resistant F_2 plants did not display the expected 999 bp band size. Consequently, a total of 39 recombinants were observed between Y15_999Fw and the *Fw* locus. The goodness-of-fit analysis for these markers yielded a χ^2 value of 2.7 (P = 0.10–0.20) in the F_2 progeny plants (Table 3).

2.3 Polymorphism survey in parents and BSA

Due to the moderate efficiency of the earlier marker (Y15_999Fw) in predicting desired genotypes because of high number of recombinants and dominant nature, this study was extended for the identification of new molecular markers and their possible association with Fusarium wilt resistance. A total of 122 markers, comprising 60 ISSR and 62 SSR markers, were employed to identify polymorphisms between the parental lines 'Arkel' and 'GP-6'. Among these markers, 55 (45.08%) exhibited amplification of one or more bands from each parental line.

Remarkably, all SSR markers displayed monomorphic bands in both parents. Specifically, ISSR markers UBC-847, UBC-856 UBC-848, UBC-855, and UBC-854, demonstrated polymorphic band sizes of approximately 1200, 1250, 1200, 1250, and 800 bp, respectively, in the resistant parent 'GP-6'. These polymorphic ISSR markers were utilized in bulked segregant analysis (BSA) alongside the parents (Figure 4). Following repeated PCR, it was noted that only one ISSR marker, UBC-812, exhibited a clear amplicon size of 600 bp in the resistant bulks, specific to the resistant parent. Individual F_2 plants from both resistant and susceptible bulks were genotyped using the UBC-812 marker, revealing that all eight F_2 resistant plants from the resistant bulk displayed a 600 bp band, while it was absent in the eight susceptible F_2 plants from the susceptible bulk (Figure 5).

2.4 Genotyping of F2 plants using polymorphic ISSR marker UBC-812 and Linkage analysis

The genotyping of 160 F_2 plants was carried out using polymorphic ISSR marker UBC-812 (Figure 6A-E). Among 160 F_2 plants, 600 bp band was found in 125 F_2 plants ('+' sign in figure) and absent in 35 plants ('–' sign in figure). It was also found that four phenotypically susceptible F_2 plants showed a resistant parent-specific band of 600 bp and three phenotypically resistant F_2plants did not show the expected size (~ 600bp). Out of 160 F_2 plants, the number of recombinants for the marker was 7. The segregation of UBC-812 marker showed 3:1 Mendelian ratio with χ^2 value of 0.833 (P = 0.361) at 5% level of significance in 160 F_2 plants (Table 3).

The marker linkage analysis with Fusarium wilt resistance locus was done. The segregation data of the marker were obtained by analyzing the entire F_2 population. Two markers (SCAR; Y15_999*Fw* and ISSR UBC-812) along with resistance locus covered a distance of 30.77 cM. The distance of SCAR marker Y15_999*Fw* and UBC-812 from *Fw* gene was 25.76 cM and 5.01 cM, respectively (Table 4 and Figure 7).

3. Discussion

Fusarium wilt poses a major threat to pea production, significantly impacting both yield and quality. While fungicides offer a temporary solution for controlling outbreaks, they can have environmental drawbacks [16]. Genetically resistant pea cultivars, on the other hand, provide a more sustainable and eco-friendly approach for long-term disease management. This approach eliminates the need for fungicides, reducing potential harm to beneficial insects and minimizing chemical runoff into the environment. In the present investigation, the resistance gene derived from the resistant parent 'GP-6' was characterized through phenotypic screening. 'Arkel', a highly susceptible parent known for its high yield and desirable pod quality, was selected based on diverse phenological traits. These parents were involved in crosses to isolate transgressive segregants, resulting in 160 F_2 progenies. The Fusarium wilt-resistant progenies were selected under both epiphytotic and artificial conditions, ensuring they also met the high-yield preferences of farmers.

Extensive research [15,16,32,33] has demonstrated that resistance to Fusarium wilt race 1 in peas is controlled by a single dominant gene. This finding is further validated by the present study, where all F_1 progeny derived from the cross Arkel × GP-6 displayed resistance to Fusarium wilt disease. These observations provide robust support for the dominant inheritance of resistance in this pathosystem. The segregation of resistant and susceptible plants in F_2 plants showed Mendelian ratio which confirmed the monogenic dominant nature of the resistance gene. The non-significance of the χ^2 test (*P* value > 0.05) revealed a close union between the expected and observed ratio of resistant: susceptible plants. To validate the F_2 data, backcross generations of the cross were also evaluated which supported the monogenic inheritance of the resistant gene. The plant groups used in this study displayed a clear separation between resistance and susceptibility to the fungus in a 1:1 ratio. This finding supports previous research on the inheritance pattern of the Fw gene, which governs resistance to Fusarium wilt disease. Marker-assisted breeding (MAB) for this pathogen necessitates the use of robust, reliable, and tightly connected markers that may be used in a variety of genetic backgrounds. Several studies have investigated the chromosomal location of the *Fw* gene. Grajal-Martin and Muehlbauer [34] placed it at a distance of 14 map units from Td (leaf dentation gene), 13 map units from b (pink flower color gene), and 13 map units from the isozyme marker, Lap1, all on linkage group (LG) III. Further mapping efforts refined the position. McClendon et al. (2002) reported Fw to be 1.4 cM away from ACG: CAT 222, an amplified fragment length polymorphism marker. Loridon et al. [38] found Fw flanked by markers AA5 225 and AD134 213 at distances of 2.7 cM and 2.5 cM, respectively. Kwon et al. (2013) employed target-region amplified polymorphic (TRAP) marker technology to develop three sequence-characterized amplified region (SCAR) markers (Fw Trap 480, Fw Trap 340, and Fw Trap 220) positioned very close to Fw (1.2 cM) in a population derived from PI 179449 and 'Green Arrow' using bulk segregant analysis. Most recently, Jain et al. (2015) identified a CAPS marker on LG3 at a mere 0.9 cM from the Fw locus, potentially serving as a valuable tool for screening pea cultivars for Fusarium wilt resistance. However, use of these markers in the present study was not satisfying may be due to altogether different population used or major chromosomal change in parent. In this study resistant line 'GP-6' was used after screening 5 year in field as well as wilt sick plot condition. Therefore, the resistant line GP-6 will serve as a useful donor for transferring resistant gene into 'Arkel' commercial susceptible varieties using advanced breeding strategies. Selection from generation to generation only for various economic traits in garden pea such as long pod size, a greater number of seed per pod and increased sweetness lead to increase in susceptibility to Fusarium wilt in Indian condition. The high value of DI in the commercial variety 'Arkel' reduces the yield and quality of pods and the application of harmful chemicals further decrease the quality. Therefore, the only possible way to manage the disease is growing resistant variety (s) which can provides a safe product to the consumer without disturbing environmental health.

The use of pure isolate of *Fop* race 1 in the present study facilitated the analysis of inheritance of resistance very easily as previously reported by Saha et al. [49], Patil et al. [50]. In the present study most virulent and widely distributed across India *Fop* race 1 was used. The monogenic nature of the gene will facilitate the transfer of the resistance gene through the simple backcross method which is most widely used to transfer one or two major resistant gene and followed in many crops to develop variety (s). Hybridization followed by selection can also be carried out to accumulate the genes for other agronomical traits along with the Fusarium wilt resistance gene. During the process of selection focus should be given to identifying plants with higher pod yield along with Fusarium wilt resistance.

In India, no reports are available on DNA markers linked to *Fusarium* wilt resistance gene in garden pea which may be due to lack of data on pathogenic races or the use of mixed isolates.

Therefore, the present study was required to identify closely linked *Fop* race 1 specific markers using 160 F_2 plants from cross of Arkel × GP-6. The F_2 mapping population size was appropriate as previously reported by Kashyap [51]; Saha et al. [49]. AFLP [35], SCAR [39], SSR [38], and TRAP [41] genetic connections with *Fw* have been reported in pea.

Prior to this study, known markers were unable to differentiate between phenotypically resistant and susceptible parents. All the SSR markers reported in Linkage Group III were included in this study, as the *Fw* gene is reported to reside in Linkage Group III. However, no polymorphisms were found between resistant and susceptible lines. Subsequently, ISSR markers were employed due to the lack of sequence knowledge of the parents. Various studies have highlighted the advantages of ISSR markers over SSR markers. ISSR markers are known for their rapidity, simplicity, power, reproducibility, and cost-effectiveness. They are developed in a manner that does not necessitate prior sequence knowledge for evaluating genetic diversity, differentiating closely related individuals, and selecting suitable parents for cultivar development [52-55]. To identify potentially linked markers, Bulk Segregant Analysis (BSA) was conducted followed by genotyping of the entire F_2 population, as outlined by Michelmore et al. [47]. BSA has been widely used in various studies to identify markers linked to resistance genes in other crops [56-58]. In this study, one ISSR marker, UBC-812, was found to be polymorphic during BSA. The repeated amplification of respective bands in parents, bulks, and individual F_2 plants confirmed the polymorphism. Our findings suggest that marker UBC-812 is potentially linked to the Fusarium wilt resistance locus. Notably, UBC-812 amplified an expected ~600 bp band in both the resistant parent and resistant bulk, indicating the presence of the amplified band as dominant over its absence. This polymorphic marker was subsequently used to analyze all 120 F_2 plants. The marker UBC-812 amplified the expected size in ~600 bp F_2 plants, further supporting its linkage with the resistant gene. The recombinants were identified based on comparing the phenotypic and genotypic data of the F_2 individuals. The co-segregation of resistance in individual F_2 plants and dominant UBC-812 markers showed a Mendelian segregation pattern (3:1 ratio) which suggests the monogenic resistance. The genotyping data of F_2 plants were analyzed by Joinmap software (version 3.0) and a total map distance of 30.77 cM was generated. It is clear from the map that the marker UBC-812 marker was closest. The SCAR marker Y15_999*Fw* was far from the resistant locus and therefore, did not show satisfactory accuracy due to its far distance (25.76 cM) from the *Fw* locus. The ISSR marker, UBC-812 was closely linked to the *Fw* locus at 5.01Cm (Figure 7). Marker-assisted selection have been reported to be most widely used method to transfer resistant gene (s) in many crop species [59-62].

4. Conclusion

Fusarium wilt poses a significant threat to pea production, affecting both yield and quality. While fungicides offer a temporary solution, genetically resistant pea cultivars present a more sustainable and eco-friendly approach to disease management. Our investigation characterized the resistance gene derived from the resistant parent 'GP-6' and identified potential markers linked to the Fusarium wilt resistance locus. Through phenotypic screening and genetic analysis, we confirmed the monogenic dominant nature of resistance in the studied population. Marker-assisted selection, facilitated by the identification of closely linked markers such as UBC-812, holds promise for breeding resistant pea cultivars. Our findings contribute to the development of effective strategies for managing Fusarium wilt in peas, reducing reliance on chemical treatments, and promoting environmental sustainability in agriculture.

Data availability statement

The original contributions featured in the study are contained within the article and supplementary materials. For additional inquiries, please contact the corresponding authors.

Ethics statement

This article does not include any research involving human participants or animals carried out by the authors.

Funding

We also acknowledge the DST Inspire Fellowship provided to the first author by the Department of Science of Technology, Govt. of India for funding this research.

Acknowledgment

The authors extend their gratitude to the Head of the Division of Vegetable Science and the Director of ICAR-Indian Agricultural Research Institute (IARI) for their assistance and support.

Declaration of competing interest

The authors declare that the research was carried out without any commercial or financial affiliations that could be interpreted as a potential conflict of interest.

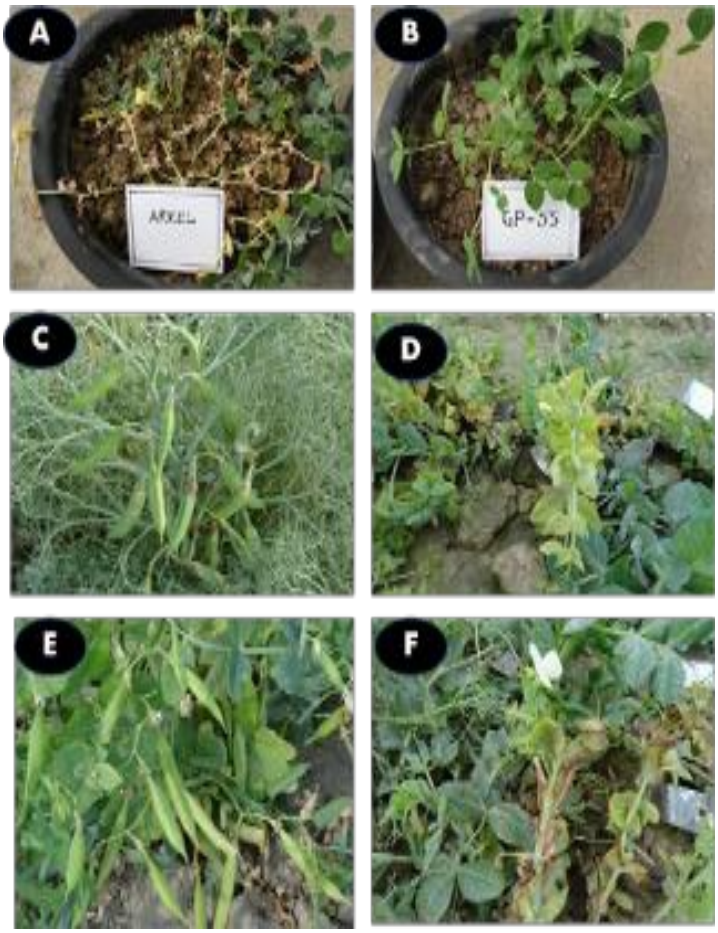


Figure 1: (A) Preliminary screening of Fusarium wilt (A): Arkel (Susceptible); (B): GP-55 (Resistant); (C): GP-6 (Resistant); (D): Pusa Pragati (Susceptible); (E): GP-17 (Resistant), (F): F_2 population of cross Arkel × GP-6

Figure 2: (A): Patchy appearance of Fusarium affected plants in artificial screening (B): wilt incidence in Arkel at field condition

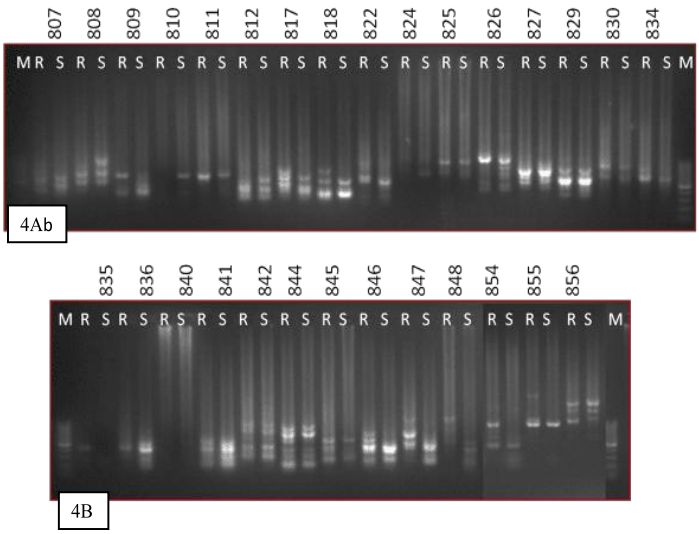


Figure 3: Screening of ISSR Primers with resistant, GP-6 (R) and susceptible parent Arkel (S)

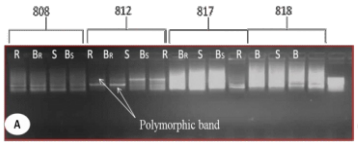


Figure 4: Bulk segregant analysis with polymorphic ISSR markers

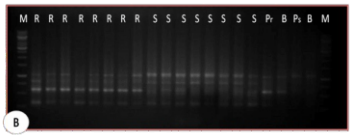
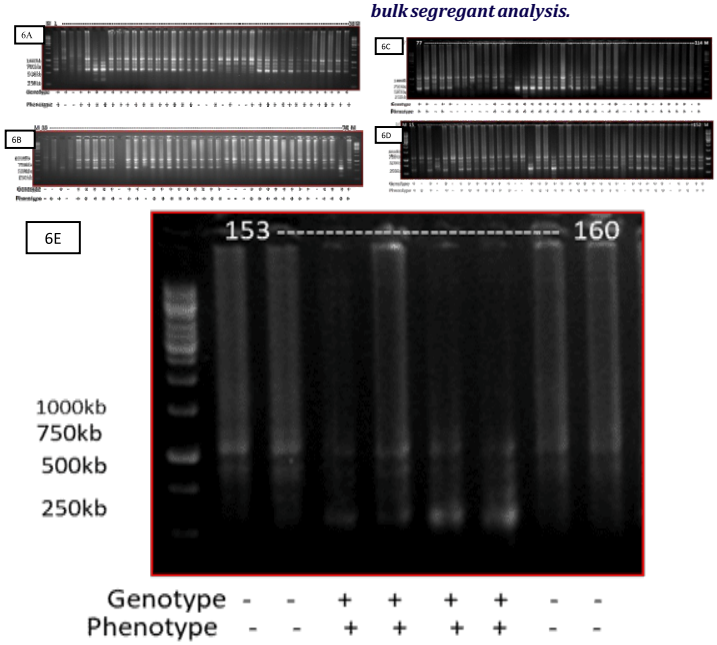


Figure 5: UBC-812 analysis displaying individual lines, categorized into 8 susceptible and resistant lines, utilized in bulk segregant analysis.



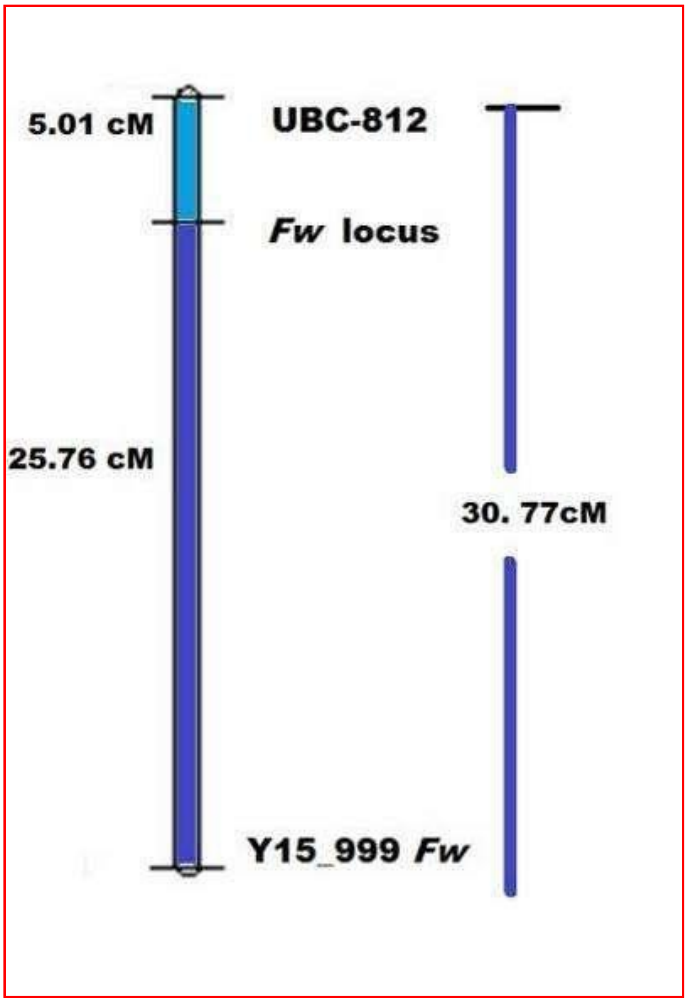


Figure 7: Map of Fw locus conferring resistance to Fusarium oxysporum f. sp. pisi. The markers and Fw locus are depicted on the right side of the estimated map

Table 1. Segregation of garden pea genotype response against Fusarium wilt and their probability in the F2 and back-cross generations in cross Arkel × GP-6

Cross	Total plant		Observed plants	Observed ratio	Expected ratio	χ^2 (Cal.)	χ^2 (Tab.)	P-value at 5%
Arkel × GP-6		**R**	**S**					
Arkel	35	0	35					
GP-6	35	35	0					
F_1	30	30	0					
F_2	160	124	36	3.44:1	3:1	0.533	3.84	0.25-0.50 (0.465)
BC_1P_1	35	19	16	1:1	1:1	0.850		0.356
BC_1P_2	35	35	0	1:0				

R : resistant; S: susceptible

Table 2. Molecular markers linked to Fusarium wilt locus in Pisum sativum L.

Markers	SCAR; Y15_999*Fw*	ISSR; UBC-812
Nucleotide sequence	**F-**ATGAGGGTAGCGCTTCATTG **R-** GCCCTTTGTTGTCTCACCTG	GAGAGAGAGAGAGAGAA
Genetic distance from *Fw* locus (cM)	25.768	5.011
Annealing temperature (°C)	60°C	50°C
Size (bp)	999	~600
Scoring pattern	Dominant	Dominant

Table 3. Segregation analysis of molecular markers with resistance locus Fw

Marker	Observed F_2 plants		Expected ratio	Calculated χ^2 value	p value
	Resistant	Susceptible			
Phenotype	124	36	3.1	0.533	0.25-0.5 (0.465)
Y15_999	129	31	3.1	2.71	0.1-0.25 (0.140)
UBC812	125	35	3.1	0.833	0.25-0.5 (0.361)

**Tabulated value for the P = 0.05 significance level is 3.84, ** Figures in parentheses are exact probability values*

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