



Genetic variability and association study of SSR markers for yield and powdery mildew disease in pea (*Pisum sativum*)

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ABSTRACT

Makhyatmubi, one of the most popular local varieties of field pea (*Pisum sativum* L.) in Manipur has morpho-physiological bottlenecks such as tall nature and high susceptibility to fungus *Erysiphe pisi* causing powdery mildew disease. Therefore, an experiment was conducted during winter (*rabi*) seasons of 2018–19 and 2020–21 at College of Post-Graduate Studies in Agricultural Sciences (Central Agricultural University, Imphal, Manipur), Umiam, Meghalaya to generate genetic variability by hybridization of Makhyatmubi with Prakash variety which is a well-known powdery mildew resistant variety in India and possesses several contrasting characters. Fruitful heterosis was estimated with highest record in number of pods per plant (114.75%) and seed yield per plant (108.04%). These two characters also showed high PCV, GCV, heritability and GAM. Number of pods per plant (0.95) was found to have highest positive significant correlation with seed yield per plant. MP-5 and MP-119 recorded superior performance in several characters under study. For the association study of simple sequence repeats (SSR) markers, easily scorable polymorphic markers were selected for surveying association with the traits. Results revealed that SSR marker AA122 may be associated with seed yield and number of pods per plant while marker AA136 with number of seeds per pod. A5 marker was found to be associated with the powdery mildew disease. Hence, it can be employed for marker assisted selection (MAS) to identify and select pea genotypes exhibit resistance to powdery mildew disease.

Keywords: Genetic analysis, *Pisum sativum*, Powdery mildew, Yield

Field pea (*Pisum sativum* L.) is one of the major legume crops widely grown around the globe including north-eastern hill states of India. In Manipur, it occupies about 70% of the area under winter (*rabi*) pulses. It contains protein, carbohydrates, fibre constituents, minerals, phytochemicals and vitamins (Burstin *et al.* 2011). It has the ability of converting atmospheric nitrogen for economic purposes (Macwilliam *et al.* 2014). However, the potential yield of field pea has been hindered by various biotic and abiotic stresses (Sunil *et al.* 2022). Among which powdery mildew caused by *Erysiphe pisi* DC, is identified as the most threatened disease in field pea (Dixon 1987) causing yield reductions up to 26 to 47% (Munjal *et al.* 1963). The extent of improvement of crop depends on magnitude of genetic variability and heritable traits of interest. So, availability of genetic variation is an essential pre-requisite. As pea is a self-pollinated crop, variability is limited requiring to create genetic variability. Hybridization programme

is considered as one of the most important methods to generate genetic variability. Therefore, this study aimed to create variability by hybridizing the two contrasting parents, Makhyatmubi and Prakash Variety, and explore genetic variability, heritability and genetic advance for yield and its related traits. The programme is also done to generate transgressive segregants.

An attractive alternative method for screening of important traits is marker assisted selection (MAS). It provides the breeding process with lesser resource demand and efficient result. Identifying molecular markers which are associated or closely linked to disease allows performing MAS at the initial phases of plant growth with no environmental influence and without disease exposure of the plants. SSR markers are considered best suited for MAS due to their various advantages over other genetic markers such as abundance, highly polymorphic, co-dominant and inexpensive etc. In this aspect, the study includes screening of SSR markers associated to powdery mildew resistance in the progenies of the cross.

MATERIALS AND METHODS

Present study was carried during winter (*rabi*) seasons

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of 2018–19 and 2020–21 at College of Post-Graduate Studies in Agricultural Sciences (Central Agricultural University, Imphal, Manipur), Umiam, Meghalaya. Two contrasting cultivars, Makhyatmubi and Prakash were chosen as parents to generate segregating population. Makhyatmubi is one of the most popular field pea varieties in Manipur state of India because of its good taste and higher yield. However, the variety could not be cultivated widely because of some morpho-physiological bottleneck such as its tall nature which demands staking as well as highly susceptible nature to various biotic and abiotic stresses including powdery mildew disease. On the other hand, Prakash is a variety collected from ICAR-Indian Institute of Pulses Research (IIPR), Kanpur, Uttar Pradesh has short to intermediate height and is resistant to powdery mildew disease (ICAR-IIPR 2020). The two parents were screened for important characters in December 2018 and some contrasting characters were recorded (Table 1).

Phenotyping: The crossing programme was carried out during December, 2019. The F₁ seeds were collected and planted for advancing the generation during off-period season in the month of April 2020. A total of 132 F₂ were selected which was planted with 30 cm × 20 cm spacing. Makhyatmubi (disease susceptible) was planted alongside the progeny plants as spreader rows after every 5 rows. Every bed of the field was surrounded by two rows of susceptible parents to establish heavy inoculum. The spores were collected from diseased plants and dusted over other healthy plants to ensure sufficient inoculum. Partial shade was made through net shading and irrigation was given in 2-days interval till maturity stage to create favourable environment for the pathogen. A total of 71 F₃ progenies possessing desirable traits were collected and sown (24 November 2021) along with the parents in a similar manner as in F₂. For F₃ families, average values per line were taken for evaluation. Observations were recorded for 9 quantitative traits and powdery mildew disease was scored on 0–9 scale based on visual symptom (Saari and Prescott 1975). The scoring of the disease was done at two different stages i.e. pod setting stage and maturity stage.

Fruitful heterosis: Fruitful heterosis (H_F) was estimated for the superior performing progenies within both F₂ and F₃ populations as:

$$H_F (\%) = [(F_2 - MP)/MP] \times 100$$

where F₂, Average data of top 10 superior progenies in both F₂ and F₃ populations; MP, mean of the two parents.

Genotyping: Leaf samples of 45 days old seedlings were collected from the field and genomic DNA was isolated using CTAB method with slightly modified protocols. A total of

112 SSR primers were selected from previously reported SSR map (Loridon *et al.* 2005) located across all 7 linkage groups (LGs) for polymorphism survey in parental plants. The polymorphic markers were then analysed on DNA of F₁ hybrids for confirmation of true hybrids and F₂ plants for Bulk Segregant Analysis.

The Polymerase Chain Reaction (PCR) amplification program is as follows: Initial denaturation for 7 min at 94°C followed by 35 cycles of denaturation at 94°C for 30 sec., annealing ranges from 58°C to 61°C for 30 sec. and extension at 72°C for 30 sec. This was followed by primer extension step with 72°C for 10 min. The PCR amplified products were resolved on 2.5% agarose electrophoresis gel and visualized under gel documentation system. F₂ plants consisting of 15 individuals that were extremely susceptible were bulked separately from 15 individual plants that showed extremely resistant to powdery mildew disease for Bulk Segregation Analysis. The analysis was done on parental lines, resistant bulk and susceptible bulk using SSR primers that exhibited polymorphism between the parental genotypes. However, in the present study, each selected 30 individual plants were genotyped to avoid false positives. The SSR markers that showed polymorphism among the two contrasting bulks were used to genotype all F₂ plants for further confirmation.

RESULTS AND DISCUSSION

The success of crop improvement programme crucially relies on the ability to accumulate the variability by hybridisation and selecting of best performing genotypes from the segregating population. Therefore, this study aimed to develop superior lines based on yield and its related traits as well as powdery mildew resistance. The cross generated 132 F₂ individuals and 71 F₃ families were selected from F₂ based on desirable superior progenies. A diverse array of variation was recorded among the progenies for the characters under study.

Fruitful heterosis: Estimation of fruitful heterosis for 10 most superior progenies in both F₂ and F₃ revealed highest heterosis value in number of pods per plant with 114.75% followed by 108.04% in seed yield per plant. Similar results were observed by Singh *et al.* (2018) in chickpea. Meanwhile, negative heterosis values were recorded in some characters such as days to 50% flowering (-1.86%), days to maturity (-0.05%) and plant height (-9.04%). The consistent result of fruitful heterosis in both the generations of F₂ and F₃ could potentially arise from the accumulation of favourable additive alleles. Therefore, selecting elite progenies from the derived population might be suitable for developing the superior genotypes.

Table 1 The parental genotypes, their sources and few important contrasting characters

Parent	Source	Powdery mildew	Plant height (cm)	100-seed weight (g)	Days to maturity	Hilum colour
Makhyatmubi	Imphal, Manipur	Susceptible	150.6	30.23	131.9	Black
Prakash	IIPR, Kanpur, UP	Resistant	71.3	23.03	126.9	White

Genetic variability: High estimate of Phenotypic Coefficients of Variation (PCV) and Genotypic Coefficients of Variation (GCV) was recorded for plant height, primary branches per plant, number of seeds per pod, number of pods per plant and seed yield per plant (Table 2). Saxena *et al.* (2014), Singh *et al.* (2019) and Cazzola *et al.* (2020) reported similar result in regards to coefficient of variation. PCV and GCV showed a decreasing trend from F₂ to F₃ generation for all the characters, which reiterated the substantial decrease of variability in F₃ generation (Patil *et al.* 2023) due to practice of selection in F₂ generation. Characters with high heritability combined with high genetic advance were recorded in plant height, primary branches per plant, number of pods per plant and seed yield per plant. This implied the preponderance of additive gene action and selection depending on such characters would be effective for further crop improvement. Shivakumar *et al.* (2013), Lakic *et al.* (2019), Singh *et al.* (2019) and Cazzola *et al.* (2020) reported similar conclusion in field pea.

Correlation coefficient study indicated number of pods per plants as the most significant trait that is positively associated with seed yield. Other characters that were strongly associated with seed yield in both or either of the population (F₂ and F₃) were days to 50% flowering, plant height, primary branches per plant, number of seeds per pod, 100-seed weight and harvest index. Therefore, selection of such traits individually or simultaneously would be quite efficient in improving seed yield.

Screening of powdery mildew: Under natural field condition at experimental farm of College of Post-Graduate Studies in Agricultural Sciences (Central Agricultural University, Imphal, Manipur), Umiam, Meghalaya, powdery mildew appeared by the end of February to early March. The lines or individuals that were susceptible, developed symptoms such as white floury specks on the stems and underside of leaves. When the crop attained full podding stage by the end of March, white powdery mass of the mycelium covered the pods, leaves, stem and branches in the susceptible cultivars. The tips parts of the plants were even covered with the mycelium at its maturity stage. The susceptible plants convert from white powdery to pale yellow and dull brown to finally blackish. Similar symptoms of the disease were also observed by Srivastava *et al.* (2012) and Sulima and Zhukov (2022). The powdery mildew was screened visually in the natural field condition following the 0–9 scale as given by Saari and Prescott (1975).

Table 2 Parameters of genetic variability for yield and its components in biparental population

Character	Gen	GCV	PCV	H ² (bs)	GAM
Days to 50% flowering	F ₂	4.20	6.29	66.74	8.65
	F ₃	3.78	4.68	64.98	6.27
Days to maturity	F ₂	3.52	4.02	87.60	7.26
	F ₃	1.68	2.83	35.38	2.06
Plant height (cm)	F ₂	28.47	32.98	86.33	58.65
	F ₃	25.04	30.06	69.39	42.97
Primary branches/plant	F ₂	40.03	143.82	27.83	82.45
	F ₃	37.24	56.24	43.84	50.79
Number of pods/plant	F ₂	89.48	99.25	90.16	184.33
	F ₃	27.55	40.19	47.00	38.90
Number of seeds/pod	F ₂	18.63	23.49	79.32	38.38
	F ₃	11.02	15.06	53.56	16.61
100-seed weight (g)	F ₂	8.16	19.61	41.61	16.81
	F ₃	6.07	12.34	24.18	6.14
Seed yield/plant (g)	F ₂	82.77	97.74	84.68	170.50
	F ₃	36.04	43.17	69.71	61.98
Harvest index (%)	F ₂	22.34	32.18	69.42	46.02
	F ₃	11.12	18.04	37.88	14.08

In F₂ generation, 34 lines were recorded to show resistant reaction out of 132 total individual plants. Bobkov and Selikova (2021) had similar ratio of the progenies that were showing symptoms. The disease score of 3 to 9 were considered as susceptible and 98 F₂ individuals were phenotyped under this category. This result suggested that

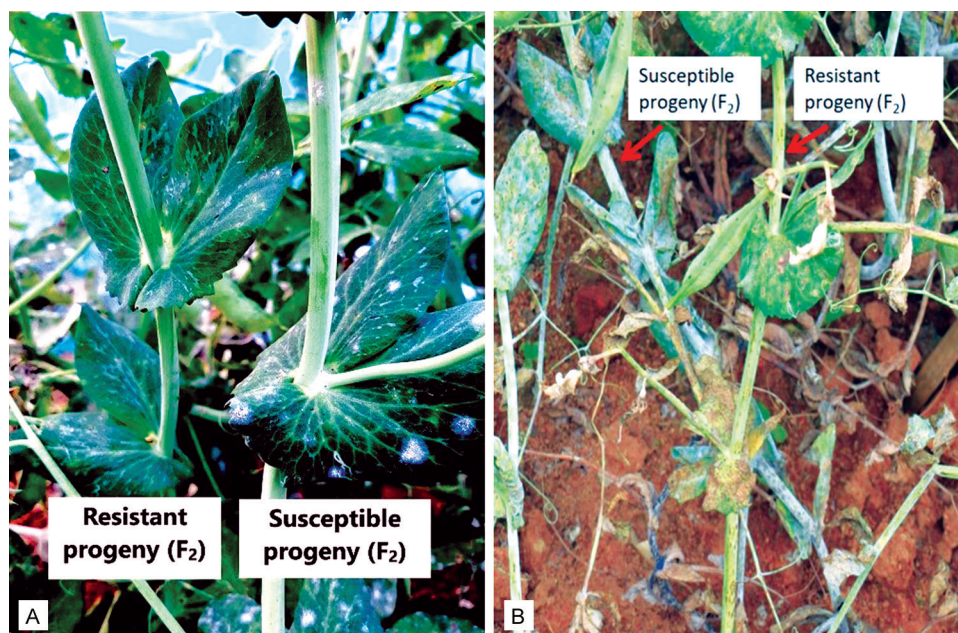


Fig. 1 Powdery mildew symptoms in the progenies of the cross, Makhyatmubi × Prakash. Pea resistant and susceptible to *Erysiphe pisi* at (A) flowering stage and (B) late podding stage.

powdery mildew resistance (*erer*) gene present in Prakash variety is monogenic recessive in nature as it fitted closely to a 3:1 (Susceptible vs Resistant) ratio. Ek *et al.* (2005), Srivastava *et al.* (2012), Reddy *et al.* (2015) and Sun *et al.* (2022) disclosed the monogenic and recessive nature of resistance gene of the disease. Progenies such as MP-5 and MP-119 possessed several desirable characters such as plant height, 100-seed weight, and larger pod size as well as resistant to powdery mildew (Fig. 1 A and B). Such plants may need further investigation and use for incorporating in the program of crop improvement. Estimation of heritability for powdery mildew revealed high heritability along with high genetic advance per cent over mean (GAM) indicating the potential for further improvement of the crop.

Marker trait association: Marker assisted selection requires identification of molecular markers in the vicinity of the gene/s of interest. SSR markers are still viewed as one of the most ideal molecular markers available so far (Abdel-Rahman *et al.* 2016). Therefore, the two contrasting parents for powdery mildew disease in this study i.e. Makhyatmubi and Prakash, were screened for 112 SSR markers, of which 30 markers were found polymorphic. Bulk segregant analysis (BSA) was employed to find the markers associated to the gene of interest from the identified polymorphic SSR markers. 30 individuals (15 susceptible and 15 resistant) in F₂ that showed extreme reaction to powdery mildew disease were selected. Each individual was separately genotyped further to avoid false positives and employed for analysis along with the two parents. The analysis identified that A5 marker is associated with powdery mildew disease. Furthermore, this study conducted a simple regression analysis and chi square test for goodness of fit to analyze the A5 marker in the total F₂ population and confirmed its association with powdery mildew disease. Ek *et al.* (2005) reported this marker to be located at the distance of 14.9 cM from the *er1* gene. The SSR marker A5 is the only marker that gave consistent result in all the studies of SSR markers for *er1* gene till date. Thus, this marker may be used to identify and employed for marker assisted selection in pea.

Polymorphic SSR markers were also analyzed for other characters that are under study, which revealed that marker AA122 may be associated to number of pods per plant with a linear regression value of 0.33 while marker AA136 to number of seeds per pod with a linear regression value of 0.59. Further confirmation of these results would be needed to use these markers for future breeding purpose.

SSR markers are considered to be more effective and inexpensive as compared to other kinds of genetic marker systems making them an attractive choice for MAS of powdery mildew disease resistance in field pea. Hence, SSR marker A5 can be deployed for breeding purpose in field pea for powdery mildew which is governed by *er1* gene. The present study also demonstrated that the resistance gene present in Prakash variety is *er1* gene.

Hybridization generates a diverse range of genetic variability evidenced with high heritability as well as

correlation coefficients. MP-5 and MP-119 should be maintained to hold promise as great variety showing superior performance by combining desirable characters from both the parents. It is also advisable to go for backcrosses to introgress other desirable characters from either of the parents. A5 marker can be employed in MAS for powdery mildew, since it showed reliable and consistent results in various studies that have investigated the SSR markers with powdery mildew disease in field pea.

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