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Identification of SSR Markers Linked to Powdery Mildew Resistance in Table Pea (*Pisum sativum* var. *Hortense* L.)

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ABSTRACT

Background: Powdery mildew is one of the major diseases of pea which leads to severe crop losses and also affects the quality of pods and seeds. Therefore, it is necessary to screen for resistant sources and introduce the resistant gene into cultivars. So, for marker-assisted pea breeding, rapid screening of pea germplasm for powdery mildew resistance is required.

Methods: A total of twenty-eight pea lines were generated using half-diallel mating design with eight pea lines (AP-3, Kashi Nandini, Arkel, VL-7, PMR-53, Kashi Uday, PC-531 and AP-1). These hybrids and parental lines were screened for powdery mildew resistance phenotypically by PDI and genotypically by SSR and SCAR markers.

Result: Based on screening for powdery mildew resistance, the genotypes PMR-53, Kashi Nandini, Kashi Uday, VL-7, AP-1 and hybrids Kashi Nandini × VL-7, Kashi Nandini × PMR-53, Kashi Nandini × Kashi Uday, Kashi Nandini × AP-1, VL-7 × PMR-53, VL-7 × Kashi Uday, VL-7 × AP-1, PMR-53 × Kashi Uday, PMR-53 × AP-1 and Kashi Uday × AP-1 were found relatively resistant to powdery mildew disease. Two SSR markers (AD237 and AD141) showed polymorphism between resistant and susceptible lines and can be used for genetic improvement of pea germplasm.

Key words: Disease screening, Pea, Powdery mildew, SCAR markers, SSR.

INTRODUCTION

Pea (*Pisum sativum* L.; Fabaceae) is a significant cool-season vegetable crop grown for green pods in temperate zones and tropical high lands around the world (Ali *et al*., 1994; Azmat *et al*., 2010). Pea is typically grown in summer as an off-season crop in the high hills and during the winter season in the Indian plains (Rana *et al*., 2010; Bala *et al*., 2011). Powdery mildew of pea, caused by *Erysiphe pisi* DC, is one of the most devastating diseases, causing up to 50% yield losses globally (Munjal *et al*., 1963; Singh *et al*., 1978; Warkentin *et al*., 1996; Katoch *et al*., 2010). Powdery mildew has been found in the majority of the pea varieties grown here. There are germplasm lines that have shown resistance to powdery mildew, but the majority of them are not agronomically superior (Ghafoor *et al*., 2005). As a result, it is critical to identify germplasm lines that may contain genes for both disease resistance and high yielding (Tiwari *et al*. 2004; Zong *et al*., 2008; Bing *et al*., 2011). This could help conventional plant breeders to overcome the challenges of linkage drag while transferring powdery mildew resistance from germplasm lines with poor agronomic performance.

Traditionally, disease screening in the field or greenhouse is to select resistant plants. This is more often a time-consuming method that also involves handling and maintaining the pathogen. Use of Molecular markers is the best alternative in screening powdery mildew resistance lines and making the breeding process more efficient. Gene specific markers that are closely linked to powdery mildew resistance must be identified for rapid screening of resistant lines using marker-assisted selection (MAS) at early stages of plant development, thereby avoiding selection through disease exposure (Rakshit *et al*., 2001). MAS can be useful not only for qualitative traits controlled by single genes but also for quantitative traits (Lande and Thompson, 1990). The complex genetic architecture of quantitative traits, on the other hand, may limit the efficiency of MAS for such traits (Edwards and Page, 1994). Powdery mildew resistance is achieved by transferring the disease resistant gene from a resistant donor line to a susceptible recipient line. Earlier studies have established that powdery mildew resistance is controlled by a single recessive gene, designated as per

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according to Gupta (1987), Kumar and Singh (1981) and Tiwari *et al*. (1997), two genes, er1 and er2, are involved. The current study aimed to identify powdery mildew resistant lines using simple sequence repeats (SSR) markers for genetic improvement of pea germplasm.

MATERIALS AND METHODS

This experiment was conducted at the Horticulture Research Centre (HRC) of the SVP University of Agriculture and Technology, Meerut during the *rabi* season, of 2018-19 and 2019-20. Twenty-eight cross combinations were generated with eight parental lines (AP-3, Kashi Nandini, Arkel, VL-7, PMR-53, Kashi Uday, PC-531 and AP-1) using half-diallel mating design (Table 1) during *Rabi* 2018-19. Under open field conditions, all eight parents and twenty-eight F1 hybrids were screened for powdery mildew resistance and disease scoring was done according to Munjal *et al*. (1963) (Table 2 and Table 3). The per cent disease index (PDI) was calculated using Wheeler's formula (Wheeler, 1969).

Genotyping for disease resistance using molecular markers for all the parental lines and F_1 hybrids was done at Molecular Biology Lab, Department of Genetics and Plant Breeding. The leaf samples were collected and stored at -80°C. The genomic DNA was isolated based on a modified protocol of CTAB (Cetyl- Trimethyl-Ammonium Bromide) by Doyle and Doyle (1990) and stored at -20°C for further experiments.

Molecular screening was performed using 27 SSR and 3 SCAR primer pairs (Merk Bioscience, USA) (Table 4). SSR and SCAR markers that were found to be linked to *er* gene, (Loridon *et al*., 2005) were tested for linkage to the candidate gene within the parental lines. PCR amplification was done in 25 µl reaction mixtures, containing 1 µl of diluted template DNA (50 ng/µl), 0.5 µl (5 µM) of each forward and reverse primer, 0.5 µl of 10 mM dNTPs, 2.5 µl of 10× buffer, 0.8 µl (U/µl) of *Taq* polymerase and 19.2 µl of ddH_2O. PCR reactions were carried out on a PTC Peltier Thermal Cycler (MJ Research) with Initial denaturation at 94°C for 3 min then denaturation at 94°C for 1 min, annealing at 45°C to 60°C for 1 min, extension at 72°C for 1 min (35 cycle) and then final extension at 72°C for 10 min and termination at 4°C. The Amplified PCR products were separated by ethidium bromide stained gel electrophoresis, which was further visualized using Alpha Imager 1200 TM (Alpha Innotech Corporation, USA).

RESULTS AND DISCUSSION

A total of eight parents used in the present study, were grouped as immune, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible category based on disease score using 0-5 scale (Munjal *et al*., 1963). The genotypes PMR-53, Kashi nandini, Kashi Uday, VL-7 had shown relatively high powdery mildew resistance . The genotype AP-1 was found to be relatively moderately resistant. Whereas, the genotypes AP-3 and PC-531 were found to be moderately susceptible and susceptible to powdery mildew respectively. Furthermore, the genotype Arkel was adversely affected with the disease among the screened genotypes which was scored as most susceptible line.

The above results suggested that the screened lines against powdery mildew disease provide information on new resistant varieties as well as few good sources of resistance that could be useful to researchers for developing powdery mildew resistant varieties. However, there is need for further evaluation of more numbers of lines against powdery mildew to find more resistant materials. The findings of screening is similar to Tiwari *et al*. (1997); Singh *et al*. (1988); Pandey *et al*. (1999); Shiwani and Sharma (2022). Similarly, Davidson *et al*. (2004) screened for 88 lines, of which 19 lines were showed powdery mildew resistance. Furthermore, Mishra *et al*. (2013) evaluated nine accessions for powdery mildew resistance and found one variety as moderately resistant, two entries were found as moderately susceptible, one entry was found susceptible and two entries were found highly susceptible. Moreover, Rana *et al*. (2013) screened 701 accessions of garden and field pea originating from 60 countries for powdery mildew resistance under natural epiphytotic conditions. Among them 64 accessions were found resistant in field screening. Nag *et al*. (2018) evaluated fifteen Pea varieties for powdery mildew resistance, among them two varieties were found resistant (R), two entries and one variety was found moderately resistant (MR), two varieties were found susceptible (S) and one variety was found highly susceptible.

It is also observed that among 28 F_1 hybrids, 10 hybrids (PMR-53 × AP-1, VL-7 × PC-531, Kashi Nandini × VL-7, AP-3 × PMR-53, VL-7 × PMR-53, AP-3 × AP-1, Kashi Nandini ×

Table 1: List of pea genotypes used as parents for resistance screening.

Genotypes	Source
AP-3	CSAUAT, Kanpur, Uttar Pradesh
Kashi Nandini	IIVR, Varansi, Uttar Pradesh
Arkel	IARI, New Delhi
VL-7	VPKAS, Almora, Uttarakhand
PMR-53	GBPUAT, Pantnagar, Uttarakhand
Kashi Uday	IIVR, Varansi, Uttar Pradesh
PC-531	PAU, Ludihana, Punjab
AP-1	CSAUAT, Kanpur, Uttar Pradesh

Table 2: Disease scale description for powdery mildew.

Scale	PDI-Per cent disease index	Reaction	Genotypes
0	0	I-Immune	
1	1-10	R-Resistant	PMR-53, Kashi Nandni, Kashi Uday, VL-7
2	10.1-25	MR- Moderately resistant	AP-1
3	25.1-50	MS-Moderately susceptible	AP-3
4	50.1-75	S-Susceptible	Pc-531
5	>75.1	HS-Highly susceptible	Arkel

Table 3: List of twenty-eight cross combinations generated using half-diallel mating design with eight parental lines.

Crosses	PDI	Category
AP-3 × Kashi Nandini	07.46	R
AP-3 × Arkel	56.21	S
AP-3 × VL-7	72.34	S
AP-3 × PMR-53	03.68	R
AP-3 × Kashi Uday	14.17	MR
AP-3 × PC-531	26.89	MS
AP-3 × AP-1	04.75	R
Kashi Nandini × Arkel	83.39	HS
Kashi Nandini × VL-7	03.45	R
Kashi Nandini × PMR-53	18.45	MR
Kashi Nandini × Kashi Uday	16.34	MR
Kashi Nandini × PC-531	05.35	R
Kashi Nandini × AP-1	65.82	S
Arkel × VL-7	76.33	HS
Arkel × PMR-53	44.23	MS
Arkel × Kashi Uday	78.52	HS
Arkel × PC-531	83.67	HS
Arkel × AP-1	60.15	S
VL-7 × PMR-53	04.55	R
VL-7 × Kashi Uday	18.45	MR
VL-7 × PC-531	03.22	R
VL-7 × AP-1	33.15	MS
PMR-53 × Kashi Uday	14.85	MR
PMR-53 × PC-531	05.86	R
PMR-53 × AP-1	02.41	R
Kashi Uday × PC-531	50.76	S
Kashi Uday × AP-1	06.51	R
PC- 531 × AP-1	13.16	MR

PC-531, PMR-53 × PC-531, Kashi Uday × AP-1, AP-3 × Kashi Nandini) were found to be resistant to powdery mildew *i.e.* which were categorised as resistant on 0 to 5 scale. Six hybrids *i.e.* PC- 531 × AP-1, AP-3 × Kashi Uday, PMR-53 × Kashi Uday , Kashi Nandini x Kashi Uday, VL-7 × Kashi Uday, Kashi Nandini × PMR-53 were found moderately resistant to powdery mildew. Three hybrids AP-3 × PC-531, VL-7 × AP-1, Arkel × PMR-53 were found moderately susceptible. Five hybrids Kashi Uday × PC-531, AP-3 × Arkel, Arkel × AP-1, Kashi Nandini × AP-1, AP-3 × VL-7 were classified as susceptible category. Four hybrids Arkel × VL-7, Arkel × Kashi Uday, Kashi Nandini × Arkel, Arkel × PC-531 were highly susceptible for powdery mildew as they exhibited maximum PDI range. The overall results indicated that out of 28 crosses, 16 hybrids were found resistant and 12 hybrids were found susceptible to powdery mildew. Out of 16 resistant hybrids, PMR-53 × AP-1, VL-7 × PC-531 were found highly resistant for powdery mildew as they exhibited minimum PDI. Out of 12 susceptible hybrids, Arkel × PC-531 were found highly susceptible for powdery mildew. This is evident that among all the parents and hybrid combinations, we found the variety Arkel and hybrids in which Arkel is used as one of the parents showed susceptibility to powdery mildew. That indicates the susceptibility is governed by dominant gene. The similar findings were reported earlier by Janila *et al*. (2001), screening for powdery mildew resistance in pea in 10 crosses, involving 16 different parents for inheritance studies in natural epidemic conditions was used for disease screening.

The eight parental lines and their derived 28 hybrids of pea were further screened using 27 SSR markers and 3 SCAR markers linked to powdery mildew resistance. Out of thirty, only 2 SSR markers (AD237 and AD141) had shown polymorphism between resistant and susceptible parents (Fig 1 and Fig 2). AD237 marker produced an amplicon of

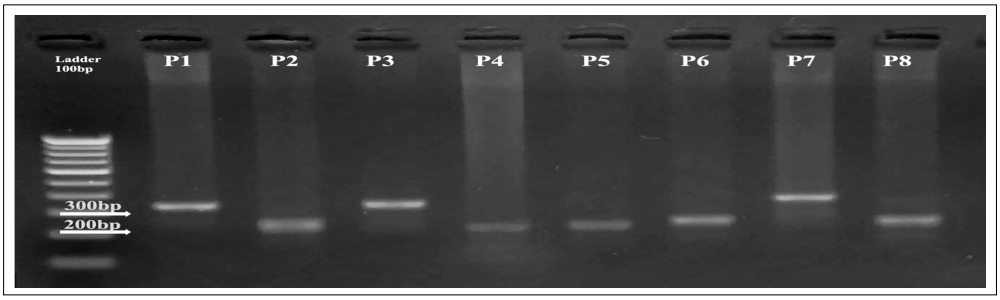


Fig 1: Molecular profyling of eight pea parental lines using AD237 SSR marker.

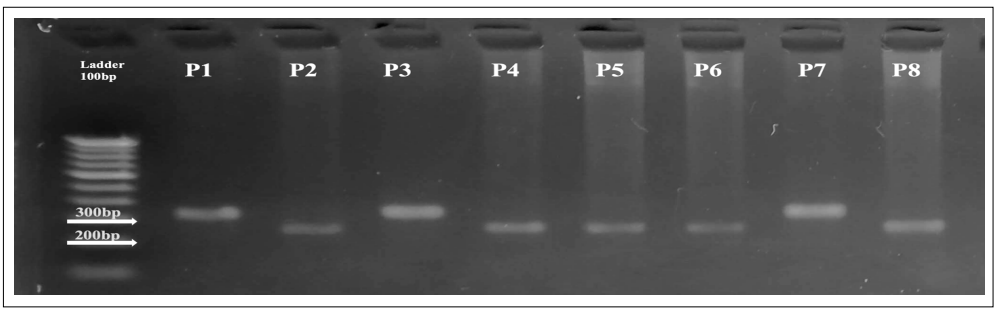


Fig 2: Molecular profyling of eight pea parental lines using AD141 SSR marker.

approx. 300 bp in all the susceptible/ moderately susceptible genotypes. Likewise, an amplification product of approx. 200 bp was observed in all the resistant genotypes. The AD141 primer uniformly produced an amplification product of approx. 300 bp in all the susceptible moderately susceptible genotypes. Similarly, an amplification product of approx. 200 bp was observed in all the resistant genotypes. These results were in concurrence with earlier studies by Loridon *et al*., 2005., Katoch *et al*. (2010) reported a RAPD marker OPX17 1400 (2.6 cM) linked to the *er*-2 gene which was successfully converted to a SCAR marker, ScX 17 1400. The ScX17 1400 marker will ensure precise introgression of *er*-2 gene into susceptible cultivars by permitting selection of *er*-2 in the backcross generation without progeny tests and resistance screening. Similarly, Pereira *et al*. (2010) identified DNA markers linked to induced mutated genes (*er*1mut1 and *er*1mut2) conferring resistance to powdery mildew in pea (Aziz-ur-Rahman *et al*., 2021). Pavan *et al*. (2013), Sun *et al*. (2014) constructed SSR genetic linkage map of pea (*Pisum sativum* L.) based on Chinese native varieties for powdery mildew resistance. Reddy *et al*. (2015) screened parental genotypes and F_2 hybrids using 12 SCAR markers and 5 SSR markers. None of the SCAR markers could distinguish between the resistant or susceptible genotypes. The SSR marker A5 clearly differentiate the homozygous resistant and susceptible parents and F_2 progeny from crosses of Arka Priya × IP-3, Arka Pramod × IP-3 and Arka Ajit ×Azad-Pea. Thus, even if none of the markers is close enough to the er gene, two SSR markers can considerably improve the likelihood of correct identification and may thus be successfully employed in MAS for powdery mildew resistance in pea.

In both primers, hybrids i.e. Kashi Nandini × VL-7, Kashi Nandini × PMR-53, Kashi Nandini × Kashi Uday, Kashi Nandini × AP-1, VL-7 × PMR-53, VL-7 × Kashi Uday, VL-7 × AP-1, PMR-53 × Kashi Uday, PMR-53 × AP-1 and Kashi Uday

Table 4: Random SSR and SCAR primers used to amplify the pea genotypes in this study.

Primer code	Primer sequence		Annealing temp. (°C)
	Forward	Reverse	
SSR markers			
SSR A5	GTAAAGCATAAGGGGATTCTCT	CAGCTTTTAACTCATCTGACACA	51
SSR AA369	CCCTTCGCACACCATTCTA	AGTCGTTTTGGAGATCTGTTCA	59.0
SSR AA374	GTCAATATCTCCAATGGTAACG	GCATTTGTGTAGTTGTAATTTCT	59.0
SSR AD51	ATGAAGTAGGCATAGCGAAGAT	GATTAAATAAAGTTCGATGGCG	51.0
SSR AD60	CTGAAGCACTTTTGACAACTAC	ATCATATAGCGACGAATACACC	60.0
Mlo1	ACTTGGCATCCTTGTTCCAC	ATGACTCGACACCCGCTATT	51.0
Mlo2	CCAATCACAAGCCTGGAAC	GATCCGTGCCCTTGAAGAT	49.0
Mlo3	CTTTCTCTTTCCCCGGAATC	TGGGTTTGTCTTGCAGTGAG	45.1
Mlo4	AGCACGGATTGAAGCTAGGA	TCGGATGATCTGACCTGACA	51.0
AD270	CTCATCTGATGCGTTGGATTAG	AGGTTGGATTTGTTGTTTGTTG	51.0
AD237	AGATCATTTGGTGTCATCAGTG	TGTTTAATACAACGTGCTCCTC	48.0
AD141	AATTTGAAAGAGGCGGATGTG	ACTTCTCTCCAACATCCAACGA	48.0
AA278	CCAAGAAAGGCTTATCAACAGG	TGCTTGTGTCAAGTGATCAGTG	51.0
AD186	TCAATGACGTGTTGATCGAGGA	CCATGCTTTGCACCGAAAGTAA	51.0
AA67	CCCATGTGAAATTCTCTTGAA	GCATTTCACTTGATGAAATTTCG	53.0
AA5	TGCCAATCCTGAGGTATTAACAC	CATTTTTGCAGTTGCAATTTCGT	52.0
PSMPSAD146	TGCTCAAGTCAATATATGAAGAR	CAAGCAAATAGTTGTTTTGTTA	48.1
PSMPSAD134	TTTATTTTTCCATATATTACAGR	ACACCTTTATCTCCCGAAGACTTAG	53.0
SSR 1	GACTTGCATTTCTATGTTATATAG	AATATAAGGAAATTTGATCGAATAT	58.0
SSR 2	AAATTGACTTGCATTTCTATGTT	TACTACTAGGTTACATTAATTACTA	58.0
SSR 3	AAATTGACTTGCATTTCTATGTT	AGAAATTGCCTATGATTTGACT	56.0
PSAS	GGTGATAACTATTTGGCTCATC	GTAGATTTCTCCATTCACCTG	51.6
S144	TTTTCTCACCGCGCTTATTT	AACAACCACCGAAGACGAAG	55.0
AA335s	ACGCACACGCTTAGATAGAAAT	ATCCACCATAAGTTTTGGCATA	49.0
PSMPA5	GTAAAGCATAAGGGGTTCTCAT	CAGCTTTTAACTCATCTGACA	56.2
PsMLO1	AAAATGGCTGAAGAGGGAGTT	TCCACAAATCAAGCTGCTACC	52.0
PSMPSAA374	GTCAATATCTCCAATGGTAACG	GCATTTGTGTAGTTGTAATTTCAT	59.2
SCAR markers			
$ScW4_{637}$	CAGAAGCGGATGAGGCGGA	CAGAAGCGGATACAGTACTAAC	51.6
ScX17-1400	GGACCAAGCTCGGATCTTTC	GACACGGACCCAATGACATC	51.6
$Sc\text{-}AB1_{874}$	CCGTCGGTAGTAAAAAAAACTA	CCGTCGGTAGCCACACCA	51.6

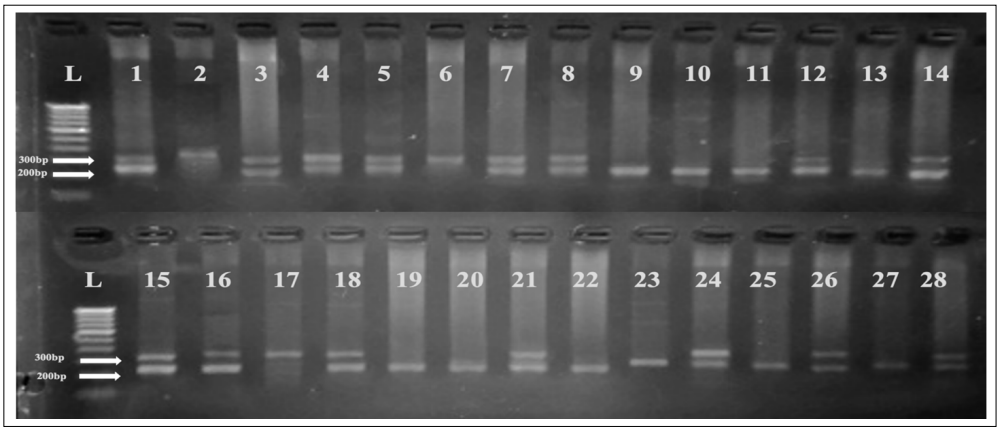


Fig 3: Molecular screening of 28 hybrid combinations lines using AD237 SSR marker. (100 bp ladder).

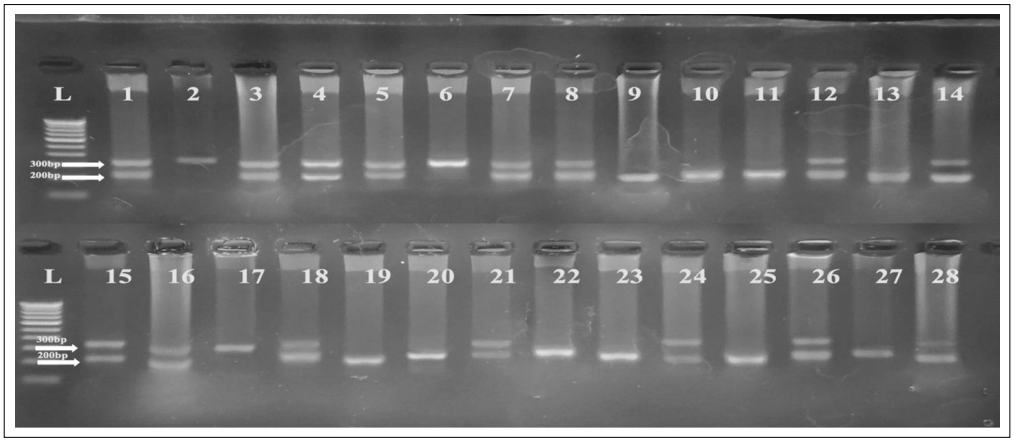


Fig 4: Molecular screening of 28 hybrid combinations lines using AD141 SSR marker. (100 bp ladder).

× AP-1 are resistant it means that the parents used for crossing was resistance that's why it was heritable to the off spring while AP-3 × Arkel, AP-3 × PC-531, Arkel × PC-531 are susceptible. Moreover, some hybrids AP-3 × Kashi Nadini, AP-3 × VL-7, AP-3 × PMR-53, AP-3 × Kashi Uday, AP-3 X AP-1, Kashi Nandini × Arkel, Kashi Nandini × PC-531, Arkel × VL-7, Arkel × PMR-53, Arkel × Kashi Uday, Arkel × AP-1, VL-7 × PC-531 PMR-53 × PC-531and Kashi Uday × PC-531are heterozygous as they are showing both resistant and susceptible bands (Fig 3 and Fig 4). Similar screening results were reported by Ek *et al*. (2005) who used microsatellites (SSR) to find markers linked to powdery mildew resistance, using bulked segregant analysis. Pereira *et al*. (2010) found that, screening of 360 decamer primers enabled the identification of two RAPD markers linked to one of the mutant resistant genes (Frilene mutant), which need further confirmation in a segregant F_2 progeny in Pea. These primer pairs (AD237 and AD141) can help in rapid screening of powdery mildew resistant genotypes for pea breeding. That in turn help in identifying the QTLs linked for powdery mildew resistance for marker assisted selection and gene introgression for genetic improvement pea germplasm.

CONCLUSION

Genotypes PMR-53, Kashi Nandni, kashi Uday, VL-7, AP-1 and hybrids, Kashi Nandini × Vl-7, Kashi Nandini × PMR-53, Kashi Nandini × Kashi Uday, Kashi Nandini X AP-1, VL-7 × PMR-53, VL-7 × Kashi Uday, VL-7 X AP-1, PMR-53 × Kashi Uday, PMR-53 × AP-1and Kashi Uday × AP-1 were found resistant to powdery mildew which could be further used in identifying QTLs associated with powdery mildew for the development of resistant genotypes using markers assisted selected (MAS). SSR Markers (AD237 and AD141) gave polymorphism between resistant and susceptible parents and hybrids and further these primers can be used for rapid screening of powdery mildew resistance.

Conflict of interest

Authors declare that there is no conflict of interest.

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