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Screening of Indian wild apple accessions with scab and powdery mildew disease resistant gene specific molecular markers

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ABSTRACT

Investigations on resistance to pathogens within local wild populations is the most efficient way for developing resistant cultivars in breeding programmes. In this study, Indian wild crab apple (*Malus baccata* Borkh var Himalaica) accessions maintained at two field gene banks in Himachal Pradesh were screened to find out the resistant sources. Individual trees were tested for the presence/absence of *Vf*, *Vrl* and *Vbj* scab resistant and Pl_1 and Pl_2 mildew resistant genes with eight and one SCAR markers respectively tightly linked to these genes. The potted plants were evaluated after the inoculation of pathogen. All the accessions showed diversity in relation to resistant gene to both the pathogens. All the SCAR markers K08, T06, Al07, Am19, ACS07, ACS09, Z13 and S22 were present in eight crab apple accessions. PLBJ SCAR marker linked with major genes Pl_1 and Pl_2 was detected in six accessions. Molecular marker assessment results almost corresponded to the data received in pot assay experiments thus were in good agreement. The study confirmed that these accessions carry scab and mildew resistant genes which could play important role in selection of desirable genotypes for apple breeding as well as use as suitable rootstock.

Keywords: Crab apple; SCAR markers; disease; resistance; genes

INTRODUCTION

Apple scab is a widespread and one of the most harmful diseases of apple caused by an ascomycetes fungus *Venturia inaequalis.* Powdery mildew, caused by the obligate biotrophic ascomycete fungus *Podosphaera leucotricha*, is another main fungal disease in commercial apple production. Large quantities of fungicides are applied to reduce the damage caused by these pathogens. Use of fungicides gives rise to numerous ecological problems and consumer health concerns. At present, majority of apple cultivars of world-wide economic importance are still susceptible to these diseases. The development of apple cultivars displaying durable resistance to *V inaequalis* and *P leucotricha* is one of the major aims in apple resistance breeding worldwide. Investigations on genetic diversity and resistance to pathogens within local wild populations is the most efficient way for developing resistant cultivars in breeding programmes. They control the epidemic of fungal diseases and eliminate economic losses caused by them. Many other desirable traits like abiotic and biotic stress resistance are also found in wild *Malus* species (Volk et al 2015). Thus a number of cultivars are being developed that contain ancestry from several wild relatives. Therefore it is necessary to identify and evaluate valuable sources of resistance within wild genetic resources (Patzak et al 2011) because they provide an invaluable resource for improving perennial crops through disease resistance, fruit quality and rootstocks (Migikovsky and Myles 2017).

More than 17 different resistant genes have been identified for apple scab (Bus et al 2011) and among them *Vf* gene originated from wild crab apple *Malus floribunda* 821 has been used throughout the world for breeding purposes to create new scab resistant cultivars (Liebhard et al 2003). Various other sources of major scab resistant genes were originated

from small fruited Asiatic *Malus* spp (Gygax et al 2004, Patzak et al 2011), eg the genes *Vbj* from *M baccata jackii*, *Vb* from *M baccata*, *Vm* from *M* × *micromalus* and *M* × *atrosanguinea* 804, *Vr* from *M pumila* and *Vh* from Russian seedling R12740-7A of *M sieversii* (Bus et al 2005, Boudichevskaia et al 2006).

Very little is known about the variability of *P leucotricha* in the wild apple. Breeding programme on resistance phenotyping of apple cultivars has identified resistant genes to powdery mildew such as Pl_1 from *M robusta* and Pl_2 from *M zumi* (Markussen et al 1995, Dunemann et al 1994), *Plw* from the ornamental crab apple White Angel (Evans and James 2003), *Pld* from the D12 clone (James et al 2004) and *Plm* from mildew immune seedling (Dayton 1977). More than 40 RAPD and SSR-SCAR markers linked to scab resistant genes and a few linked to powdery mildew have been developed from wild apple species (Gessler et al 2006). Cultivars with multiple resistance genes can be easily selected with molecular markers associated with the resistance genes. MAS is the simplest method of increasing the efficiency of a breeding programme by identification of resistance genes and creation of resistant cultivars (Patzak et al 2011). It utilizes markers closely linked to a gene of interest to select for desirable traits that may not be expressed in an existing environment and, therefore, eliminating the error due to poor phenotyping (Tanhuanpaa and Vilkki 1999).

M baccata is a widespread wild species of apple native to temperate and tropical Asia that occurs in mixed forests on slopes. It is a secondary genetic relative to the cultivated apple *M domestica*. Collection of accessions of Indian wild crab apple (*M baccata* Borkh *himaliaca*) since 1966 included mostly from Jammu and Kashmir, Shimla hills and northeast was maintained at IARI and NBPGR regional research stations at Shimla, Himachal Pradesh. These have already been demonstrated as valuable sources of diversity for important horticultural and environmentally adapted traits (Kishore and Randhawa 1993). They have also shown different levels of resistance against various diseases like apple scab, powdery mildew and white root rot at phenotypic level (Sharma et al 2006). The untapped genetic diversity in these accessions/ biotypes has recently been reported (Vikrant 2014, Vikrant and Modgil 2019) which can be considered useful source for genetic variation to be used in apple improvement and conservation programmes. Six specific diagnostic RAPD-SCAR markers for scab and rosy aphid resistant genes have also been developed which were found to be able to discriminate the resistant from susceptible (Vikrant 2019). These SCARs revealed similarity with *M floribunda HcrVf1*, *2*, *3*, *4* genes which can overcome the races up to 5 but not 6 and 7. This has prompted to test the small fruited wild crab apple accessions with molecular markers associated with other scab resistant genes in order to find out the natural resistance. Molecular marker-based screening of wild apple accessions can be of importance to protect the germplasm sources for apple breeding and a more ingenious and cost effective approach than the field phenotyping (Kaymak et al 2013).

This work was aimed at assessing the genetic resources of apple to find out the accessions carrying resistant genes of scab and powdery mildew diseases using previously reported tightly linked molecular markers. For this, primers of nine SCAR markers were used to identify DNA fragments associated with *Vf*, *Vbj* and *Vrl* scab resistant and Pl_1 and Pl_2 powdery mildew resistant genes.

MATERIAL and METHODS

Plant material and pot screening for scab and mildew: Fourteen accessions of seven biotypes of *M baccata* maintained at field gene bank of IARI and NBPGR regional research stations, Dhanda and Phagli, Shimla, Himachal Pradesh were used (Table 1). *M floribunda* (C) of known resistance collected from field of Department of Fruit Science, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh was taken as control. Potted plants of crab apple biotypes were screened for resistance against apple scab (*V inequalis*) by spreading the conidial inoculums of *Venturia* taken from the infected leaves, whereas, powdery mildew (*P leucotricha*) bioassay was done by spraying the pathogen using standardized methods (Sharma et al 2006, Sharma and Pramanick 2012) and maintained under controlled conditions of glasshouse. The assessment of lesions related to the diseases was made according to the above methods. Biotypes were identified as tolerant/ resistant/ susceptible on the basis of time taken (number of days) for appearance of disease symptoms. Motility

Table 1. Field gene banks and origin of indigenous crab apple accessions used in the study

Name of the accession/ biotype (sample)	Code	Field gene bank	Origin
M baccata Kashmir (A)	I/1	ICAR- Indian Agriculture Research Station (IARI-RS), Dhanda, Shimla, HP	Kashmir valley, J&K
M baccata Khrot	I/2	-do-	Khrot, Dist Shimla, HP
M baccata Kinnaur	I/3	-do-	Kinnaur, Dist Kinnaur, HP
M baccata Kinnaur (Dhack)	I/4	-do-	Dhack, Dist Kinnaur, HP
M baccata Pangi	I/5	-do-	Pangi, Dist Chamba, HP
M baccata Rohru	I/6	-do-	Rohru, Dist Shimla, HP
M baccata Shillong	I/7	-do-	Shillong, Meghalaya
M baccata	N/1	ICAR- National Bureau of Plant Genetic Resources Regional Station (NBPGR-RS), Phagli, Shimla, HP	Meghalaya
M baccata Assam	N/2	-do-	Assam
M baccata Chamba	N/3	-do-	Chamba, Dist Chamba, HP
M baccata J&K	N/4	-do-	Srinagar, J&K
M baccata Kashmir (1)	N/5	-do-	Kashmir valley, J&K
M baccata Khrot	N/6	-do-	Khrot, Dist Shimla, HP
M baccata Shillong	N/7	-do-	Shillong, Meghalaya

rate was observed at variable disease pressures and at the end of the growing season.

Screening for the presence of resistance gene

DNA extraction and PCR analysis: Freshly grown young leaves of above mentioned sample accessions were collected from the fields and stored at –80°C until use. Total genomic DNA was isolated by CTAB method following the protocol given by Virscek-Marn et al (1999). After isolation of the genomic DNA,15 µl of PCR reaction volume was set up by making mixture cocktail with the components Taq DNA polymerase (1U), PCR buffer (10X), dNTPs (0.8 mM) and genomic DNA (60-80 ng) and forward and reverse SCAR primers (10 pmoles each). Eight SCAR markers (PCR molecular markers) K08, T06, Al07, Am19, ACS05, ACS09, S-22 and Z-13 linked to resistant genes of apple scab and one marker *Plbj* to powdery mildew were used for molecular marker analysis. Forward and reverse primers for SCAR markers not mentioned in Table 1 were obtained from published reports with product size from original RAPD/SSR. Eppendorf® thermal cycler was set at 5 min at 94°C (1X) followed by 35 cycles for 45 sec at 94°C, annealing temperature for 1 min (specific to T_m of primer), 2 min at 72°C for elongation and a final extension step of 8 min at 72°C. Amplified products were resolved on 2 per cent agarose gel under BioVis gel documentation system and scored for the presence and absence of bands in each sample and control.

RESULTS

All the fourteen plants of seven biotypes were phenotypically evaluated for apple scab and powdery mildew in 2018-19 (Table 2). The results showed that *M baccata* J&K, *M baccata* Kashmir (1), *M baccata* Khrot and *M baccata* Shillong collected from NBPGR field bank and *M baccata* Kashmir, *M baccata* Khrot, *M baccata* Rohru and *M baccata* Shillong from IARI field were moderately resistant to apple scab. *M baccata* from NBPGR field bank and *M baccata* Kinnaur and *M baccata* Kinnaur (Dhack) collected from IARI field bank showed moderate tolerance. The accessions of *M baccata* Assam and *M baccata* Chamba of NBPGR and *M baccata* Pangi collected from IARI field were found susceptible. For powdery mildew phenotyping, *M baccata* Shillong collected from both the sites were found resistant. *M baccata*, *M baccata* J&K and *M baccata* Kashmir collected from NBPGR field bank and *M baccata* Kashmir and *M baccata* Khrot from IARI showed moderate resistance whereas all other samples were found susceptible.

It was observed that DNA analysis using scab resistant gene *Vf* sequence specific molecular marker Al-07 identified the presence of *Vf* like sequences of the size between 750-1,000 bp in *M baccata* Kashmir, *M baccata* Khrot, *M baccata* Rohru and *M baccata* Shillong of IARI, RS and *M floribunda* (C). The same fragment was obtained in *M baccata* J&K, *M baccata*

Table 2. Presence or absence of SCAR markers in wild crab apple accessions/biotypes and their relationship with apple scab and powdery mildew pot bioassay data

Code	Biotype/accession	Pot bioassay data		Presence/absence of SCAR								
		Apple scab	Powdery mildew	AL-07	ACS-09	ACS-07	Am-19	K-08	T-06	S-22	Z-13	PLBJ
I/1	*Mb* Kashmir (A) (I/1)	MR	MR	P	P	P	P	P	P	P	P	P
I/2	*Mb* Khrot (I/2)	MR	MR	P	P	P	P	P	P	-	P	-
I/3	*Mb* Kinnaur (I/3)	MT	S	-	-	-	-	-	-	-	-	-
I/4	*Mb* Kinnaur (Dhack) (I/4)	MT	S	-	-	-	-	-	-	-	-	P
I/5	*Mb* Pangi (I/5)	MS	S	-	-	-	-	-	-	-	-	P
I/6	*Mb* Rohru (I/6)	MR	S	P	P	P	P	P	P	P	P	-
I/7	*Mb* Shillong(I/7)	MR	R	P	P	P	P	P	P	P	P	P
N/1	*Mb* (N/1)	MT	MR	-	-	-	-	-	-	-	-	P
N/2	*Mb* Assam (N/2)	S	S	-	-	-	-	-	-	-	-	-
N/3	*Mb* Chamba (N/3)	S	S	-	-	-	-	-	-	-	-	-
N/4	*Mb* J&K (N/4)	MR	MR	P	P	P	P	P	P	P	P	-
N/5	*Mb* Kashmir(1) (N/5)	MR	MR	P	P	P	P	P	P	P	P	-
N/6	*Mb* Khrot (N/6)	MR	S	P	P	P	P	P	P	-	P	-
N/7	*Mb* Shillong (N/7)	MR	R	P	P	P	P	P	P	P	P	P
C	*M floribunda*	R	S	P	P	P	P	P	P	P	P	P

Mb= *Malus baccata*, S= Susceptible, MS= Moderately susceptible, MT= Mild tolerant, R= Resistant, MR= Moderately resistant, P= Presence, - = Absence

Kashmir, *M baccata* Khrot and *M baccata* Shillong of NBPGR, RS (Table 2, Fig 1a).

The results showed that accessions *M baccata* Kashmir, *M baccata* Khrot, *M baccata* Rohru and *M baccata* Shillong of IARI, RS as well as *M baccata* J&K, *M baccata* Kashmir, *M baccata* Khrot and *M baccata* Shillong of NBPGR, RS (Fig 1b) which were showing scab resistance in pot screening experiment had a DNA fragment of approx 500 bp specific to the *Vf* gene sequence specific marker ACS-09. This fragment was also detected in *M floribunda* (C). Marker primer ACS-07 linked to the *Vf* scab resistant gene resulted in amplification of two bands of 750 and 500 bp in the same accessions as with SCARACS-09 and *M floribunda* (Fig 1c). Similarly, the amplification results with SCAR primer Am-19 (Fig 1d) linked to *Vf* gene clearly detected a single band of approx 1,000 bp in accessions *M baccata* Kashmir, *M baccata* Khrot, *M baccata* Rohru and *M baccata* Shillong of IARI, RS. This fragment was also present in *M baccata* J&K, *M baccata* Kashmir, *M baccata* Khrot and *M baccata* Shillong of NBPGR, RS and *M floribunda*. Other SCAR markers used in the study were K08 and T06 linked to scab resistant genes *Vbj* of source *M baccata jackii* amplified single band of 750 and 1,000 bp respectively in *M baccata* Kashmir, *M baccata* Khrot, *M baccata* Rohru, *M baccata* Shillong of IARI, RS and *M baccata* J&K, *M baccata* Kashmir, *M baccata* Khrot and *M baccata* Shillong of NBPGR, RS and in control *M floribunda* (Table 2, Figs 1e, 1f).

The accessions *M baccata* Kashmir, *M baccata* Rohru and *M baccata* Shillong of IARI, RS and *M baccata* J&K, *M baccata* Kashmir and *M baccata* Shillong of NBPGR, RS and control crab apple *M floribunda* (Figs 1g, 1h) detected a DNA fragment of less than 1,000 and 1,300 bp size respectively with SCAR primers S-22 and Z-13 specific to scab resistant genes *Vr1* and *Vbj* sequences respectively. Z-13 SCAR primer also showed amplification in *M baccata* Khrot collected from both the gene banks.

The above mentioned DNA fragments were not obtained in *M baccata* Kinnaur and *M baccata* Kinnaur (Dhack) which were reported to be mildly tolerant to apple scab in pot assay experiment. On the other hand, *M baccata* Pangi collected from IARI and *M baccata* Assam and *M baccata* Chamba of NBPGR which showed the susceptibility to apple scab in pot assay experiments (Table 2) did not detect the presence of any of the fragments.

SCAR marker *Plbj* linked to two major powdery mildew resistant genes Pl_1 and Pl_2 detected a single fragment of approx 240 bp in *M baccata* Kashmir, *M baccata* Kinnaur (Dhack), *M baccata* Pangi and *M baccata* Shillong of IARI, RS and *M baccata* and *M baccata* Shillong of NBPGR, RS (Table 2, Fig 1i). The accessions *M baccata* J&K and *M baccata* Kashmir of NBPGR and *M baccata* Khrot of IARI which were found moderately resistant in pot assay did not show the presence of this fragment while susceptible accessions *M baccata* Kinnaur (Dhack) and *M baccata* Pangi detected its presence. Screening of *M baccata* biotype collection showed that *M baccata* Shillong of both the gene banks and *M baccata* Kashmir of IARI, RS showed all the nine SCAR markers,

DISCUSSION

In most apple breeding programmes, resistance to diseases remains to be a significant target (Kumar et al 2014) because pathogen resistance to certain chemicals is a persistent problem for the apple industry. Environmental sustainability and the safety of food products is one of the major concerns to worry for the consumer. Several resistances are difficult to use because the diagnostics tests are hard to develop due to the challenge posed by inoculum production and maintenance. Therefore, markers tightly linked to resistance genes can be useful for disease resistance programme. Availability of a number of molecular markers linked to *Vf* gene made it possible to optimize marker assisted selection and to investigate its advantages with respect to phenotypic selection methods. In the present study, screening of indigenous wild apple genetic resources were carried out by RAPD and AFLP converted SCAR markers linked to resistant genes of apple scab and powdery mildew by earlier workers (Gygax et al 2004, Tartanini et al 1999, Huaracha et al 2004, Hemmat and Brown 2002, Dunemann and Schuster 2009). The present study for the first time identified the sources of resistance to these two main apple pathogens.

While testing the samples of wild apple accessions and *M floribunda* with apple scab disease resistant gene specific molecular markers, the presence of three different genes *Vf*, *Vbj* and *Vr1* were detected with eight SCAR markers K08, T06, Al07, Am19,

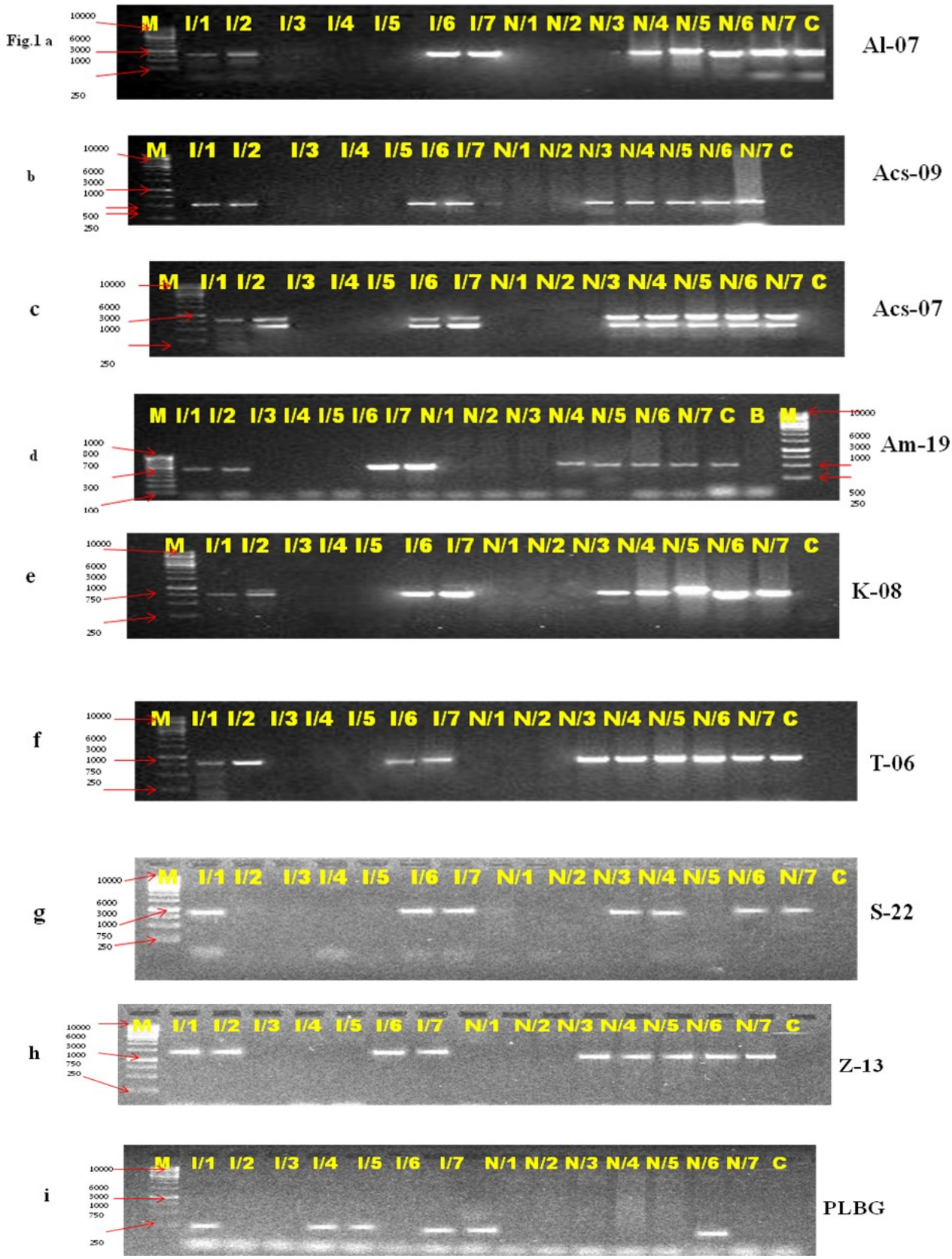

Fig 1. a, b, c, d, e, f, g, h & i: Presence/absence of previously developed SCAR markers in wild apple biotypes and *Malus floribunda* (C) where I/1- *M baccata* Kashmir, I/2- *M baccata* Khrot, I/3- *M baccata* Kinnaur, I/4- *M baccata* Kinnaur (Dhack), I/5- *M baccata* Pangi, I/6- *M baccata* Rohru, I/7- *M baccata* Shillong, N/1- *M baccata*, N/2- *M baccata* Assam, N/3- *M baccata* Chamba, N/4- *M baccata* J&K, N/5- *M baccata* Kashmir (1), N/6- *M baccata* Khrot, N/7- *M baccata* Shillong

ACS07, ACS09, Z13, S22 in *M baccata* Kashmir and *M baccata* Shillong of both gene banks, *M Baccata* Rohru of IARI, RS and *M baccata* J&K of NBPGR, RS and *M floribunda*. SCAR primer AL07 and AM19 derived from RAPD markers $OPAM19_{2200}$ and $OPAL07_{580}$ closely linked to *Vf* gene derived from the wild species *M floribunda* 821. Linkage of the RAPD primer with *Vf* gene was confirmed by bulked segregant analysis (Tartanini et al 1999). ACS-07 and ACS-09 derived from AFLP primers by Xu et al (2001) co-segregate with the *Vf* gene were found to be useful for the identification of scab resistance. Huaracha et al (2004) used these markers for screening of the scab resistant cultivars Liberty and Florina. Three RAPD-SCAR markers Z13, K08 and T06 linked to scab resistant gene *Vbj* originating from *M baccata jackii*- USA were first identified by Gygax et al (2004) using bulk segregant analysis and proved to be codominant. RAPD derived S22-SCAR linked with *Vx* locus was identified for resistance against apple scab in R12740-7A by Hemmat and Brown (2002). The results agree with presently done pot bioassay as well as the previously published data (Sharma et al 2006) on these biotypes/accessions. The independent segregation of *Vbj* from *Vf* in a cross of Golden Delicious and Hansen's baccata #2 was already confirmed by Erdin et al (2006).

Seven markers out of eight scab resistant markers were detected in *M baccata* Khrot of both the gene banks. Absence of SCAR S-22 in *M baccata* Khrot showed the absence of scab resistant gene *Vrl*. Pot bioassay carried out in these investigations confirmed the all above accessions as moderately resistant. Gelvonauskiene et al (2007) suggested that it is important for selecting trees that combine two or more genes for scab resistance because traditional methods for selection can be inefficient. The presence of markers associated with several *Vf* and other scab resistant genes in above accessions may be useful in resistance breeding by pyramiding of several resistance genes against the same pathogen to achieve more durable resistance. Kaymak et al (2013) also used ACS-07, ACS-09 and AL-07 SCAR markers to select for the presence of *Vf* gene in crossed progenies of scab resistant Williams and Priscilla with Golden delicious. The *Vf* locus originated from wild apple species, *M floribunda* 821 is an important source of resistance to apple scab disease caused by *V inaequalis*. It has been introduced into various domesticated apple cultivars (Afunian et al 2004). Researchers of the University of Illinois crossed a generous amount of commercial cultivars with crab apples (Janick 2002, Gessler and Pertot 2012). Two siblings (F226829-2-2 and F226830-2) distinguished as scab resistant after several crosses, were applied in further crosses as well as were the starting material for their breeding programmes. The inheritance of scab resistance was analyzed and called *Vf* (Bianca et al 2006). A number of inherited resistance sources mainly from wild species of apple have been identified for introgression into high quality of breeding material (Duneman et al 2004).

M baccata Kinnaur and *M baccata* Kinnaur (Dhack) from IARI, RS and *M baccata* of NBPGR, RS showed mild tolerance to apple scab disease despite the absence of all SCAR markers used in the study. The possible reason may be that they may contain a high level of polygenic resistance which is not related to *Vf*, *Vrl* and *Vbj* genes. This assumption is based on the observation of Patzak et al (2011) who did not find amplification of markers for *Vf* and *Va* resistant genes in *Va* scab resistant genotypes under their study and suggested a polygenic resistance based on another alleles of resistance genes *Va*, *Vd* etc. *M baccata* Kinnaur and *M baccata* Kinnaur (Dhack) were collected from same district Kinnaur. It may be possible that both belonged to same location Dhack.

First SCAR marker AT20-450 was developed for Pl_1 (Markussen et al 1995) followed by the N18-SCAR for a major gene involved in Pl_2 resistance (Seglias and Gessler 1997). Later several Plmis markers were identified (Gardiner et al 2003) as well as SSR and SCAR markers for *Plw* and *Pld* were developed (Evans and James 2003, James et al 2004). The primers which produced markers linked to Pl_1 gene from *M robusta* also generated markers linked to Pl_2 gene thus a putative linkage between the two has been suggested.

Because of a few resistance sources of powdery mildew (Pl_1 from *M robusta* and Pl_2 from *M zumi*) used in apple breeding programmes were reported to be not fully active against the whole race spectrum of the fungus. Therefore, Dunemann and Schuster (2009) mapped *Plbj* gene from *M baccata* accession 419 var *jackii*. The *Plbj* locus is the first major mildew resistance gene found on LG 10. They developed specific AFLP-based SCAR marker (called as PLBj-SCAR) for this gene as alternate mildew resistance sources. In the present study, its presence was found in six accessions. Out of these, *M baccata*

Shillong showed the presence of marker and resistance in pot assay, whereas, *M baccata* Kashmir and *M baccata* showed moderate resistance. However, two accessions *M baccata* Pangi and *M baccata* Kinnaur detected the presence of SCAR marker but in pot bioassay, they were found to be susceptible. A more durable resistance is achieved by combining at least two different resistance factors eg major genes Pl_1 and Pl_2. However, it was not detected in some of the biotypes like *M baccata* J&K and *M baccata* Kashmir of NBPGR and *M baccata* Khrot of IARI which were observed to be moderately resistant to powdery mildew disease in presently done pot assay experiment as well as in previous studies (Sharma et al 2006). This may be attributed to the presence of different races of the mildew fungus, the lower inoculum dose in the greenhouse, the physiological status of the apple trees or the changing climatic conditions being more favourable in the greenhouse (Dunemann et al 2004). Regarding the race spectrum, it is probable from the molecular studies on the pathogen (Urbanietz and Dunemann 2005) that in the greenhouse experiment, only a single or a few genetically similar races were present, whereas, outside in the field a more complex race spectrum occurred.

In the present study, most of the crab apple accessions have shown to carry scab and mildew resistant genes thus showing gene diversity in particular, *M baccata* Shillong of both field gene banks in terms of presence of all resistant genes (*Vf*, *Vbj*, *Vrl* and *Plbj*) to both the pathogens, which may be a target for future breeding programmes. A high level of scab resistant gene diversity has already been reported in wild apples (*M orientalis*) populations from Black Sea in Turkey and Russia and Caucasian in Iran (Volk et al 2015, Amirchakhmaghi et al 2018). According to them, presence of marker or their alleles does not confirm the resistance, therefore, phenotyping the resistance or susceptibility of wild populations must be considered. However, in the present study, the biotypes/ accessions detecting the presence of SCAR markers for scab and mildew resistance also showed resistance to apple scab disease in bioassay studies except a few while their presence was also shown in resistant *M floribunda*. PCR results almost correspond to the data received in pot assay experiments, thus are in good agreement and clearly confirm that most of the accessions carry scab and powdery mildew resistant genes. The results are similar to the previous studies based on phenotypic expression of natural resistance which revealed different levels of resistance against various diseases among these *Malus baccata* biotypes/strains while high level of resistance was observed for powdery mildew and apple scab in *M baccata* Shillong (Sharma et al 2006). It may further be suggested that there will be a practical advantage to introduce strong resistance to these two pathogens from the same donor like *M baccata* Shillong in a single cross. If a scab and mildew resistant variety with good horticultural characteristics is evolved, it will have more acceptability to the orchardists because they will be saved from heavy spray schedule of fungicides. The combination of different resistance genes is a possible way of obtaining durable fungal resistance for a long time (Urbanovich and Kazlovskaya 2008).

It is concluded that these new discovered sources showed a wide variability of resistance genes to fungal diseases. This study would be useful for the development of new resistant varieties through marker assisted breeding for improving resistance to both the fungal diseases in India. PCR molecular markers are important tools for detection/identification of resistant genes within wild apple germplasm collection and can be used to supplement the number of disease resistance sources. Further, it demonstrates the usefulness of wild apple collection from different locations at these gene banks which could play important role in the selection of desirable genotypes for apple breeding as well as for use as suitable rootstock.

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Compliance with ethical standards

The authors declare that no ethical approval was needed for the study as it did not involve the use of animals or human subjects.

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