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Short Communication

Mining new scab resistance alleles in apple (*Malus* × *domestica* Borkh.) germplasm of Kashmir: Towards breeding scab free apple cultivars

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Abstract

Apple an important fruit crop in Kashmir ranks first in all the fruits grown here. It is commercially the most important horticultural crop grown in temperate parts of the world. To control scab in Apple a lot of fungicides are being used however, an alternative approach to fungicide application is the use of scab resistant cultivars as a genetic stock. Therefore, there is an urgent need to identify more cultivars resistant to scab with maximum genes and can be used for apple breeding. In view of this, one hundred apple cultivars grown in Kashmir Valley were screened for the presence of *Vf* gene conferring resistance against apple scab. Eight trait linked markers were used so for and among them three markers like AL07, AM19, and CH05e03 proved to be useful to distinguish the resistant cultivars from the other apple germplasm. These three markers have amplified resistant fragments in five cultivars. Thus out of the total of 100 cultivars investigated in the present study, only five cultivars namely Shalimar I, Shalimar II, Dubhour, Gold Spur, and Crab apple were found to harbour resistant alleles which may be resistant to some of the races of *V. inaequalis*. Among the five cultivars harbouring the scab resistant alleles for some races of *V. inaequalis,* three cultivars namely, Shalimar I, Shalimar II, and Dubhour, which contain the maximum number of scab resistance alleles can be used as parents in further breeding programms to develop more resistant apple cultivars with high market acceptance.

Keywords: *Malus* × *domestica*, Apple scab, *Vf* gene, *Venturia inaequalis*

Apple is one of the most important horticultural crops of the temperate regions (Brown 2012). In terms of production, apple ranks third with the annual production of approximately 85 million tons (FAOSTAT 2014). The hybridization events of different Malus species have given rise to the present day cultivated appple (Velasco et al. 2010; Cornille et al. 2012). The origin of the diploid apple can be explained on the basis of wide hybridization hypothesis in which allopolyploidization event occurred between spireoid (x=9) and amygdaloid (x=8) (Phipps et al. 1991; Velasco et al. 2010). Most of the apple cultivars growing in Kashmir proved to be diploid with 2n=34 (Jahangir et al. 2018) and the estimated genome size of apple is about 750 Mb.

Apple is attacked by a large number of pathogens, resulting in the inferior quality as well as reduction in overall production. One of the serious fungal pathogens that cause a drastic apple scab is the *Venturia inaequalis* (Benaouf and Parisi 2000) which has invaded almost all the apple growing regions worldwide. The disease causes great economic losses to the growers (Machardy 1996). Although, infection may occur on leaves and fruits however, it affects stem or green twigs also. About more than 15 fungicide sprays are being used to control the disease during a particular fruit growing season. This poses direct threat to human beings and the environment. Alternative

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approach to fungicide application is the use of scab resistant cultivars as a genetic stock to breeding scab resistance cultivars. Therefore, there is an urgent need to identify more cultivars resistant to scab with maximum genes and can be used for apple breeding. Keeping in view the present scenario, an initiative was taken to screen the apple germplasm of Kashmir Valley on the basis of scab resistance genes and to utilize those genes in apple breeding in future research programmes.

Leaf samples for DNA extraction were collected from the previously established apple germplasm repository at Zakura Campus, University of Kashmir, Hazratbal, Srinagar. However, few samples that are not present in germplasm repository were collected from the respective orchards and Govt. horticultural nurseries of the Kashmir Valley. The DNA was extracted from 100 apple cultivars by using the protocol of Doyle and Doyle (1990) with little modifications. PVP was added to the extraction buffer to remove the polyphenolics. DNA samples were further processed for PCR amplification.

Amplification reactions were carried out in volumes of 10ìl containing 1 µl (50 ng) of template DNA, 0.2mM each dNTP, 20 pico molar primer, 2mM $MgCl_2$, 1XPCR buffer, 1U Taq DNA polymerase. Amplification reactions were carried out by using Eppendorf thermal cycler programmed as follows: 90 sec at 94^{o}C for initial denaturation step, 30sec at 94^{o}C (denaturation), 30 sec at 55^{o}C (annealing) and 60 sec at 72^{o}C (extension), followed by 10 min at 72^{o}C for final extension of the single strands. The SSR products were resolved by electrophoresis in 3% agarose gel using 1XTAE buffer, and stained with ethidium bromide. A 100 bp ladder (Promega Inc.) was used as a molecular weight marker, to estimate the size of the amplification products. The gels were visually examined under UV and documented using gel documentation system.

The use of marker-assisted selection is an excellent instrument for identification of resistance genes and creation of resistance cultivars. The trait linked markers used in the present study to screen 100 apple cultivars are given in Table 1. About 8 markers including SCAR and SSR (T06 SCAR, Z13 SCAR, K08 SCAR, AL07, AM19, Vf2ARD, OPL-19, and CH05e03) were used and out of these, three markers (AL07, AM19, and Ch05e03) have shown resistant fragments in few apple cultivars (Table 1). Marker AL07 amplified two fragments of about 800bp and 570bp. The 800bp fragment was produced in all the 100 investigated cultivars including both resistant and susceptible ones. On the otherhand, the 570bp fragment was amplified in three apple cultivars only *viz*., Shalimar I, Shalimar II, and Dubhour (Fig. 1). Similar results have also been reported by Khujuria et al. (2014) in other cultivars. Hence, this marker can be used for identification of homozygous and heterozygous genotypes in different cultivars. Similarly the marker AM19 has amplified about 520bp resistant fragment in four apple cultivars (Shalimar I, Shalimar II, Dubhour and Gold Spur.). However, due to dominant nature of the marker AM19, it cannot distinguish homozygous and heterozygous cultivars containing *Vf* gene, and the same was confirmed by Khujuria et al. (2014). In addition to these two markers, Ch05e03 has also produced the resistant band of 150bp in crab apple only (Gel pic not shown). Thus out of 8 used

Table 1. Markers used in apple cultivars and their resistant fragments

	Shalimar Apple I	Shalimar Apple II	Dubhour	Crab Apple	Gold Spur
AL07	570bp	570bp	570bp		
AM19	520bp	520bp	520bp		520bp
CH05e03				150bp	
K08SCAR					
T06SCAR					
Z13SCAR					
OPL-19					
Vf2ARD					

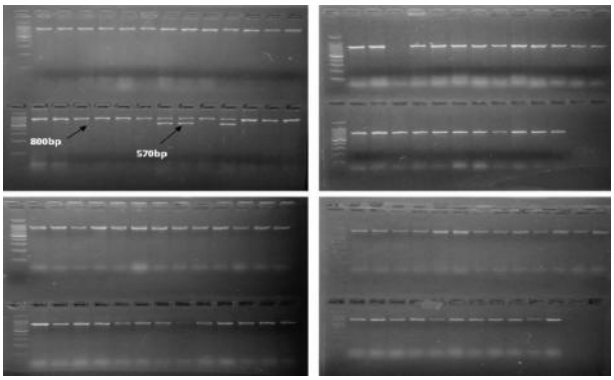

Fig. 1. AL07 primer produced 800bp band in all 100 apple cultivars. Further, a resistant band of about 570 bp was produced in three cultivars - Shalimar Apple I, Shalimar Apple II, and Dubhour

markers, the three markers (AL07, AM19, and CH05e03) that have amplified the resistant fragment in few apple cultivars proved to be very useful because of their ability to distinguish resistant and susceptible cultivars on the basis of presence and absence of a single band on the gel. Out of five cultivars containing the scab resistance alleles, three cultivars that contain the maximum number of scab resistance alleles against some races of *V. inaequalis* include Shalimar Apple I, Shalimar Apple II, and Dubhour. The cultivars like Shalimar Apple I and Shalimar Apple II are new to the apple market released by SKUAST Kashmir few years ago while as the cultivar Dubhour is among the old apple germplasm from Kashmir. These three important cultivars based on scab resistance can be used in future breeding programmes as parental stock to develop more resistant apple cultivars with high market acceptance.

The markers used in the present investigation can be utilised directly for screening of large apple germplasm growing in other regions worlwide for developing marker assisted resistant apple cultivars against scab. The results of the presents study can be used easily for designing the future breeding programmes for the development of high quality disease resistant commercial apple cultivars by introgression of scab resistant genes. Since the Kashmir Valley has a very rich apple germplasm of about 200 cultivars besides some unknown genotypes growing wild in the orchards and among them only 20-30 cultivars are commercially successful which are circulated in the apple market at national and international level. But it is noteworthy to mention that most of the commercially important apple cultivars are highly susceptible to scab which causes great economic losses to growers. Therefore, introgression of scab ressistant genes in commercially important apple cultivars of Kashmir will be a commendable work to increase the economy of people directly engaged in apple growing in various regions of the valley.

Authors' contribution

Conceptualization of research (JAD, RNR, AAW, SMZ); Designing of the experiments (SMZ, JAD); Contribution of experimental materials (AAW); Execution of field/lab experiments and data collection (JAD, RNR); Analysis of data and interpretation (JAD, RNR, AAW, SMZ); Preparation of manuscript (JAD, SMZ).

Declaration

The authors declare no conflict of interest.

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