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Marker Based Screening of F1 (Rosalie x Fuji) Mapping Population for Major Scab Resistance Gene Rvi6 in Apple (Malus × Domestica)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Apple (*Malus domestica* Borkh.) being the most significant and profitable crop cultivated in temperate areas is extremely susceptible to the apple scab disease. It becomes problematic when pathogens develop resistance and farmers spend a lot of money on fungicides. These widespread worries reasonably call for a different disease resistance management strategy in order to use fungicides less frequently. As a result, the current study focused on scab resistance gene

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introgression in cultivar 'Fuji' using phenotypic and marker-assisted screening in hybrids created by crossing scab succeptible cultivar 'Fuji' with a scab resistant cultivar 'Rosalie'. There was a 1:1 Mendelian segregation ratio between susceptible and resistant hybrids. From our study, the resistant hybrids obtained have the potential to be utilized as commercial varieties after evaluation for important pomological traits and also as resistant gene sources in downstream breeding programs.

Keywords: Apple; scab; resistance; introgression; screening; marker.

1. INTRODUCTION

According to FAO STAT [1], the apple *(Malus x domestica* Borkh.) is the second most widely grown fruit crop in the world, behind bananas. It is also one of the most recognizable and common fruit crops in temperate climates. According to reports, apples are home to over 70 infectious diseases, the majority of which are brought on by pathogenic fungi, followed by bacteria, viruses, mycoplasmas, and nematodes. Famous illnesses like root rot, leaf spot, leaf blight, scab, blossom blight, fruit decay, fruit spot, canker, and post-harvest decay are caused by Fungi. Similarly apple scab, which is also brought on by a fungus, *Venturia inaequalis* (Cke.) Wint (anamorph Spilocaea pomi Fr.) is the disease that causes the most yield loss. . Due to considerable financial losses of up to 70% in apple production, this disease has a significant negative economic impact on apple output and quality Mac Hardy [2]. Managing apple scab disease necessitates 10-15 fungicidal sprays annually, which present a number of ecological issues, consumer health concerns, and financial costs [3]. The general public is very interested in reducing fungicide use and supporting an alternative disease resistance management strategy as a result of these public concerns. The use of pesticide-resistant cultivars could lower costs for growers, improve the environment, and lessen fungicide residues on apples for consumers. These cultivars can be cultivated with significantly less pesticide application. Breeding for scab resistance has recently increased all over the world. An essential factor in apple breeding is to find cultivars resistant to apple scab because the climatic conditions in temperate areas of India, like Kashmir, are very suitable for the development and spread of this disease. Numerous scab-resistant cultivars have been made available,the majority of these are descended from *Malus floribunda*, the wild crab apple [4]. For more than 40 years, apple breeding programs have used the Vf (Rvi6) resistance gene extensively, and it has been introduced into a significant number of apple varieties. The gene has been remarkably resilient in apple orchards, conferring resistance to five of the seven known races of V. inaequalis [5]. Resistance breeding programs should prioritize efforts to create cultivars that are long-lastingly scab resistant and marketable. Apple genotypes must be tested for scab resistance with fungal races found in a certain region in order to successfully design such programs [6,7]. The resistance to apple scab can be controlled by a single gene (monogenic) or multiple genes (polygenic) may be involved [8]. Nevertheless, pyramiding of resistance genes in apple varieties through breeding approaches is of utmost importance in order to create durable resistance against V. inaequalis. In addition, resistance alleles against apple scab must also be related to adequate phenotypic resistance evidence. Use of molecular markers which are closely linked with scab resistance genes is an important tool for screening of germplasm on a large scale with much more accuracy [9]. Based on this background information and keeping in view the importance of management of scab disease using resistant cultivars in India, the study was devised to introgress Vf (Rvi6) scab resistant gene from scab resistant cultivar 'Rosalie' in the F1 hybrids derived from the cross between 'Rosalie' and commercial cultivar 'Fuji'.

2. MATERIALS AND METHODS

30 F1 individuals produced from the cross between Fuji and Rosalie were employed in the current experiment for phenotypic and molecular screening. The F1 plants were initially grown in trays and ultimately transferred to the experimental field, of Division of Fruit Science SKUAST-K, Shalimar. Rosalie, a commercial apple cultivar that is widely produced, is predicted to have the Vf (Rvi6) gene from the wild species M. floribunda, and hence was used as the donar parent whereas Fuji was used as the recipient parent owing to its succeptiblity to scab.

2.1 Screening for Resistance to Scab

The individuals in F1 mapping population were inoculated with four races [(0), (1), (2) and (1, 2)] of Venturia inaequalis. The fungal culture preparation was carried out following the method of Barbara et al., [10]. The suspension was adjusted to 5×10^5 conidia per milliliter and the spore suspension was applied till runoff with a manual atomizer. Inoculation was done under artificial conditions when the seedlings were 45 days old at 3-5 leaf stage. Conditions were maintained by covering the seedlings with black polythene and ensuring complete leaf wetting for 48 hours by time to time spraying of water. Disease scoring on plants was performed after 14 days after inoculation and was classified on 0-4 scale as given by Chevalier et al. [11]. Plants with score of 0 (Hypersensitive), 1to2(resistant), 3a (moderately resistant) were graded as resistant and plants recorded at a score of 3b (weakly succeptible) and 4 (succeptible) were categorized as susceptible.

2.2 DNA Isolation and PCR Amplification

“Genomic DNA was isolated from fresh and young leaves of F1 progenies and parents using a CTAB (cetyl trimethylammonium bromide) method as elaborated” by Doyle and Doyle [12]. “The DNA was then purified by RNase A treatment using standard methods” [13]. “DNA was quantified on 0.8% agarose gel stained with ethidium bromide and the quality of the DNA was verified spectrophotometric ally on NanoDrop. The F1s were tested for presence of Rvi6 gene using the linked SCAR markerAL-07 (F: 5`-TGGAAGAGAGATCCAGAAAGTG-3’; R: 5’-CATCCCTCCACAAATGCC-3). PCR assay was performed in a reaction volume of 20µL containing 25-50 ng genomic DNA, 1x PCR buffer (20 mMTris-Cl pH 8.4, 50mM KCl), 1.5 mM MgCl2, 0.2 mM dNTPs and 1.0 unit of Taq DNA polymerase” [14]. “PCR amplifications were performed in a gradient thermal cycler (Make TAKARA, Japan) with the following thermal regimes: Initial denaturation at 94°C for 5 min was followed by 35 cycles at 94°C for 1 min, 58–60°C for 1 min and 72°C for 2 min. The final extension was carried out at 72°C for 7 min. Amplified fragments were resolved in 3% agarose gel in a 1X TAE buffer. The gels were stained with ethidium bromide (0.5µg/ml) and visualized under UV light and documented in gel documentation system (Bio Rad, U.S.A)” [14].

3. RESULTS AND DISCUSSION

3.1 Phenotype Screening

The "Rosalie x Fuji" F1 population was tested against four monoconidial strains of V. inaequalis. Different phenotypes were seen, including immunological response in individual F1 plants, chlorotic signs in some plants, and obvious sporulation on leaves in others. The minor genes or modifiers of the Vf gene itself may be responsible for this significant heterogeneity in symptom expression [15]. The illness score divided the progenies into 9 vulnerable and 8 resistant groups. Marker evaluation 17 F1 individuals and their two parents were subjected to a Polymerase Chain Reaction (PCR) assay utilising the SCAR marker AL-07 connected to the Rvi6 gene, which confers resistance to the apple scab disease. The codominant marker AL07 has been located by Tartarini et al., 2000, 0.9 cM distant from the Rvi6 (VF) gene. The marker AL07 amplified two alleles, 820 bp and 570 bp, corresponding to susceptibility and resistance, respectively, from the resistant parent, Rosalie, and a single 820 bp allele from a susceptible parent, Fuji (Fig. 1). The allelic distribution pattern and the phenotypic response to diagnostic races correlated in a way that was consistent with research conducted by Suat et al [16]. It was particularly helpful to distinguish between progenies who were homozygous and heterozygous for the Rvi6 (Vf) gene thanks to the co-dominant character of marker AL07. The offspring would be anticipated to segregate given that one of the parents employed in this investigation appeared to be heterozygous. It would be expected that the progeny will segregate as 1/2 (VFvf): 1/2 (vfvf) for resistance and susceptibility, respectively, as one of the parents employed in this investigation appeared to be heterozygous Fig. 2. A single dominant gene ratio was validated by the Chi-square test (2 = 0.58), which also provided evidence for Rosalie's Rvi6 (Vf) resistance. Mac Hardy used apple segregation ratio to look into the existence of a significant gene resistance [2]. Using genetic markers closely linked to the Rvi6 resistance gene, the conventional selection procedures for apple scab resistance can be improved. A great tool for locating resistance genes and creating resistant cultivars is the use of marker-assisted selection [17,18].

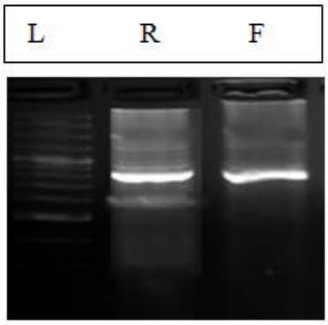

Fig. 1. Molecular marker AL07 for scab resistance gene, R=Rosalie (Scab resistant); F=Fuji (Scab susceptible Ladder: 100bp, Trait: Scab resistance, 570bp (R) and 820 bp (S)

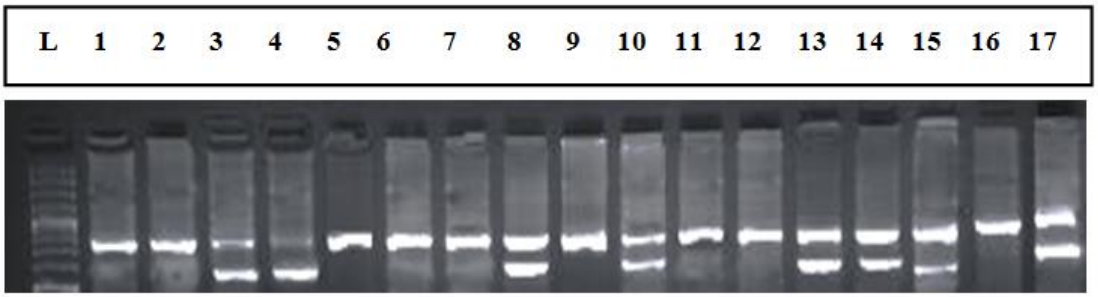

Fig. 2. Molecular marker AL07 for scab resistance gene, R=Rosalie (Scab resistant); F=Fuji (Scab susceptible); 1-17 = F1 resulting from "Fuji x Rosalie" Ladder: 100bp, Trait: Scab resistance, 570bp (R) and 820 bp (S)

4. CONCLUSIONS

From our study the resistant hybrids viz., 3, 4, 8, 10, 13, 14, 15 & 17 (Fig. 1) obtained can be further evaluated for other pomologically important traits like color, size, TSS etc. and have the potential for being utilized as commercial varieties. In addition these hybrids lines can be further used in future resistance breeding programmes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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