Int.J.Curr.Microbiol.App.Sci (2019) *8*(12): 2641-2646

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 8 Number 12 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.812.308

Marker Based Screening of F_1 (*Firdous x Gala*) Mapping Population for Major Scab Resistance Gene *Rvi6* in Apple (*Malus* × *Domestica*)

Saba Mir[1], Khalid Z. Masoodi[1], Aafreen Sakina[1], Khalid M. Bhat[2], Bilal A. Padder[3], Imtiyaz Murtaza[4], Nagina Nazir[5] and Asif B. Shikari[1*]

[1]Division of Plant Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shalimar, J&K-190025, India
[2]Division of Fruit Science, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shalimar, J&K-190025, India
[3]Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shalimar, J&K-190025, India
[4]Division of Basic Sciences and Humanities, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shalimar, J&K-190025, India
[5]Division of Agricultural Statistics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shalimar, J&K-190025, India

**Corresponding author*

Keywords

Apple, Scab, Markers, Resistance, Gene, *Rvi6*

Article Info

Accepted:
20 November 2019
Available Online:
10 December 2019

A B S T R A C T

Scab caused by *Venturia inaequalis* (Cke.) Wint. is the most important fungal disease of apple. The development and deployment of genetic resistance is reliable and a safe option to combat the dreaded disease with palpable advantage as compared to chemical control. A variety *Firdous* developed by SKUAST-K has been known for its resistance, however, genetic basis of resistance has not been proved or established till date. An initiative was taken to cross the scab resistant variety, *Firdous* to susceptible cultivar *Gala* that resulted into population of 110F_1 plants. Among these derived F_1s 46 were tested for presence of *Vf* gene using the linked SCAR marker. The marker analysis helped us to identify resistance specific alleles for gene *Rvi6* in 25 out of 46 F_1s. The F_1 population was also screened using a mono-conidial mixture of four races viz., (0), (1), (2) and (1, 2) under controlled conditions. A fair degree of correlation existed between allelic information and reaction phenotype that indicated the presence of *Rvi6* in *Firdous*. Further, the F_1 population was screened for the markers linked to fruit firmness. The combined information of both the markers may help us to identify individuals which are resistant to scab and carry favourable alleles for fruit firmness required for better storage and quality.

Introduction

Apple (*Malus x domestica* Borkh.) is the second largest fruit crop produced in the world after banana (FAO STAT, 2012) and is also one of the most emblematic and widespread fruit crops of temperate regions. It has been reported that apple is host to over 70 infectious diseases, many of them being caused by pathogenic fungi followed by bacteria, viruses, mycoplasmas and nematodes. The class Fungi is responsible for notorious diseases like root rot, leaf spot, leaf blight, scab, blossom blight, fruit decay, fruit spot, canker and post- harvest decay.

Among them apple scab caused by the hemi-biotrophic ascomycete *Venturia inaequalis* (Cke.) Wint. Is a cosmopolitan and one of the most serious diseases of apple which causes huge economic losses. Depending upon the weather conditions and fungal inoculum available for infection, as many as 20 to 30 fungicide applications may be necessary in a season to control the disease (Soriano *et al.,* 2009). Such an intensive and indiscriminate use of fungicidal applications raises ecological and human health concerns apart from escalating the cost of production and tedious management. Development of resistant cultivars is the most effective, economically sustainable and environmental friendly option to control the disease. The later requires precise identification and utilization of effective donors carrying genes for resistance.

In Jammu and Kashmir State of India, Apple is grown across 61,773 ha with an annual production of 1,966,417 metric tons. Number of varieties is grown across with varied quality and consumer acceptability. Unfortunately, most of the commercially important varieties like *Red Delicious* are susceptible to scab. *Firdous* is a scab resistant apple variety released by Sher-e-Kashmir University of Agricultural Science and Technology of Kashmir (SKUAST-K), India (Feza and Verma, 2011) in the year 1995. It was developed from a cross between Golden Delicious x Rome beauty x *Malus floribunda*. Therefore, by descent, *Firdous* is expected to carry *Vf* gene derived from wild crab apple *Malus floribunda*. The resistance gene *Rvi6*formerly known as *Vf* (Bus *et al.,* 2009) and initially identified in the wild relative *Malus floribunda*, has been the most widely introgressed gene in commercial varieties released by plant breeders. It confers resistance to five out of seven known races of *V.inaequalis*. Characterization of successful cultivars and varieties for scab resistance and its genetic basis is going to be of prime importance at least against prevalent fungal races present in the region (Didelot *et al.,* 2007, Le Van *et al.,* 2011). Molecular markers can reliably detect the presence of gene in target genotypes, though, the mere presence of marker allele in a germplasm is not an unambiguous proof to conclude that the line carries a gene of interest. Simple way to test the hypothesis would be to evaluate the mapping population for establishment of marker-trait relation and possibly the presence of gene. The objective of our study was to determine the inheritance of *Rvi6* (*Vf*) in F_1 mapping population derived from *Firdous* x *Gala* that aims at establishment of genetic basis of scab resistance in variety *Firdous*.

Materials and Methods

Plant materials DNA isolation

In the present experiment, 46F_1 individuals derived from across between apple cultivars *Firdous* and *Gala* were used for phenotypic and molecular screening. The apple cultivar *Firdous* is expected to carry *Vf* (*Rvi6*) gene donated from the wild species *M. floribunda*, whereas *Gala* susceptible is susceptible to scab. Both these parents are widely grown commercial varieties.

Screening for resistance to scab

The individuals in F_1 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.,* (2008).

The suspension was adjusted to 5×10^5 conidia per milliliter and the spore suspension was applied till runoff with a manual atomizer. Disease scoring on plants was performed after 14 days after inoculation and was classified on 0-4 scale as given by Chevalier *et al.,* (1991). Plants with score of 0, 1, 2, 3aor 3b were graded as resistant and plants recorded at a score of 4 were categorized as susceptible.

DNA isolation and PCR amplification

Genomic DNA was isolated from fresh and young leaves of F_1 progenies and parents using a CTAB (cetyl trimethylammonium bromide) method as elaborated by Doyle and Doyle (1990).The DNAwas then purified by RNaseA treatment using standard methods (Sambrook *et al.,* 1989). 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 F_1s were tested for presence of *Rvi6* gene using the linked SCAR markerAL-07 (F: 5`-TGGAAGAGAGATCCAGAAAGTG-3'; R: 5'-CATCCCTCCACAAATGCC-3).Further, the F_1 population was screened for ethylene biosynthesis potential using Md-ACS1 specific SSR marker. 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. 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)

Statistical analysis

Segregation pattern of the F_1 population was judged through chi-square analysis. T-test with unequal variance was performed to detect an association between marker and trait. Both the statistical tests were performed following the method of Gomez and Gomez (1976).

Results and Discussion

Phenotype screening

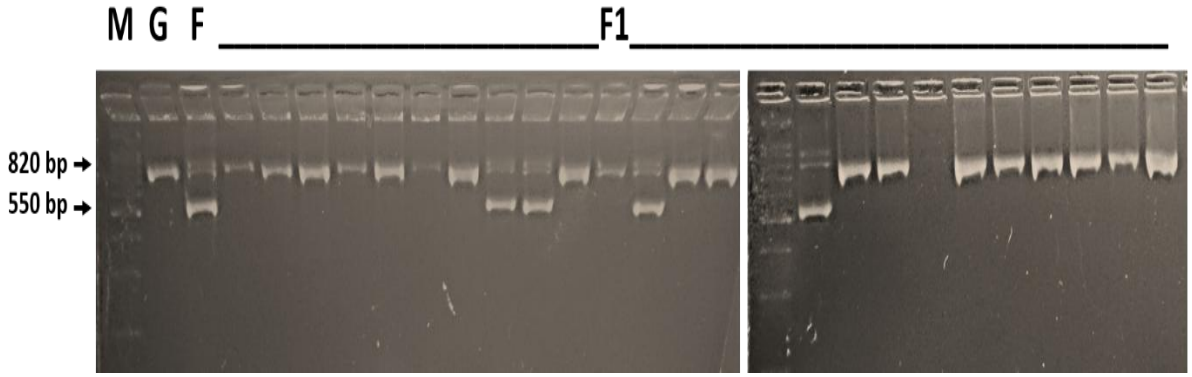
The F_1 population derived from *Firdous x Gala*was screened against four monoconidial strains of *V. inaequalis*. Different phenotypes were recorded with individual F_1 plants showing immune reaction followed by some plants with chlorotic symptoms and others having clear sporulation on leaves. This large variation in symptom expression may be attributed to minor genes or modifiers of the *Vf* gene itself (Gessler, 1989). Based on the disease score, 21 progenies were classified as resistant and 25 as susceptible.

Marker analysis

Polymerase Chain Reaction (PCR) assay was carried out on $46F_1$ individuals along with two parents using a SCAR marker AL-07 linked to*Rvi6* gene conferring resistance against apple scab disease. AL07 is a codominant marker and has been mapped by Tartarini *et al.,* (2000) at a genetic distance of 0.9 cM

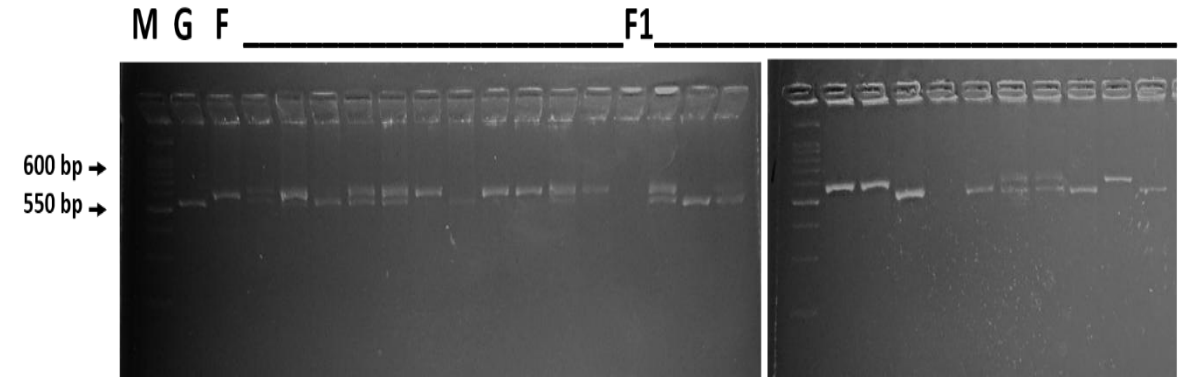
away from *Rvi6 (VF)* gene. The marker AL07 amplified a single 820 bp allele from a susceptible parent, *Gala* and two alleles: 820 bp and 570 bp corresponding to susceptibility and resistance, respectively, from resistant parent, *Firdous* (Fig. 1a). The correlation of allelic distribution pattern vis-à-vis the phenotypic response to diagnostic races was in concurrence with the studies carried out by Suat*et al.*, (2013).The codominant nature of marker AL07 was very useful to discriminate homozygous from heterozygous progenies for the *Rvi6* (Vf) gene. Since one of the parents used in this study appeared to be heterozygous, the progeny would be expected to segregate 1/2 (*VFvf*): 1/2 (*vfvf*) for resistance and susceptibility, respectively. The Chi-square test (χ^2=0.58) confirmed a single dominant gene ratio and established an evidence in support of the presence of *Rvi6* (Vf) resistance in Firdous. Segregation ratio in apple has been applied to investigate the presence of a major gene resistance by MacHardy (1996). The classical selection methods for apple scab resistance can be refined using genetic markers tightly linked to the *Rvi6* resistance gene. The use of marker-assisted selection is an excellent instrument for identification of resistance genes and development of resistant cultivars.

Fig.1a Marker analysis of F_1 population for scab resistance gene Rvi6



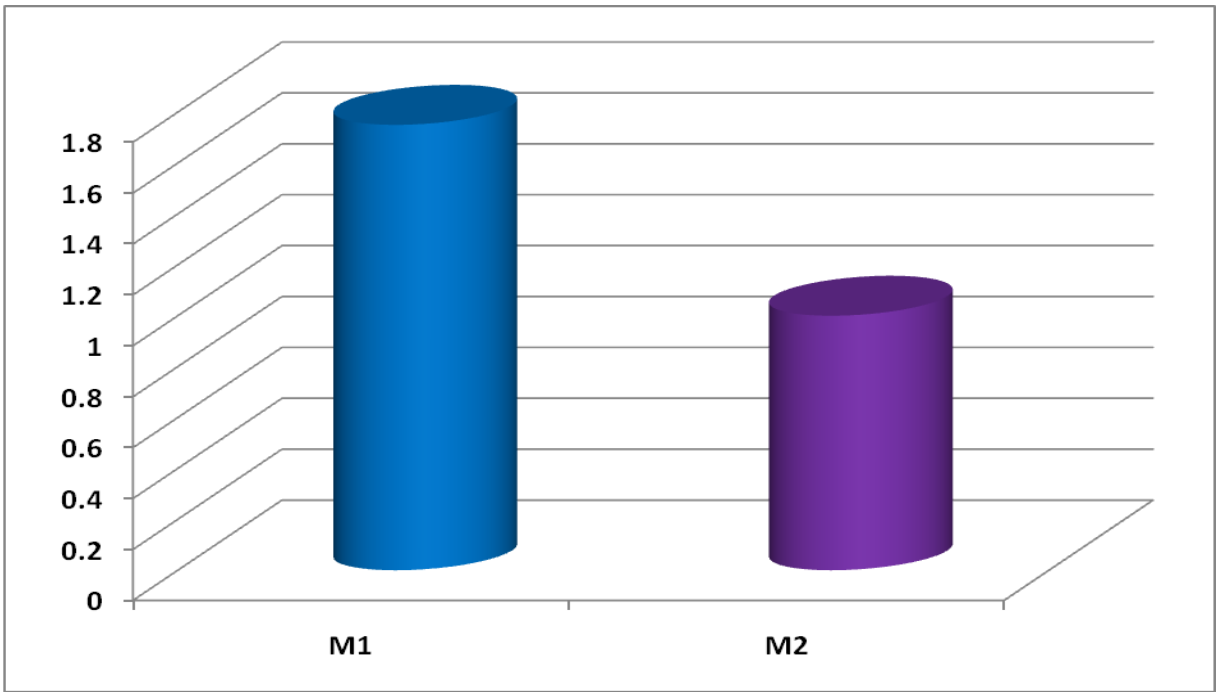
Primer name: AL07; M: 100 bp Ladder (Fermentas, U.S.A), G: Gala, F: Firdous, F_1: F_1 progenies derived from Firdous x Gala

Fig.1b Marker analysis of F_1 population for Md-ACS1 gene



Primer name: Md-ACS1; M: 100 bp Ladder (Fermentas, U.S.A), G: Gala, F: Firdous, F_1: F_1 progenies derived from Firdous x Gala

Fig.2 Mean disease score of individuals in a mapping population representing marker classes M1 and M2



M_1: Mean of progenies with resistant allele, M_2: Mean of progenies with susceptible allele.

The mapping population was divided into two marker classes, M_1 and M_2. A significant difference was observed between the means of two groups (Figure 2). We tested the null hypothesis that if the mean disease score between two groups is same or different using a t-test. The marker AL07 was found significantly associated with *Vf* resistance at P = 0.08316. Shelf life determines the economic life time of mature apples. Good shelf life is associated with a slow decrease of fruit firmness at room temperature. Apple is a climacteric fruit, in which loss of firmness seems to be physiologically related to ethylene. Ethylene's biosynthetic pathway is controlled by two large gene families coding for 1-aminocyclopropane-1-carboxylate synthase (ACS) and 1- aminocyclopropane-1-carboxylate oxidase (ACO). The marker ACS1 was used to amplify two alleles of 550bp (ACS1-1) and 600bp (ACS1-2) (Fig. 1b). Of the 46 progenies evaluated, 14 were homozygous for ACS1-1/1, 11were homozygous for ACS1-2/2 and 14 were heterozygous for ACS1-1/2.The screening of F_1 population for the markers linked scab resistance and ethylene biosynthesis, helped us to identify resistant individual having favourable alleles for low ethylene production and better fruit storage quality alleles for fruit firmness. The identified plants can be grafted on appropriate rootstocks and further validated for phenotypic response to disease and fruit quality and subsequently may be used for development of scab resistant varieties with wide commercial acceptability. Further, the validation for *Rvi6* locus needs to be performed in Firdous using genomics based approaches.

References

Barbara, D. J., Roberts, A. L. and Xu, X. M. 2008. Virulence characteristics of apple scab (*Venturia inaequalis*) isolates from monoculture and mixed orchards. *Plant*

Pathology 57: 552-561.

Bus, V. G. M., Rikkerink, E., Aldwinckle, H. S., Caffier, V., Durel, C. E. and Gardiner, S., A. 2009. Proposal for the nomenclature of *Venturia inaequalis* races. *ActaHortic* 814(2): 739-746.

Chevalier, M., Lespinasse, Y. and Renaudin, S. 1991. A microscopic study of the different classes of symptoms coded by the Vf gene in apple for resistance to scab (*Venturia inaequalis*). *Plant Pathology* 40: 249–256.

Didelot, F., Brun, L. and Parisi, L. 2007. Effects of cultivar mixtures on scab control in apple orchards. *Plant Pathol* 56: 1014-1022.

Doyle, J. J. and Doyle, J. L. 1990. Isolation of plant DNA from fresh tissue. *Focus*; 12: 13-15.

FAOSTAT: http://faostat3.fao.org/browse/Q/QC/E

Feza, M. A. And Verma, M. K. 2011. Temperate Fruit Scenario in Jammu and Kashmir: Status and Strategies for Enhancing Productivity. *Indian Horticulture Journal*. 1: 001-010.

Gardiner, S. E., Bassett, H. C. M., Noiton, D. A. M., Bus, V. G., Hofstee, M. E., White, A. G., Ball, R. D., Forster, R. L. S., and Rikkerink, E. H. A. 1996. A detailed linkage map around an apple scab resistance gene demonstrates that two disease classes both carry the *Vf* gene. Theor. Appl. Genet. 93: 485– 493.

Gessler, C. 1989. Genetics of the interaction *Venturia inaequalis*- Malus: the coflict between theory and reality. In: Gessler C, Butt DJ, Koller B (eds) Integrated control of pome fruit diseases II. OILB-WPRS Bulletin XII/6: 168±190

Gomez, A.K. and Gomez, A.A. 1976. Statistical procedures for agricultural research. International Rice Research Institute Book, John Willy & Sons.

Le Van., A. L., Durel, C. E., LeCam, B. and Caffier, V. 2011. The threat of wild habitat to scab resistant apple cultivars. *Plant Pathol*. 60: 621-630.

MacHardy, W. E. Apple scab: Biology, epidemiology, and management. APS Press, St. Paul MN, 1996.

Sambrook, J., Fritsch, E. F. andManiatis, T. Molecular Cloning - A Laboratory Manual, 2nd Edition. Cold Spring Habour Laboratory Press, New York, 1989.

Soriano, J., Joshi, S., van Kaauwen, M., Noordijk, Y., Groenwold, R., Henken, B., van de Weg, W. and Schouten, H. 2009. Identification and mapping of the novel apple scab resistance gene Vd3. *Tree Genetics & Genomes*, 5: 475-482.

Suat, K., Emel, K. and Yusuf, Ö. 2013. Screening breeding apple progenies with *Vf* apple scab (*Venturia inaequalis* (Cke.) Wint.) Disease resistance gene specific molecular markers, integrated protection of fruit crops. *IOBC-WPRS Bulletin* 91: 361-365.

Tartarini, S., Sansavini, S., Vinatzer, B. A., Gennari, F., and Domizi, C. 2000. Efficiency of marker assisted selection (MAS) for the *Vf* scab resistance gene. ISHS. Acta Hort. 538: 549–552.

How to cite this article:

Saba Mir, Khalid Z. Masoodi, Aafreen Sakina, Khalid M. Bhat, Bilal A. Padder, Imtiyaz Murtaza, Nagina Nazir and Asif B. Shikari. 2019. Marker Based Screening of F_1 (*Firdous x Gala*) Mapping Population for Major Scab Resistance Gene *Rvi6* in Apple (*Malus* × *Domestica*). *Int.J.Curr.Microbiol.App.Sci.* 8(12): 2641-2646.
doi: https://doi.org/10.20546/ijcmas.2019.812.308