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Screening breeding apple progenies with vf apple scab (*Venturia inaequalis* (Cke.) Wint.) disease resistance gene specific molecular markers

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Abstract: Apple scab (*Venturia inaequalis* (Cke.) Wint.) is the most important fungal disease of apples. Since commercially grown apple cultivars are sensitive to this disease, fungicides are extensively used for controlling apple scab disease. Using resistant varieties is the most efficient way of controlling the disease and reduction of pesticide applications. In this study, Williams' Pride (Co-op 23) and Priscilla (Co-op 4) varieties known to have resistant Vf gene were crossed with Golden Delicious to get resistant new varieties in Isparta Fruit Research Station. Apple progenies were tested for presence of Vf gene with three different SCAR markers linked to the Vf gene. For this purpose, samples were tested for the presence of Vf gene by SCAR PCR using primers specific to ALO-7, ACS-7 and ACS-9 SCAR markers. Also crossed progenies were evaluated in natural inoculation field conditions. According to results natural inoculation and markers were compared and sameness was determined. In Golden Delicious x Williams' Pride combination resistivity was transferred 46%, and Golden Delicious x Priscilla combination resistivity was transferred 44%.

Key words: Apple, *Venturia inaequalis*, resistance, SCAR, Vf

Introduction

Apple has a wide distribution in the world and can be produced in different ecological conditions. Today, 70 million tons of apples were grown worldwide. Turkey is the third leading producer, accounting for 2.600.000 tons of world production (Anonymous, 2012). Pome fruit production in Turkey accounts for 24% per year of total fruit production and apple production is accounted for 84% in pome fruits (Anonymous, 2003).

Apple scab, caused by the fungal pathogen *Venturia inaequalis* (Cke), is the most important apple disease, and is present in all apple growing regions. Pathogen damaged to leaves and fruits. Leaves attacked by scab have a lower assimilation rate. They are falling off earlier; fruits do not develop to normal size and do not have the full quality (Jha *et al*., 2009). It causes 70% reduction in yield as well as leads to loss of 30-60% reducing the market value (Agrios, 1997; Turkoglu, 1978).

Controlling apple scab requires the application of numerous fungicide treatments during the growing season. Most of the commercial apple cultivars are susceptible to the diseases, and growers have to spray 20-30 times with fungicides in a season (Boyraz *et al*., 2005; Soriano *et al*., 2009; Patocchi *et al*., 2004). The use of resistant cultivars could reduce the cost to the growers and may also contribute to a cleaner environment. The development of integrated pest management has contributed to a considerable reduction of pesticide use, but further reduction seems only possible with the introduction of resistant cultivar. Using resistant varieties is the most efficient way of controlling the disease and reduction of

pesticide applications. Therefore, several apple scab resistance gene were identified from wild apples and these genes were attempted to introduce into cultivated apple by classical breeding methods. Studies which were done on apple scab showed 17 resistance genes for the disease. Vf gene identified from *Malus floribunda* 821 is the most commonly used apple scab resistance gene (Hough, 1944; Lespinasse *et al*., 1989).

A more elegant approach than the field phenotyping, is to identify or mark the genome carrying the resistance information and to select progeny by the presence of such DNA markers. Marker- assisted breeding is thought to have a high feasibility and cost-benefit ratio (Gessler & Blaise, 1994; Maric *et al.*, 2010).

In Turkey, the first studies were initiated in 2008 related to breeding resistant varieties in Fruit Research Station. In this project has been aimed to determination of apple genotypes to scab resistance with molecular markers.

Material and method

This study was initiated in 2008 in the Fruit Research Station obtained for high quality apple varieties resistant to scab. Golden Delicious (maternal), Priscilla and Williams' Pride (paternal) varieties, which are carrying the Vf gene, are used as parents in the crosses. Seedlings were planted at the spacing 2 x 0.2 m. in the orchard for scab and first fruit evaluations.

All seedlings were assessed for their susceptibility to scab both natural inoculation in the orchards and molecular markers. Scab evaluations were performed on the two hundred apple progenies in the field. Natural inoculation assessments were initiated at the beginning of August and leaves were graded as 0 or 1 (healthy or infected).

Marker assisted selection applied for seedling progenies in their young leaves. DNA was isolated from these leaves using QIAGEN® DNA easy genomic DNA isolated Kit in spring. After determination of the DNA concentration spectrofotometrically, samples were tested for the presence of Vf gene.

A total of 3 SCAR markers linked to the Vf gene were used in this study (Table 1). These SCARs included ACS-7 and ACS-9. Two of the SCARs were derived from amplified fragment length polymorphism (AFLP) markers (Xu *et al.*, 2001), while SCAR OPAL07 was derived from random amplified polymorphic DNA (RAPD) markers (Tartarini *et al*., 1999).

All PCR reactions were performed in 96-well microtitre plates using a Thermal Cycler (BIORAD C1000). The reaction volume of 25 µl contained, 10x PCR buffer (50 Mm KCl, 10 mM Tris HCl 25 °C pH 9.0, 1% Triton X-100), 2 µl DNA (approx. 100 ng), 0.5 mM dNTP, 2.5 U Takara Ex Taq and 20 pmol forward and reverse primers of each SCAR marker. The following PCR reaction was used for all SCARs, one cycle of 2 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 60 °C, and 2 min at 72 °C; and 1 cycle of 5 min at 72 °C. PCR products were size-fractionated by electrophoresis through a 1.5% agarose gel in 0.5 × TBE buffer.

All 200 individuals from the 2 crosses were tested for their genotypes using each of the 3 SCARs. For genotyping of resistant individuals, the PCR banding pattern for each SCAR marker must be consistent with that of the positive control, Prima (Co-op 2). A scab-susceptible cultivar, Golden Delicious, was used as a negative control. Therefore, all SCAR markers should be present in resistant individuals and Prima, but absent in cv. Golden Delicious. Eliminating PCR artifacts and experimental errors and to confirm reliability of the banding profiles, we screened all samples two times with each SCAR marker.

Table 1. Length and nucleotide sequence of SCAR markers.

SCAR markers	Sequence (5′→3′)	Length (base pairs)
AL07 For	TGG AAG AGA GAT CCA GAA AGT G	580bp
AL07 Rev	CAT CCC TCC ACA AAT GCC	
ACS-7 For	GTG CCA ATG TAA CTA GAG TGA CGT G	256 bp
ACS-7 Rev	ATG TAG GTG GTG ATG TAT CTG GAT T	
ACS-9 For	ACA TGG AAG ATG AAG GAG AAG GAG	469 bp
ACS-9 Rev	GAT AAA TTG AGT GAC TGC AAA GCG	

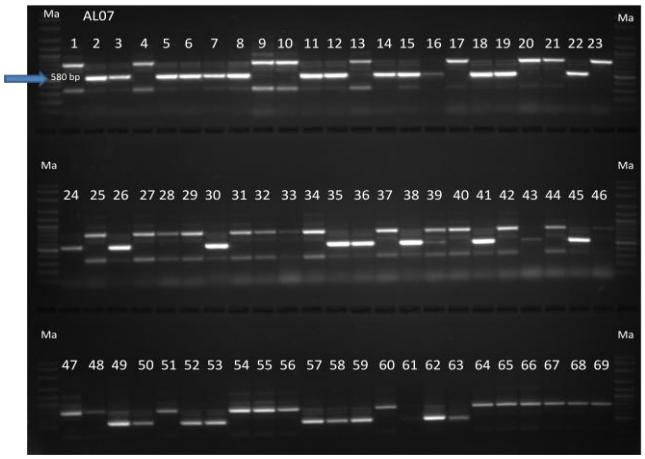

Figure 1**.** Segregation of co-dominant ALO7 marker of *Vf* gene in cross Golden Delicious X Williams' Pride; Ma) Size Marker GeneRuler 100 bp DNA ladder (Fermentas, St. Leon-Rot, Germany) 1) Golden Delicous, 2) Williams' Pride, 3) Priam, and 4 to 69) progeny plants

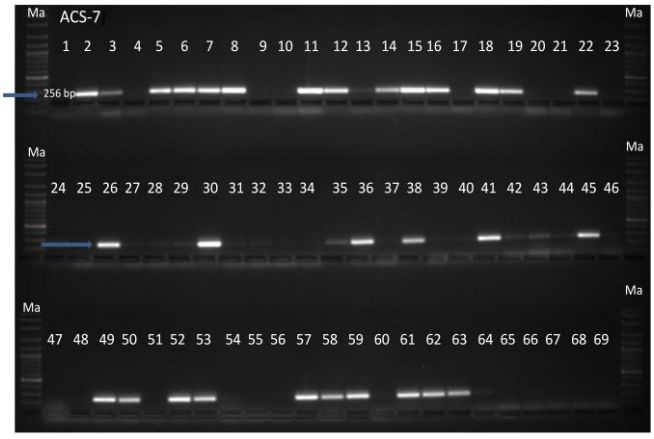

Figure 2. Segregation of dominant ACS-7 marker of *Vf* gene in cross Golden Delicious X Williams' Pride; Ma) Size Marker GeneRuler 100 bp DNA ladder (Fermentas, St. Leon-Rot, Germany), 1) Golden Delicous, 2) Williams' Pride, 3) Priam, and 4 to 69) Progenies

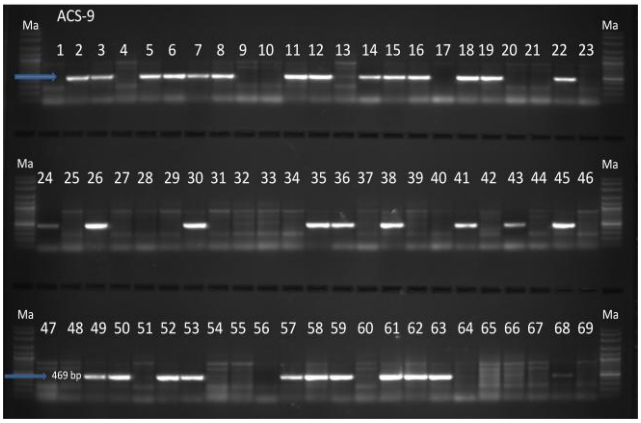

Figure 3. Segregation of dominant ACS-9 marker of *Vf* gene in cross Golden Delicious X Williams' Pride; Ma) Size Marker GeneRuler 100 bp DNA ladder (Fermentas, St. Leon-Rot, Germany) 1) Golden Delicous, 2) Williams' Pride, 3) Priam, and 4 to 69) Progenies

Results and discussion

The program has so far resulted in over 2000 seedling, of which about 200 are still under observation and 90 have been selected to be resistant. They have been found to segregate nearly 1 : 1 for the Vf gene from *Malus floribunda* 821. There were differences between progeny groups not only depending on resistant but on non–resistant parents, among progenies originated from Williams' Pride the combination of Golden Delicious x Williams' Pride was the best one. This combination resistivity was transferred 46% and Golden Delicious x Priscilla combination resistivity was transferred 44%.

The results of natural inoculation were compared to the genotyping results, and two progenies of Golden Delicious x Williams' Pride combination were found to be resistant using SCARs markers, while this progenies were found to be susceptible under field condition. Meanwhile from the Golden Delicious x Priscilla combination three progenies determined as genetic resistant were susceptible in field condition.

The results revealed that selection of plants with markers made the selection genotypes earlier practical, marker based selection was accepted as earlier, practical useful method.

The risk of resistance being overcome by a new race of scab can be very high, particularly when resistant varieties are cultivated as monocultures. Therefore it is very urgent to discuss these recent results to set up experiments to examine the genetics, to draw conclusions for the durability of resistances in the orchard and to propose new breeding strategies. The best planting strategy is to mix varieties with resistance from different genetic sources (Fischer *et al*., 1994).

This study was the first step for breeding studying which will continue for years. It is aimed to develop new varieties for organic production and conventional production and also for less pesticide consumption. These varieties will be preserved as resistant source for the next studies.

References

Anonymous 2003: Tarimsal Yapi ve Üretim 2003, DIE Yayinlari, Ankara.

Anonymous 2012: FAOSTAT data, http://www.fao.org/corp/statistics/en/

Agrios, G. N. 1997: Plant pathology (IV Edition), 12-044564-6, California, USA, 619 pp.

Boyraz, N., Kaymak, S. & Yigit, F. 2005: General Evaluation of applications Chemical Control of Apple Growers in Eðirdir County. Selçuk University. The Journal of Agricultural Faculty 19(36): 37-51.

Fischer, C., Bondarenko, A. & Artamonova, E. 1994: Results on the stability of scab resistance in apple breeding. In: Progress in Temperate Fruit Breeding (eds. H. Schmidt and M. Kellerhals): 81-85. Kluwer Academic Publishers, The Netherlands.

Gessler, C. & Blaise, P. 1994: Differential resistance in apple against scab and its use in breeding and in orchard planting strategies to control the disease. In: Progress in Temperate Fruit Breeding (eds. H. Schmidt and M. Kellerhals): 99-104. Kluwer Academic Publishers, The Netherlands.

Hough, L. F. 1944: A suvey of the scab resistance of the foliage on seedling in selected apple progenies. Proc. Amer. Soc. Hortic. Sci. 44: 260-272.

Jha, G., Thakur, K. & Thakur, P. 2009: The Venturia apple pahosystem: pathogenicity mechanisms and plant defense responses. Journal of Biomedicine and Biotechnology: 1-10.

Lespinasse, Y. 1989: Breeding pome fruits with stable resistance to disease: 3 genes, resistance mechanisms, present work and prospects. IOBC-WPRS Bull. 12: 100-115.

Maric, S., Lukic, M., Cerovic, R., Mitrovic, M. & Boskovic, R. 2010: Application of molecular markers in apple breeding. Genetika 42: 259-375.

Patocchi, A., Bigler, B., Koller, B. & Kellerhals, M. 2004: Vr2: a new apple scab resistance gene, Theor. Apple. Genet. 109: 1087-1092.

Soriano, J. M., Joshi, S., Groenwold, R., Noordijk, Y., Henken, B., van Kaauwen, M. & Schouten, H. J. 2009: Identification and mapping of a new apple scab resistance gene. I. International Symposium on Biotechnology of Fruit Species, BIOTECHFRUIT 2008, Acta Horticulturae 839.

Tartarini, S., Gianfranceschi, L., Sansavini, S. & Gessler, C. 1999: Development of reliable PCR markers for the selection of the Vf gene conferring scab resistance in apple. Plant Breed 118: 183-186.

Turkoglu, K. 1978: Karaleke (*Venturia inaequalis* (Cke.) Wint.) Epidemisinin önceden saptanmasý ve hastaligin eradikasyonu üzerine arastirmalar. Gida, Tarim ve Hayvancilik Bakanligi Izmir Bölge Zirai Mücadele Arastirma Enstitüsü Müdürlügü Arastirma Servisi, No: 030-465.

Vinatzer, B. A., Patocchi, A., Tartarini, S., Gianfranceschi, L., Sansavini, S. & Gessler, C. 2004: Isolation of two microsatellite markers from BAC clones of the Vf scab resistance region and molecular characterization of scab-resistant accessions in *Malus* germplasm. Plant Breed 123: 321-326.

Xu, M. L., Song, J. Q., Cheng, Z. K., Jiang, J. M. & Korban, S. S. 2001: A bacterial artificial chromosome (BAC) library of *Malus floribunda* 821 and counting construction for positional cloning of the apple scab resistance gene Vf. Genome 44: 1104-1113.