Scientia Horticulturae 232 (2018) 107–111

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Short communication

Searching for resistance genes to *Venturia inaequalis* in wild and domestic apples in Iran

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ARTICLE INFO

Keywords:
Apple scab
Resistance genes
Rvi6
Apple genotypes
SCAR and SSR molecular markers

ABSTRACT

Scab caused by the fungus *Venturia inaequalis* (Cook) G. Winter is the most devastating disease of the apple worldwide. To control the disease most efforts have been directed toward the breeding programmes by introgression of scab resistance genes from wild apples into commercial cultivars. Although, various apple genotypes exist in Iran, no comprehensive data regarding the possible presence of scab resistance genes is available. In this research the presence of five *R* genes (*Rvi2*, *Rvi4*, *Rvi6*, *Rvi8* and *Rvi11*) was assessed in 28 wild and domestic apple genotypes using 13 SCAR and SSR markers. Sequencing and blastN results confirmed the identify of *Rvi2*, *Rvi6*, *Rvi8* and *Rvi11* genes. Based on the results, wild apples had more resistance genes and among foreign and autochthonous apples, 'Golden Delicious', 'Malayer-1' and 'Shahroud-6' carrying at least three *R* genes categorized as almost tolerant cultivars. However, 'Gala', 'Boshghabi-e Damavand', 'Manouchehri' and 'Golab' had just one *R* gene and classified as susceptible cultivars. The most frequent scab resistance breeding programmes rely on *Rvi6* gene (*HcrVf2* gene), which was also detected in this study in crabapple *Malus floribunda* providing a rich resource for developing resistant apple cultivars in Iran.

1. Introduction

Apple (*Malus* × *domestica* Borkh.) is universally the third most important fruit crop, following the citrus and banana. In 2014, approx. 84 million tons of apple were produced in the world and Iran with production of over 1.5 million tons ranked ninth place (FAO, 2014). Nearly 55% of commercial production of apple in Iran takes place in northeast, northwest and southwest parts (Gharaghani et al., 2016).

Different autochthonous and foreign apple cultivars exist in Iran, in which the most cultivated are 'Red Delicious' and 'Golden Delicious'. Early ripening and autochthonous cultivar 'Golab' usually covers 10% of total apple production (Mirmohammadi Meibodi, 2003). The proximity to apple origin in Central Asia is the main cause of high apple diversity in Iran. The Central Asian wild apple species, *Malus sieversii*, is considered as the primary progenitor of domesticated apple. It is suggested that Iranian apples may be an intermediate between domesticated varieties and wild apple species. Iran (Persia) could play an important role in apple domestication and its transfer from Central Asia to West via the Silk Trade Route and regards as a main center of variation for domestic apple cultivars (Gharghani et al., 2010; Gharghani et al., 2009).

The wild species *Malus orientalis* Uglitzh., as one of the probable minor apple ancestors, is the only wild *Malus* in Iran that extends widely across the north forests, southern hillside of Alborz mountains, as well as west and central parts of Iran. *M. orientalis* is usually employed as a rootstock for domestic apple and some people also cultivate it at home gardens for self-consuming (Gharaghani et al., 2016; Gharghani et al., 2009).

Scab caused by *Venturia inaequalis* (Cooke) G. Winter (anamorph: *Spilocaea pomi* Fries) is globally the most serious disease of the apple leading to extensive economic losses up to 70% in the regions with cool and humid spring and early summer (Bowen et al., 2011; Bus et al., 2011). Most apple orchards of Iran have been also heavily suffered from this disease (Ashkan and Assadi, 1980).

Deployment and modification of resistant cultivars could be an effective solution to manage apple scab and reduce the side effects of fungicides (Boudichevskaia et al., 2006; Bowen et al., 2011; Mirmohammadi Meibodi, 2003). In this respect, application of marker-assisted selection is an efficient tool to identify resistant resources. Several loci containing resistance genes to apple scab have been already identified from different genotypes. So far, about 20 major scab resistance genes (*R* gene) have been identified and mapped across linkage groups of the apple genome (Bus et al., 2011).

The main resistance source to apple scab has been *Rvi6* gene

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https://doi.org/10.1016/j.scienta.2018.01.006
Received 29 September 2017; Received in revised form 3 January 2018; Accepted 4 January 2018

indicating resistance to five *V. inaequalis* races. A cluster of four receptor-like genes homologues to *Cladosporium fulvum* resistance genes of tomato has been identified at the *Rvi6* locus which were called *HcrVf1-HcrVf4* genes (Vinatzer et al., 2001). Gene *HcrVf2* is the main candidate for apple scab resistance and has been employed in several gene transferring programmes (Barbieri et al., 2003; Belfanti et al., 2004; Joshi et al., 2011; Szankowski et al., 2009).

Several monogenic and polygenic tolerant cultivars including 'Prima', 'Priscilla', 'Redfree', 'GoldRush', 'Antonovka' and 'Ariwa' have been already available showing different resistance levels (Gessler et al., 2006; Gessler and Pertot, 2012). Nevertheless, nearly all autochthonous and economically important apple cultivars in Iran are sensitive to scab (Ashkan and Assadi, 1980). Availability of resistance genes could be aimed at development of apple breeding programmes and introduction of scab resistant cultivars in Iran. This study was carried out to determine the presence of the resistance genes *Rvi2*, *Rvi4*, *Rvi6*, *Rvi8* and *Rvi11* in 28 genotypes of Iranian wild and domestic apples.

2. Materials and methods

2.1. Plant materials

Twenty-eight apple genotypes from three wild, crabapple and domestic species were collected in 2015 (Fig. 1, Table 1). The species determination was performed at herbarium of Natural Resources faculty, Isfahan University of Technology, Iran.

2.2. DNA extraction and PCR amplification

Genomic DNA was extracted from young leaves using 3% CTAB method described by Murray and Thompson (Murray and Thompson, 1980). For detection of five apple scab *R* genes (*Rvi2*, *Rvi4*, *Rvi6*, *Rvi8* and *Rvi11*), 13 SCAR and SSR markers were employed (Table 2). PCR amplifications were performed in 30 µl reaction containing 30–50 ng of genomic DNA, 1x Ampliqon *Taq* DNA Pol. 2x Master mix Red, and 0.4 µM of each primer. All PCR reactions were performed using a Techne TC-512 thermocycler under the following conditions: initial denaturation at 94° C for 5 min, 35 cycles of denaturation at 94° C for 40 s, annealing at varied temperature based on primers (Table 2) for 1 min and extension at 72° C for 1 min 30 s with a final extension at 72° C for 10 min. PCR products were resolved on horizontal 1.2% agarose gel in 1x TBE buffer and visualized by ethidium bromide staining. SSR amplified bands were resolved on 6% denaturing acrylamide gel in 1x TBE buffer using Sequi-Gen® GT nucleic acid electrophoresis cell and stained with 0.2% silver nitrate solution and then visualized in 3% sodium carbonate solution.

Amplified fragments were cloned into competent *Escherichia coli* MC1061 and the recombinant colonies were sequenced at MacroGen, Korea. The sequences from this article were submitted to the NCBI GenBank database to which the accession numbers were assigned and presented in Table 3.

Table 1
Wild and domestic apple genotypes collected from different locations in Iran.

No.	ID	Species/Cultivar	Location
1–10	SAR1-SAR10	*Malus orientalis*	Sardasht, WA[a]
11	AL	*M. orientalis*	Zarringol forest, Golestan
12	H1	*M. orientalis*	Fereidoun Shahr, Isfahan
13	SH	*Malus floribunda*	Shiraz, Fars
14	R-De	*Malus domestica* ('Red Delicious')	UANRRC,[b] WA
15	G-De	*M. domestica* ('Golden Delicious')	UANRRC, WA
16	RO	*M. domestica* ('Romina')	UANRRC, WA
17	Ga	*M. domestica* ('Gala')	KRS,[c] Alborz
18	B-D	*M. domestica* ('Boshghabi-e Damavand')	UANRRC, WA
19	H-GH-81	*M. domestica* ('Haji- Ghermez-81′)	UANRRC, WA
20	M-1	*M. domestica* ('Malayer-1′)	UANRRC, WA
21	Sh-6	*M. domestica* ('Shahroud-6′)	UANRRC, WA
22	S-T	*M. domestica* ('Sangani-Taleghani')	UANRRC, WA
23	T-R	*M. domestica* ('Tabestaneh-Rostami')	UANRRC, WA
24	MO	*M. domestica* ('Moruti')	UANRRC, WA
25	SH-H	*M. domestica* ('Shemirani-Haghi')	UANRRC, WA
26	H-SH	*M. domestica* ('Haji Shahryar')	UANRRC, WA
27	MN	*M. domestica* ('Manouchehri')	UANRRC, WA
28	Gol	*M. domestica* ('Golab')	Semirom, Isfahan

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3. Results

This study reports a genotyping survey of wild, autochthonous and commercially important apple genotypes in Iran for five scab resistance genes (*Rvi2*, *Rvi4*, *Rvi6*, *Rvi8* and *Rvi11*) using SCAR and SSR markers. The application of three SCARs (OPB18-SCAR, OPL19-SCAR and K08-SCAR), specific marker HcrVf2 and three SSRs (CH02b10, CH-Vf1 and CH05e03) was enabled the identification of four *R* genes (*Rvi2*, *Rvi6*, *Rvi8* and *Rvi11*) in Iranian apple germplasm, which have not been published to data. Expected amplification with two SCARs and one SSR for *Rvi4* and three SCARs for *Rvi6* was not obtained in assessed apple materials.

A fragment of 620 bp was amplified using OPB18-SCAR in all genotypes. BlastN search showed that the sequenced fragments shared the closest homology of 99% with that representative sequence of *Malus sieversii* OPB18 SCAR marker sequence band 1 (AY642927.1). The allele of 125 bp linked to *Rvi2* was also amplified using CH02b10-SSR marker in all genotypes.

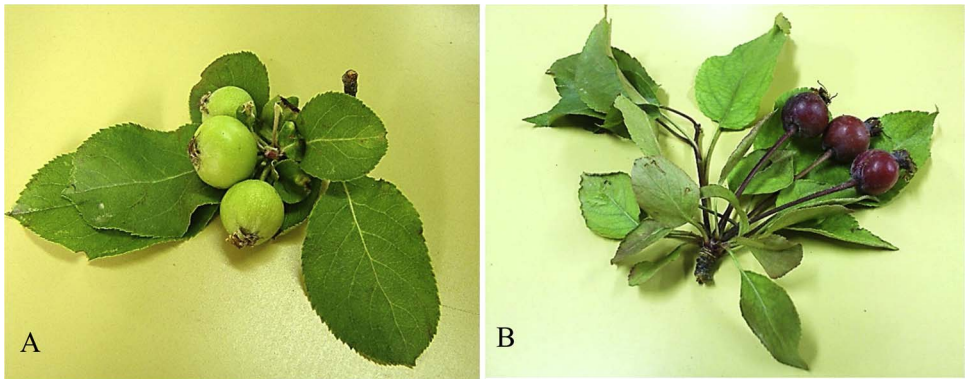

Fig. 1. Wild apple species. (A) *Malus orientalis* collected from Zarringol forest, Golestan. (B) *Malus floribunda* collected from Shiraz, Fars.