

# MOLECULAR SCREENING OF APPLE (*Malus domestica*) CULTIVARS AND BREEDING CLONES FOR THEIR RESISTANCE TO FIRE BLIGHT

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## A B S T R A C T

Three SCAR and two SSR molecular markers located on Lg 3 and Lg7 were used for early selection of apple genotypes. The purpose of the selection was to find a potential donor for resistance to fire blight (FB) in an apple breeding programme. This study was carried out on 35 breeding clones, and registered cultivars originating from seven countries. They were all characterized as having different levels of susceptibility to FB in field conditions. The number of generated markers varied from 1-2 to 4-5, depending on the genotype. For the majority of the tested genotypes, strong interactions were observed between data concerning plant behaviour in the field and the presence/number of DNA markers. For nine genotypes, however, correlations between phenotypic and molecular study were not found with selected QTLs.

**Key words:** apple, fire blight, resistance, SCAR, SSR

## INTRODUCTION

Fire blight (FB), caused by Gram-negative bacterium *Erwinia amylovora*, is one of the most harmful and destructive diseases of apple (*Malus x domestica*), pear (*Pyrus communis*) and many other members of the *Rosaceae* family. The disease

was first reported in New York State at the end of the 19<sup>th</sup> century (Van-neste, 2000). Since then, fire blight has spread over most continents (Bonn and Van der Zwet, 2000). In Europe FB appeared about one hundred years after it was first described. Since 1950s the disease has been the main reason for the heavy losses in

many apple orchards (Sobiczewski, 1984; Sobiczewski and Suski, 1988; Keck et al., 1996; Blazek, 1999; Fisher et al., 2004; Dondini et al., 2005; Toth et al., 2006; Peil et al., 2007). Over 130 strains of *E. amylovora* have been described as pathogenic populations (Donat et al., 2005). The disease can be simply transferred from ooze on the infected host to healthy plants by ants, flies, rain and hail (Thomson, 2000). On the other hand, plant protection against FB is difficult because control of bacterial diseases with antibiotics has been banned for ecological reasons and controlling strategy is limited due to the streptomycin-resistance of some *E. amylovora* populations (Jones and Schnabel, 2000). These reasons make it clear that FB can easily continue to spread despite all quarantine measures (Dondini et al., 2002; Jock et al., 2002).

According to many author's opinions, breeding for resistance is the most promising tool in the disease-management programme (Aldwinckle and Beer, 1997; Lespinasse and Aldwinckle, 2000), particularly because apple cultivars display a great variability for resistance to fire blight (Le Lezac et al., 1990; Kielak et al., 2002). However, the genetic basis of this resistance is not fully understood. In the first study on resistance inheritance for the cross of *Malus x robusta* and *Malus x sublobata*, it was suggested that resistance may be determined by dominant genes (Gardener et al., 1980). Korban and co-workers (1988), however, found evidence that FB resis-

tance in cultivated apples is quantitatively controlled. Recently, a major quantitative trait locus (QTL) containing apple genes involved in defence process was described on linkage group 7 (Calange et al., 2005; Khan et al., 2006), and some minor QTLs were reported on linkage groups 3, 12 and 13 (Calange et al., 2005). Based on this data it can be said that some specific DNA fragments can be identified in plants that would make them potential markers of resistance to fire blight (Khan et al., 2006; 2007; Peil et al., 2007).

The purpose of this paper was to estimate the interactions between the presence of molecular markers tightly linked to two important QTL regions and data concerning defence reactions of tested apple genotypes in the field. This will allow evaluating actual value of tested plants as the donors of fire blight resistance in planned apple breeding programme.

## MATERIAL AND METHODS

Plant material consisted of 2 g of leaves, collected from plants of 31 European apple cultivars and clones. They were 6 genotypes released in Belgium (CRA MaA 40, CRA MaA 68, CRA MaA 70, CRA MaB 28, CRA MaD 30, CRA MaA 4), nine German genotypes ('Reanda', 'Rebella', Pi-A 5,100, Pi A 18,24, Pi-As 12,53, Pi-As 28,68, Pi-As 33,124, Pi-as 34,100, Pi-As 50,74), four Polish genotypes ('Free Redstar', 'Gold Millennium', 'Melfree', J-79), four Swiss genotypes ('Blauacher Wändeswil', 'Bohnapfel', 'Hordapfel', 'Schneideapfel'), four Swedish genotypes

(‘Aroma’, ‘Gyllenkroks Astrakan’, ‘Kalmare Glas’, ‘Sävstaholm’), three Hungarian genotypes (MR-03, MR-10, MR-12) and one Czech genotype (‘Topaz’). Additionally, three cultivars well known as susceptible to FB (‘Idared’, ‘Spartan’ – Polish type, ‘Spartan’ – Swiss type) and one resistant genotype (‘Enterprise’) were included as the control standards.

DNA was isolated according to the Aldrich and Cullis method with CTAB buffer, preheated to 65°C. Nucleic acids were precipitated with cold isopropanol (-20°C), the pellet suspended in TE buffer (Tris-EDTA, pH = 8.0) and treated with RNA-ase. Then, DNA was purified with a mixture of phenol-chloroform-isoamyl (25:24:1), precipitated with cold 70% ethanol (-20°C), suspended in sterile water and stored at -20°C.

Amplification of DNA fragments were performed in thermocycler BIOMETRA Basic in 13µl of PCR mixture containing: 10 ng of DNA template, 10 x PCR buffer, 10 mM dNTP mix, 0.25 mM MgCl<sub>2</sub>, 0.8 mM each of primer pairs, 0.1 U of Recombinant Taq Polimerase (Invitrogen) for SCAR markers or 0.05 U of AmpliTaq Polimerase (Applied Biosystem) for SSR marker generation. Primers were synthesized on the basis of the described QTL data.

Two different thermal profiles of PCR were used to get the amplification of the desired DNA fragments. For two SSR fragments (CHO3e03 and CHO3g12), the first four cycles were performed at the following temperatures: 96°C (30 s), 65°C (90 s) with reduction of 1°C per cycle, and

72°C (120 s), followed by 35 cycles with constant annealing temperature (60°C for 90 s). For three SCAR markers (AE10, GE-8019 and CH-F7-Fb1) all 35 cycles were performed at the profile: 96°C (45 s) and at 60°C (60 s).

PCR products were analysed by electrophoresis in 1.8% agarose gel stained with ethidium bromide (SCARs) or in 7% polyacrylamide gel stained with silver nitrate (1g/L) (SSRs). Amplified fragments were size-fractionated in comparison to λ DNA, digested with *EcoV/HindIII* (Invitrogen).

## RESULTS AND DISCUSION

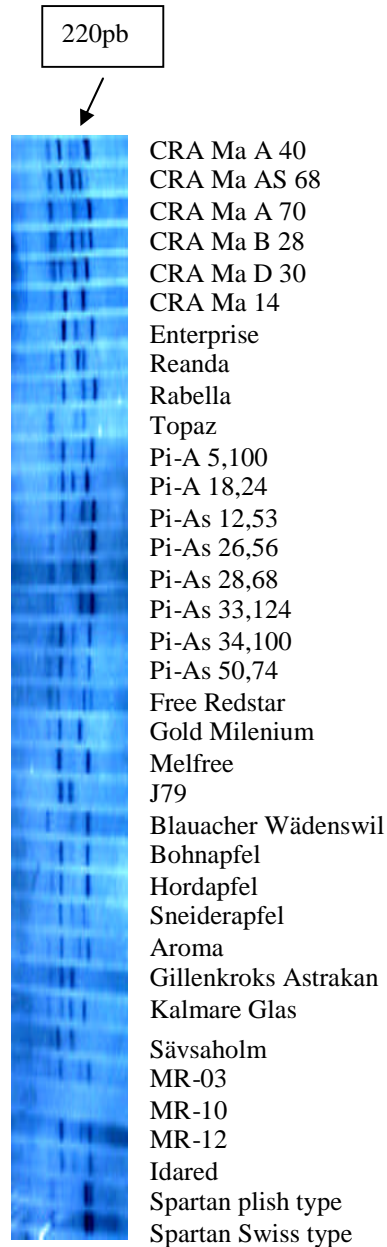
Resistance alleles relevant to the expected length of selected DNA fragments of QTLs derived from linkage groups 3 and 7, were obtained (Tab. 1, Fig. 1-3). The presence of 4 or 5 of these DNA fragments were noted for seven of the German clones considered by breeders in Dresden Pillnitz as being those with low susceptibility. The seven German clones are ‘Reanda’, ‘Rebella’, Pi-A 5,100; Pi-A 18,24; Pi-As 12,53; Pi-As 33,124; Pi-As 50-74 (Dunemann et al., 2007; A. Peil – personal communication). One Belgian genotype (CRA Ma D 30) and three Swedish genotypes (‘Aroma’, ‘Gyllenkroks Astrakan’, ‘Sävstaholm’), were also described as plants with low susceptibility to *E. amylovora* in the field (M. Lateur, H. Nybon – personal communication). In the genome of nine genotypes characterized by breeders as FB-tolerant in the field conditions (CRA Ma A 70,

Table 1. Presence of expected amplicons generated in PCR on the template of analyzed apple genotypes with primers specific for five selected QTL markers and correlation

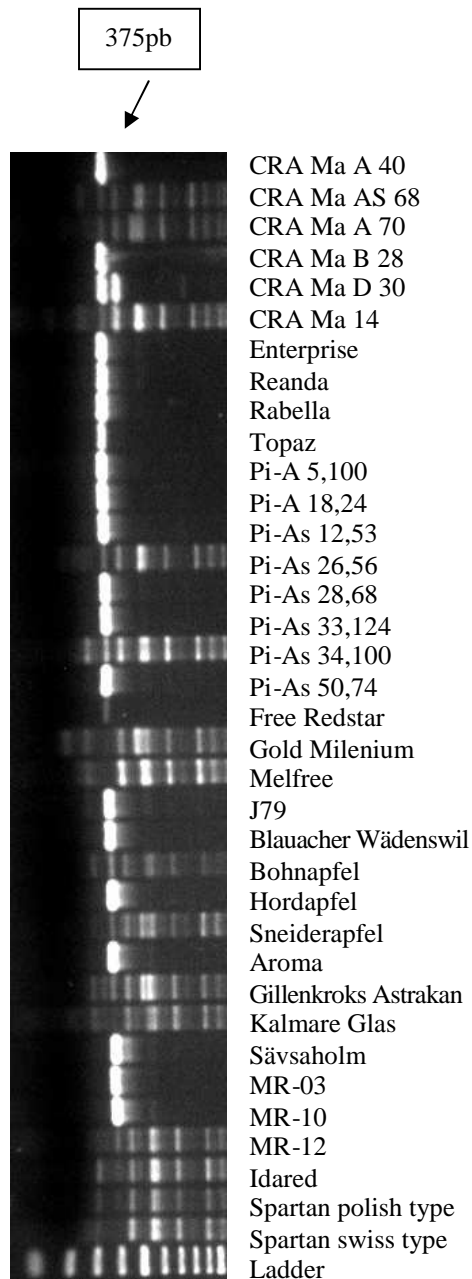
Genotype (cv, clone)	Marker/ expected molecular size (pb)					No. of alleles coding putative resistance	Evaluation of geno- type resis- tance (R)/ susceptibil- ity (S)	Field status of plant	Reference
	CHO3e 03/ 186bp	CHO3g 12/ 220bp	AE10/ 375bp	GE- 8019/ 397bp	CH-F7- Fb1/ 210bp				
	Linkage group 3		Linkage group 7						
CRA Ma A 40	-	+	+	-	+	-/+/-/+ (Rr)	partially R	Low S	M. Lateur – personal com.
CRA Ma A 68	-	+	-	-	+	-/+/-/+ (rr)	S	Low S	M. Lateur – personal-com.
CRA Ma A 70	-	-	-	-	+	-/-/-/+ (rr)	S	Low S	M. Lateur – personal com.
CRA Ma B 28	-	-	+	-	+	-/+/-/+ (rr)	S	Low S	M. Lateur – personal com.
CRA Ma D 30	-	+	+	+	+	-/+/+/+ (RR)	R	Low S	M. Lateur – personal com.
CRA Ma A 4	+	+	-	-	+	+/-/-/+ (Rr)	partially R	Low S	M. Lateur – personal com.
‘Enterprise’	+	+	+	+	+	+/+/+/+ (RR)	R	R	Dunemann et al., 2007;
‘Reanda’	+	+	+	+	+	+/+/+/+ (RR)	R	Low S	Dunemann et al., 2007;
‘Rebella’	+	+	+	+	+	+/+/+/+ (RR)	R	Low S	Dunemann et al., 2007;
Pi-A 5,100	+	+	+	+	+	+/+/+/+ (RR)	R	Low S	A. Peil - personal com.
Pi-A 18, 24	+	+	+	+	+	+/+/+/+ (RR)	R	Low S	A. Peil - personal com.
Pi-As 12, 53	+	+	+	+	+	+/+/+/+ (RR)	R	Low S	A. Peil - personal com.
Pi-As 26, 56	+	-	-	-	+	+/-/-/+ (rr)	S	Low S	A. Peil - personal com.
Pi-As 28, 68	-	-	+	+	+	-/+/+/+ (Rr)	partially R	Low S	A. Peil - personal com.
Pi-As 33, 124	+	-	+	+	+	+/-/+/+ (RR)	R	Low S	A. Peil - personal com.
Pi-As 34, 100	-	+	-	-	+	-/+/-/+ (rr)	S	Low S	A. Peil - personal com.
Pi-As 50, 74	-	+	+	+	+	-/+/+/+ (RR)	R	Low S	A. Peil - personal com.

Molecular screening of (*Malus domestica*) cultivars and breeding clones..

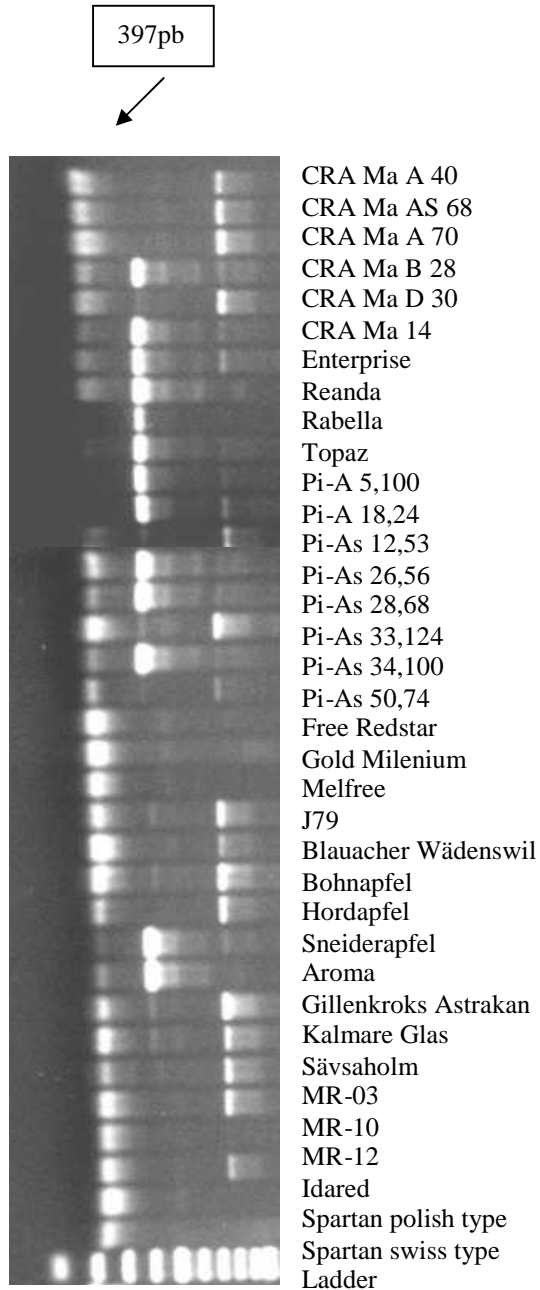
'Free Redstar'	-	+	+	-	+	-/+/-/+ (Rr)	partially R	Low S	Sobiczewski et al. 2008
'Gold Milenium'	-	+	-	-	+	-/+/-/+ (rr)	S	Low S	Sobiczewski et al. 2004
'Melfree'	+	+	-	-	+	+/+/-/+ (Rr)	partially R	Low S	Sobiczewski et al. 2004
J-79	-	+	+	-	+	-/+/-/+ (Rr)	partially R	Medium S	Sobiczewski et al.2008
'Blauacher Wädenswil'	+	-	+	-	-	+/-/+/- (rr)	S	Low S	M. Kellerhals 2004 - personal com.
'Bohnapfel'	+	+	-	-	+	+/+/-/+ (Rr)	partially R	Low S	M. Kellerhals - personal com.
'Hordapfel'	-	+	+	-	-	-/+/+/- (rr)	S	Low S	M. Kellerhals - personal com.
'Schneiderapfel'	+	+	-	-	+	+/+/-/+ (Rr)	partially R	Low S	M. Kellerhals - personal com.
'Aroma'	-	+	+	+	+	-/+/+/+ (RR)	R	Low S	H. Nybom – personal com.
'Gyllenkroks Astrakan'	+	+	-	+	+	+/+/+/+ (RR)	R	Low S	H. Nybom – personal com.
'Kalmare Glas'	-	+	-	-	+	-/+/-/+ (rr)	S	Low S	H. Nybom – personal com.
'Sävstaholm'	+	+	+	-	+	+/+/+/- (RR)	R	Low S	H. Nybom – personal com.
MR - 03	+	+	+	-	-	+/+/+/- (Rr)	partially R	Low S	M. Toth – personal com.
MR - 10	+	-	+	-	+	+/-/+/- (Rr)	partially R	Low S	M. Toth – personal com.
MR - 12	+	+	-	-	+	+/+/-/+ (Rr)	partially R	Low S	M. Toth – personal com.
'Topaz'	-	-	+	+	+	-/-/+/+ (Rr)	partially R	Low S	A.Peil – personal com.
'Idared'	+	+	-	-	+	+/+/-/+ (Rr)	partially R	S	A.Peil 2007
'Spartan' (Polish type)	-	-	-	-	+	-/-/-/+ (rr)	S	S	C.R. Hampson 2008
'Spartan' (Swiss type)	-	-	-	-	+	-/-/-/+ (rr)	S	S	C.R. Hampson 2008



**Figure 1.** Electrophoretogram obtained for DNA samples amplified with SSR primers CHO3g12 (Liebhard et al., 2003)



**Figure 2.** Electrophoretogram obtained for DNA samples amplified with SCAR primers AE10-375 (Khan et al., 2006)



**Figure 3.** Electrophoregram obtained for DNA samples amplified with SCAR primers GE-8019 (Khan et al., 2006)



CRA Ma A 68 and CRA Ma A 28, Pi-As 26,56 and Pi-As 34,100, 'Gold Millennium', 'Hordapfel', 'Blauacher Wädenswil' and 'Kalmare Glas' (Kellerhals and Meyer, 1994), only one or two loci responsible for putative fire blight resistance were identified. In genome of the other twelve tested apple cultivars and clones, single potential resistant loci from Lg 3 (MR-03) as well as from Lg 7 ('Topaz', Pi-As 28, 68) or from both Lgs (CRA Ma A 40, CRA Ma A 4, 'Free Redstar', 'Melfree', J-79, 'Bohnapfel', 'Schneiderapfel', MR – 10, MR – 12) were found. This confirms the status of these genotypes known as partially resistant plants in the field (Kellerhals et al., 2004).

Molecular markers are often used in modern breeding. They are used for identification of desired plant traits and for marker assisted selection (MAS), also in regards to genetic map construction and QTL identification (Kashkush et al., 2001; Frey et al., 2004). Co-localization of QTL with putative resistant genes suggests that some genome regions are strongly involved in pathogen recognition (Calenge et al., 2004). However, despite the explanation of the role of 4 FB-related QTLs identified in apple genome (Calenge et al., 2005), the minor QTLs on Lg 3, Lg 12 and Lg 13 were not found in the experiment performed on the small set of the same progeny as used by Calenge et al. ('Fiesta' x 'Discovery'). This suggests that the presence of minor QTLs is not necessary and additional unidentified genetic elements contributing the FB resistance can par-

ticipate in plant reaction to infection with the pathogen (Khan et al., 2006). Some additional clusters of resistance gene analogues (RGAs), linked to the major QTLs, controlling the intensity of the defence reactions in plants were found in a more recent study (Calenge et al., 2006). Moreover, many factors like pathogenesis-related proteins (PRP) can enhance the resistance in host plants (Ko et al., 2000; Malnoy et al., 2003; 2005). This type of genes should be routinely used in the study of plant resistance (Bonasera et al., 2006).

According to the results of our study, the significant FB resistance-markers from Lg 3 and 7 were identified on the DNA template of 11 of 31 investigated genotypes. This fully confirms the phenotypic data collected by breeders (Tab. 1). Three out of desired 5 markers were identified in PCR performed on the genome template of 12 genotypes described by breeders as partially resistant plants. Unfortunately, in the nine genotypes described by breeders as plants with low susceptibility to FB in the field, only single (1-2) expected markers from Lg3 and 7 were found (Tab. 1). These results suggest that FB resistance is a polygenic trait. It can be regulated by different genome regions and clusters of resistance analogues, not only by these QTLs which were characterized as the most important. With this in mind, the correlation between plant defence reactions and genotype as a donor of resistance for a breeding programme should be checked every time. Despite all these difficulties,

implementation of the FB resistance markers, supported by other resistance-related DNA fragments, seems to be a step in the right direction and a promising way to maximize chances for the proper choice of parental forms in breeding programmes. In parallel, the results of this type of molecular study can be exploited for introgression of the resistance genes detected in genomes of donors into new apple cultivars (Tartarini et al., 1999).

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# OCENA PRZYDATNOŚCI WYBRANYCH MARKERÓW MOLEKULARNYCH QTL DO SZYBKIEJ OCENY ODMIAN I KLONÓW HODOWLANÝCH JABŁONI (*Malus domestica*) JAKO DONORÓW ODPORNOŚCI NA ZARAZĘ OGNIOWĄ W PROGRAMACH HODOWLI

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## S T R E S Z C Z E N I E

Trzy markery SCAR i 2 markery SSR z grup sprzężeń Lg 3 i 7 wykorzystano do wczesnej selekcji genotypów jabłoni, będących potencjalnymi donorami genów odporności na zarazę ogniową. Badania prowadzono na 35 genotypach (klony hodowlane i zarejestrowane odmiany) z siedmiu krajów, różniących się stopniem podatności na zarazę ogniową. Liczba amplikonów generowanych na matrycy DNA pochodzących z testowanych genotypów wynosiła od 1-2 do 4-5 fragmentów. Dla większości testowanych roślin obserwowano ścisłe interakcje między liczbą uzyskanych markerów a opisanym przez różnych autorów stopniem podatności tych genotypów na zarazę ogniową w warunkach polowych. Dla 9 genotypów nie znaleziono korelacji między danymi fenotypowymi a wynikami badań molekularnych, opartych na wybranych QTL

**Słowa kluczowe:** jabłń, zaraza ogniowa, odporność, SCAR, SSR