

Identification of QTL FBF7 fire blight resistance in apple varieties germplasm

Alexander Lyzhin*, and Natalya Saveleva

Federal State Scientific Institution «I. V. Michurin Federal Scientific Center», 30 st. Michurina, Michurinsk, 393774

Abstract. This paper describes the results of marker-mediated screening of apple varieties for QTL FBF7 fire blight resistance. We detected at least one marker out of three in 12 varieties (85.7%) and two markers out of three in 5 varieties (35.7%). The target fragments of the studied markers in the Antonovka obiknovennaya and Galarina varieties are missing. Indicating the presence of QTL FBF7 three markers (GE-8019, AE10-375 and CH-F7-Fb1) were identified in the Bylina, Rozhdestvenskoe, Uspenskoe and Charodeyka varieties, which we recommend for use in breeding for *E. amylovora* resistance.

Fire blight of fruit crops (bacteria *Erwinia amylovora* (Burill) Winslow et al.) is a dangerous plant disease, including apple trees. It is widely distributed in many countries of the world [1, 2].

Due to rapid spread in fruit plantations, the harmfulness of fire blight is very high. Under favorable conditions, the mass development of *E. amylovora* can lead of entire gardens to death [3].

Using of synthetic antibiotics in fruit crops is only one way to control the spread of *E. amylovora* until now. Such an active use of chemical protective agents adversely affects the environmental situation, and can contribute to the *E. amylovora* highly resistant strains appearance [4, 5]. Therefore, using the genetically resistant to *E. amylovora* varieties is a promising way to combat the fire blight in fruit crops [6, 7].

Fire blight resistance controlled by quantitative trait loci (QTL) has been identified in various wild apple species and varieties. Many of them have also scab and powdery mildew genetic resistance, which is very important too. Individual QTLs from the original wild-growing species were subsequently introgressed into the cultivated forms germplasm [8-11].

QTL FBF7 is one of the largest QTLs that control apple trees fire blight resistance. It was mapped on the 7th chromosome of the Fiesta variety [12, 13].

This study presents the results of apple varieties marker-mediated screening for fire blight resistance. (QTL FBF7).

The study was conducted in 2020-2021. Apple varieties of the FSSI «I.V. Michurin Federal Scientific Center» genetic collection were used as biological objects (Table 1).

*Corresponding author: Ranenburzhetc@yandex.ru

Table 1. Analyzed apple varieties

Variety	Breeding combination	Origin / Originator
Antonovka obiknovennaya	-	Folk selection, Russia
Bylina	Prima × Bessemyanka michurinskaya	FSSI «I.V. Michurin Federal Scientific Center», Russia
Vimpel	Scala × Karpovskoe	
Scala	Prima × Bessemyanka michurinskaya	
Uspenskoe	Prima × Bessemyanka michurinskaya	
Fregat	Scala × Karpovskoe	
Charodeyka	12-59 × Vazak	
Kandil Orlovskii	[(F ₂ <i>M. floribunda</i> × Wealthy) × (F ₂ <i>M. floribunda</i> × Jonathan)] × free pollination	FSSI Russian Research Institute of Fruit Crop Breeding, Russia
Rozdestvenskoe	Wealthy × BM41497	
Gala	Kidds Orange × Golden Delicious	J.H. Kidd, New Zealand
Galarina	Gala × Florina	Institut national de la recherche agronomique, France
Ligol	Linda × Golden Delicious	Horticulture and Floriculture Institute, Poland
Lobo	Macintosh × free pollination	Ottawa Experimental Station, Canada
Fuji	Red Delicious × Rale Janet	Tohoku Experimental Station, Japan

Molecular markers AE10-375 (for 5'-ctgaagcgcacgttctcc-3', rev 5'-ctgaagcgcattctctgatag-3'), GE-8019 (for 5'-ttgagaccgatttctgtg-3', rev 5'-tctctcccagagcttcattgt-3') and CH-F7-Fb1 (for 5'-agccagatcacatgttttcac-3', rev 5'-acaacggccaccagtttacc-3') were used to identify the QTL FBF7 apple trees fire blight resistance [14].

AE10-375 and GE-8019 markers flank QTL FBF7: AE10-375 marker is localized at a distance of 4 cM from the QTL peak, GE-8019 marker is localized at a distance of 6 cM from the QTL peak. CH-F7-Fb1 marker is mapped on the same side of the QTL as AE10-375 and used to increase the reliability of molecular-genetic analysis [15, 16].

Total volume of 15 ml of the reaction mixture was contained: 1.5 mM Taq buffer, 2.0 mM deoxynucleoside triphosphates mixture, 2.5 mM magnesium chloride, 0.2 μm of each primer, 0.2 U Taq polymerase and 20 ng of total DNA.

The polymerase chain reaction was carried out in the T100 amplifier (BIO-RAD) in the following modes: initial denaturation of 5 min at 94°C, then 35 cycles: 1 min at 94°C, 1.5 min at 50°C, 1.5 min at 72°C; then the final elongation of 15 min at 72°C.

Amplification products separation was carried out by the electrophoretic method in an agarose gel (2% agarose concentration, 1 x TBE buffer system). The Gene Ruler 100 bp DNA Ladder (Thermo Fisher Scientific) gene line was used to amplicon size determination.

According to the conducted studies, 210 bp allele of the CH-F7-Fb1 marker (linked to QTL FBF7 fire blight resistance) was identified in 8 out of 14 apple varieties. AE10-375

marker (amplicon 375 bp) was identified in 12 forms, GE-8019 marker – in 5 forms (Table 2).

Table 2. Allele polymorphism of apple tree fire blight resistance (QTL FBF7) molecular markers

Variety	QTL FBF7 DNA markers			
	GE-8019	AE10-375	CH-F7-Fb1	
	397 bp	375 bp	174 bp	210 bp
Antonovka obiknovennaya	0	0	1	0
Bylina	1	1	0	1
Vimpel	0	1	1	0
Gala	0	1	1	0
Galarina	0	0	1	0
Kandil Orlovskii	0	1	1	0
Ligol	0	1	0	1
Lobo	1	1	1	0
Rozdestvenskoe	1	1	0	1
Scala	0	1	0	1
Uspenskoe	1	1	0	1
Fregat	0	1	0	1
Fuji	0	1	0	1
Charodeyka	1	1	0	1

At least one marker out of three was detected in most of the analyzed apple varieties (85.7%). Two markers out of three were present in 5 varieties (35.7%): Lobo variety has GE-8019 and AE10-375 markers, Ligol, Skala, Fregat, Fuji varieties have AE10-375 and CH-F7-Fb1 markers.

The target fragments of the studied markers are absent in the Antonovka obiknovennaya and Galarina varieties.

Three markers indicating the presence of QTL FBF7 fire blight resistance were identified in the genome of the Bylina, Rozhdestvenskoe, Uspenskoe and Charodeyka varieties. QTL flanking markers GE-8019 and AE10-375 (CH-F7-Fb1 marker is absent) were detected in the Lobo variety. Presumably, that also indicates the presence of QTL FBF7 with less probability than in the presence of three markers.

After analyzing apple varieties for fire blight resistance using molecular markers, we came to the following conclusions. Linked to the QTL FBF7 GE-8019, AE10-375 and CH-F7-Fb1 markers were identified in Bylina, Rozhdestvenskoe, Uspenskoe and Charodeyka varieties. We recommend these varieties for use in breeding for *E. amylovora* resistance.

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