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Genetic resistance of pear cultivars to fire blight

Abstract. The economic damage accompanying the fire blight is expressed both in the loss of crops and in the costs associated with uprooting of infected trees and restoration of fruit plantations. Pear is characterized the highest rates of susceptibility to fire blight. Development of genetically resistant cultivars is one of the crucial aspects of managing fire blight, which would allow foregoing the current economically or ecologically unsound methods such as removal of infected plants or application of antibiotics on the early stages of development. In this research, a five-year monitoring of the distribution of fire blight was performed among a collection of pear cultivars growing in a pomological garden. The cultivars 'Ajdana' and 'Kirgizskaya zimnyaya' showed the highest resistance to *E. amylovora* according to the results of field inspections. The infection occasions for every cultivar were confirmed by direct or indirect PCR. 'Ajdana' and 'Kirgizskaya zimnyaya' bear alleles of fire blight resistance for the CH02F06 marker, and 'Kirgizskaya zimnyaya' additionaly carries the resistance allele for the TsuENH017 marker. 'Talgarskaya krasavica' and 'Lesnaya Krasavitsa' are susceptible cultivars to fire blight, but at the same time their genomes include the resistance alleles for the CH02F06 marker.
Key words: pear, fire blight, cultivar, resistance, allele, marker.

Introduction

Fire blight is a perennial disease posing great danger to fruit crops, particularly from the *Rosaceae* family (1). Fire blight is caused by the Gram-negative bacterium *Erwinia amylovora*. The economic damage accompanying the disease is expressed both in the loss of crops and in the costs associated with uprooting of infected trees and restoration of fruit plantations. Moreover, the disease negatively affects growth and development of trees (1). In Kazakhstan, the disease was first detected in 2010, presumably introduced through the imported planting material (2).

Among *E. amylovora* hosts, pear is characterized the highest rates of susceptibility to fire blight (3,4). Pear was the 4th most popular fruit crop in Kazakhstan in 2020 with 14 344,9 t harvested (5). Its cultivated area spans 2054,7 ha, and more than half is situated in Almaty and Almaty region, followed by the neighboring Jambyl and Turkestan regions (6). Research conducted on the spread of fire blight in these regions demonstrated that 61% of farms were affected, with some pear cultivars infected up to 100% (cultivars 'Talgarskaya krasavitsa', 'Lyubimitsa Klappa') (4).

Development of genetically resistant cultivars is one of the crucial aspects of managing fire blight, which would allow foregoing the current economically or ecologically unsound methods such as removal of infected plants or application of antibiotics on the early stages of development (7, 8). That would require closer study of genomic regions associated with resistance to *E. amylovora*. To date, five studies have been conducted to identify quantitative trait loci (QTLs) on pear populations in Europe and in the USA (8, 9, 10, 11, 12). Dondini et al. studies crossbreed of Passe Crassane × Harrow Sweet cultivars, and identified 4 QTLs (HS2a; HS2b, HS4, HS9) on Harrow Sweet linkage groups 2 and 4 using SSRs, MFLPs, AFLPs, RGAs and AFLP-RGAs markers (10). The later study on the same population confirmed QTLs 2a and 4 using additional SSR markers (11). Bokszczanin et al. identified two

putative QTLs for fire blight resistance based on 48 AFLP and 32 SSR: no. 18 in Pyrus unsureness linkage group 11, and another on linkage group 4 in Doyenné du Comice cultivar (9). The study based on an single-nucleotide polymorphism (SNP) array revealed 6 QTLs altogether: five on PEAR3 crossbreed (PremP003, P. bretschneideri × P. communis) × Moonglow (P. communis)) LGs 7, 9, 10, 12, and 15 and one on Moonglow linkage group (LG) 2 (12). The latest study utilizing a previously developed 70 K SNP Axiom array consisting only of Pyrus SNPs considered three populations consisting of progenitors of the previously studied cultivars and identified seven QTLs in positions associated with their respective descendants (8).

The present study aims to identify resistant alleles by analyzing cultivars growing in Kazakhstan.

Materials and methods

Visual inspection of pear trees and collection of plant material. We investigated phytosanitary conditions of pear trees in Pomological Garden in Talgar (Kazakhstan), which serve as genetic pool for breeding and germplasm collection. The pear garden was established in 1992 and harbors cultivars and hybrids with important agronomical traits. Visual inspections were conducted every two years from 2014 to 2022 to identify trees infected with fire blight and to estimate the disease distribution. At least 5 pear trees having the standard phenotype of every cultivar were analyzed. Damage level was assessed in spring and autumn by 5-point scale according to the following system: 0 – asymptomatic, 1 – sporadic symptoms of blight in flowers, leaves, and shoots, 2 – tree crown affected by up to 20%, 3 – tree crown affected by 20% to 50%, 4 – 50% to 100% of tree crown damaged by infection. Index of disease development was calculated by the formula:

$$R = \frac{\Sigma\,(ab)x\,100}{kxN};$$

R – index of disease development, Σ (ab) – sum of products of the number of damaged trees multiplied by corresponding damage score; N – total number of accounting trees; k – highest score on the scale.

Leaves and twigs of every cultivar were collected in 2022 to identify alleles resistant to *Erwinia amylovora* using genetic markers.

Detection of Erwinia amylovora. Plant material of every cultivar was analyzed for presence of *E.amylovora* using PCR. Genomic DNA was extracted directly from plant material using innuPREP Plant DNA Kit (Analytik Jena) according to the manufacturer's protocol. 50 ng of genomic DNA were used in the preparation of 20 µl PCR mix containing 10 µl Luna® Universal Probe qPCR Master Mix (New England Biolabs), 10 µM forward (5′-TCCCACATACTGTGAATCATCCA-3′) and 10 µM reverse (5′-GGGTATTTGCGCTAATTTTATTCG-3′) primers, and 10 µM probe (FAM-CCAGAATCTGGCCCGCGTATACCG-TAMRA). Amplification and analysis were performed according to the protocol described by Pirc et al. (13).

SSR analysis. Pear samples were genotyped using three SSR markers (Table 1). Amplification was performed in 25 µl containing 1X Standard Taq Reaction Buffer (M0273E, New England BioLabs), 0.2 µM dNTP, 0.16 µM of each primer (Table 1), 0.15 µl Taq-polymerase (M0273E, New England BioLabs) and 40 ng of DNA template.

Table 1 – Sequences of primers and fluorescent dyes used in SSR genotyping

Marker name	Sequence (5'-3')	Fluorescent dye	Analyzed range	Reference
TsuENH017	F: ACTTCAAGTAGCCAACTATCAG	6-FAM	100-250	Montanari *et al*. (14)
	R: GGCACTCTGTTTCTTATCAAC			
CH02f06	F: CCCTCTTCAGACCTGCATATG	HEX	100-250	
	R: ACTGTTTCCAAGCGATCAGG			
CH05c07	F: TGATGCATTAGGGCTTGTACTT	TAMRA	100-250	
	R: GGGATGCATTGCTAAATAGGAT			

PCR program for amplification of every marker included the holding for 150 s at 95 °C, and then 5 cycles of 94 °C for 30 s, annealing temperature ranging from 61 °C to 57 °C for 90 s, and 72 °C for 90 s, followed by 35 cycles of 94 °C for 30 s, 57 °C for 90 s, and 72 °C for 90 s. The final step after cycling consisted of an extension for 5 min at 72 °C.

Fragment analysis was conducted on Genetic Analyzer 3500 (Applied Biosystems) according to module FragAnalysis50_POP7. First reaction mix for fragment analysis contained 1 µl of each PCR product obtained using primers for TsuENH017 and CH05c07 markers, second one contained 1 µl of PCR product for CH02f06 marker. Every reaction mix except PCR products contained 0.15 µl GeneScan 500 LIZ Size Standard and 8.85 µl HI-DI Formamide. The reaction mixes were adjusted to 40 µl by ultrapure water. Immediately before loading on analyzer, the reaction mixes were denaturized at 95 ºC for 4 min. The analysis of results, finding true peaks, evaluation of sizes of peaks were performed in GeneMapper 6.

Results and discussion

Fire blight has been ravaging pome orchards in the country, pears especially, which has led to pear shortage in the domestic market. Many pear orchards damaged by fire blight have been completely uprooted. Fire blight affects all parts of the plant including flowers, leaves, twigs, and root system. The pathogen is capable of infecting trees of different ages. The severity of damage depends on age, cultivar, and environmental conditions. Therefore, a need of studying and breeding of pear cultivars resistant to fire blight is utterly relevant. Planting resistant cultivars is the most promising disease control strategy.

Conditions suitable for development and spreading of fire blight occur in spring and autumn when high humidity prevails. To evaluate the level of damage to pear trees caused by fire blight, visual monitoring of the cultivars was performed in the above time periods against a natural background. The level of damage was calculated according to the 5-point scale, Table 2. In the result of monitoring, local cultivar ‘Ajdana’ and introduced cultivar ‘Kirgizskaya zimnyaya’ were revealed to be more resistant to fire blight. These cultivars can be grown without special measures to protect plants from the disease, only preventive measures could be applied. Local cultivar ‘Talgarskaya krasavica’ and foreign cultivars ‘Conference’ and ‘Lesnaya Krasavitsa’ were classified as moderately susceptible to *E. amylovora*. The main symptoms of infection were expressed in drying and blackening of flowers, leaves, shoots, and in release of bacterial exudate. A severe form of the disease can quickly lead to the death of the plant (15).

Table 2 – Damage level of pear cultivars induced by fire blight

Cultivar/hybrid	Index of disease development by years				
	2014	2016	2018	2020	2022
Talgarskaya krasavica	2.0	1.5	1.8	2.0	1.7
Ajdana	0.1	0	0.1	0.5	0.2
Kirgizskaya zimnyaya	0	0.1	0	0.1	0
Konferenciya	1.5	1.0	1.7	2.0	1.6
Lesnaya krasavica	1.0	1.5	1.0	1.5	1.4

Assessment of fire blight damage to trees in the field provides reliable indicators of resistance, since environmental factors affecting pathogen-host interaction can be considered.

Presence of *E. amylovora* in each studied cultivar was confirmed by direct or indirect PCR. Leaves and twigs with infection symptoms were used for detection. In the case of resistant cultivars, the pathogen was confirmed only in ‘Ajdana’ by direct PCR in 2020 and 2022. In ‘Kirgizskaya zimnyaya’ *E. amylovora* was not detected by direct PCR during the monitoring period, which may be due to low concentration of the pathogen in plant material. To confirm the pathogen presence in plant material, culture was enriched in a liquid nutrient medium in accordance with the protocol PM 7/20 (16) followed by PCR detection of *E. amylovora* in the samples. For cultivars susceptible to fire blight, pathogen was

detected every year of monitoring period by direct PCR.

To identify genetic determinants of fire blight resistance, we have genotyped pear cultivars using SSR markers TsuENH017, CH02F06, and CH05c07 associated with fire blight resistance. The selected markers were previously studied on both fire blight susceptible and resistant pear cultivars and their segregating populations (12). Markers TsuENH017 and CH02F06 display the highest association with pear resistance to fire blight and are located in LG2. Nowadays the cultivars known and described as resistant to fire blight are 'Harrow Sweet', 'Old Home' and 'Moonglow'. These cultivars carry the resistance allele 169 or 179 for marker TsuENH017. Among the studied cultivars, 'Kirgizskaya zimnyaya' carries allele 169, table 3.

Resistance allele 176 for marker CH02F06 was identified in 'Ajdana', 'Kirgizskaya zimnyaya', 'Talgarskaya krasavica', and 'Lesnaya Krasavitsa'.

The results of analyzing the marker CH05c07 associated with the minor resistance locus in the linkage group LG9 did not reveal the resistance allele 141 in the studied pear cultivars. The genome of cultivar 'Kirgizskaya zimnyaya' includes resistance alleles for two markers TsuENH017 and CH02F06 in its heterozygous form.

Table 3 – SSR profiles of pear cultivars

№	Name of cultivar	Marker							
		rbcl	**TsuENH017**		**CH02f06**		**CH05c07**	
1	Lesnaya krasavica	+	*182*	*218*	*156*	*176^r*	*117*	*149*
2	Kirgizskaya zimnyaya	+	*169^r*	*182*	*176^r*	*180*	*117*	*139*
3	Ajdana	+	*184*	*218*	*156*	*176^r*	*117*	*139*
4	Talgarskaya krasavica	+	*182*	*186*	*176^r*	*178*	*149*	*149*
5	Konferenciya	+	*182*	*182*	*156*	*178*	*no*	*no*

^{r} – resistance

The 5-year field monitoring demonstrated that 'Kirgizskaya zimnyaya' is the cultivar most resistant to fire blight, but its fruits have lower flavor properties and cannot compete with the fruit quality of the leading cultivars on the market. 'Kirgizskaya zimnyaya' can be considered a promising cultivar for marker-assisted selective breeding as a donor of genetic determinants of resistance. Moreover, resistance alleles for marker CH02F06 were found in 'Ajdana', 'Talgarskaya krasavica', and 'Lesnaya Krasavitsa'. Results of the field monitoring showed that only 'Ajdana' cultivar exhibits an increased resistance to fire blight, while 'Talgarskaya krasavica' and 'Lesnaya Krasavitsa' are susceptible to the pathogen. Presence of a resistance allele for only one of the two markers (either TsuENH017 or CH02F06) characterized by the highest association with fire blight resistance is a sufficient condition for the formation of resistance to the disease. Susceptibility of Talgarskaya krasavica' and 'Lesnaya Krasavitsa' to the pathogen may be caused by several factors, such as non-additive genetic variants, pleiotropy effect, genetic background interactions, and genotype-by-environment interactions. Environmental and host conditions have a large influence on fire blight infections, making it challenging to characterize resistance/susceptibility of cultivars reliably.

Conclusion

In summary, we report here that local cultivar 'Ajdana' and foreign cultivar 'Kirgizskaya zimnyaya' are the cultivars most resistant to fire blight, according to field inspections and genetic investigations. 'Konferenciya', 'Talgarskaya krasavica', and 'Lesnaya krasavica' cultivars are susceptible to *E. amylovora,* despite the presence of resistant allele for CH02F06 marker in the latter two cultivars. Instances of infection for every cultivar were confirmed by direct or indirect PCR. Further pear breeding programs should focus on developing new cultivars resistant to fire blight, the main factor in increasing yields, by involving donor cultivars 'Kirgizskaya zimnyaya' and 'Ajdana'. Preserving the pear genetic pool including cultivars resistant to fire blight is important for breeding as well as maintaining genetic diversity.

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