Fire Blight, Caused By The Bacterium *Erwinia amylovora*, Is A Major Threat To Walnut-Fruit Forest Ecosystems In Kyrgyzstan

Tinatin Doolotkeldieva[1], Saikal Bobushova[1], Simon Carnal [2], Fabio Rezzonico[2]

[1] *Kyrgyz-Turkish Manas University, Plant Protection Department 56 ,Prospect Ch.Aytmatov, Bishkek city, 720044, Kyrgyzstan*

[2] *Environmental Genomics and Systems Biology Research Group, Institute of Natural Resource Sciences, Zürich 6 University of Applied Sciences (ZHAW), CH-8820 Wädenswil, Switzerland*

Corresponding author: Tinatin Doolotkeldieva
fax number : +996 (312) 541935
e-mail address: *tdoolotkeldieva@gmail.com*

SUMMARY. In 2008, fire blight was first reported in Kyrgyzstan, and within a few years, the disease has spread through the northern part of the country. As fire blight has expanded in Kyrgyzstan and penetrated new areas, the disease has reached unique ecosystems with wild forests. This study presents the molecular taxonomic characterization of *E. amylovora* isolated from fruit trees grown in southern Kyrgyzstan. For this purpose, field surveys in the apple and walnut-fruit forests of the Jalal –Abad region in Kyrgyzstan, as well as in the nearby orchards and gardens, were performed to assess the current extent and impact of fire blight on the native pomaceous plant species. Phytopathological studies were conducted on wild fruit plants such as *Malus niedzwetzkiana* Dieck, *Malus sievérsii*, *Purus korshinskyi* Litv, *Crataegus turkestanica* A. Pojark, *Crataegus pontica C. Koch,* as well as on domesticated fruit plants. *Erwinia amylovora* isolates were identified by using the specific diagnostic primer pairs PEANT-1/PEANT-2 and Ea CR1 – F1/Ea CR1-sp 18R. Of the 13 *E. amylovora* isolates obtained in 2019, six were isolated from quince (*Cydonia spp)*, three from cultivated apples (*M.domestica*), two from cultivated pears (*P. communis)*, and two from hawthorn (*Crataegus turkestanica)*. Complete CRR1 and CRR2 CRISPR arrays of *E. amylovora* isolates from Kyrgyzstan indicated that the arrays were identical in spacer organization for these isolates. All tested *E. amylovora* isolates from 2019 were genotyped as A-derived, and none of the isolates showed spacer deletions typical for the genotypes Z or D, previously identified in Europe. .

Key words: *wild forest fruit trees; CRISPR; CRR1 and CRR2 arrays; A- genotyped isolates.*

Introduction

Kyrgyzstan is home of the world's largest natural, wild-growing walnut forests, situated in the mountains in the southwest of the country. The walnut forest is within the ca. 60,000 hectares (150,000 acres) forest situated between the Fergana and Chatkal Mountains. The walnut forest is located at altitudes varying between 1,500 meters (4,900 ft) and 2,000 meters (6,600 ft) above sea level on the Fergana range's south-facing slopes [Mitchell, 2008; Mayhew, 2011]. Wild-growing walnut forests have unique importance for global biodiversity and the serve as a refuge of genetic resources for walnut, apples, pears, plums, and several other species. A total number of 88 plant species from 37 botanical families were documented. The plant species reported provide food (39), medicine (51), fuelwood (28), material (14), and animal feed. According to Use reports, the most representative species were *Juglans regia, Malus niedzwetzkyana, Malus sieversii, Prunus divaricata, Prunus sogdiana, Crataegus spp., Acer semenovii, and Acer turkestanica, Pyrus korshinsky.* Simultaneously, local people derive significant economic and ecological benefits from these forests [Vlˇckova, 2019].

However, Kyrgyzstan's forests are under pressure, mainly due to heavy overgrazing, desertification, and logging of trees for firewood. Moreover, the arrival of fire blight disease in the center of origin of endangered fruit species is a major threat to the whole forest ecosystem. Fire blight, caused by the bacterium *Erwinia amylovora (Enterobacterales; Erwiniaceae)*, is the most devastating disease affecting pome fruit production globally [Thompson, 2000]. The disease is native to North America and was imported to Western Europe in the 1950's, spreading from west to east in the ensuing decades [Van der Zwet and Beer, 1995]. The most important host plants from both economic and epidemiological viewpoints are in the genera *Chaenomeles*, *Cotoneaster*, *Crataegus*, *Cydonia*, *Eriobotrya*, *Malus*, *Mespilus*, *Pyracantha*, *Pyrus*, *Sorbus* and *Stranvaesia* [Bradbury, 1986]. The *E. amylovora* pathogen infects the host through natural openings or wounds in above-ground parts of fruit trees including blossoms, fruits, shoots, and branches [Adeolu et al., 2016; Agrios, 2005]. Infected tissues typically produce a viscous

bacteria-carrying exudate (termed “ooze”) from which the pathogen is efficiently spread by insects, birds, wind, and rain [Slack et al., 2017]. Fire blight is also spread via infected plant material, and old-growth trees and wild hosts (hawthorn, cotoneaster, pyracantha, etc.) can act as inoculum reservoirs.

In 2008, fire blight was first reported in Kyrgyzstan and registered; the disease has spread within a few years through the northern part of the country, affecting apple orchards across Chuy and Issyk-kul provinces [Dootkulova et al., 2017]. The situation with fire blight worsens every year, the disease expands its spread, penetrating new areas, thereby reaching the unique ecosystems where wild forests with rich biodiversity grow. A new disease invasion is now posing a critical threat to the conservation efforts to preserve endangered pome fruit species, the associated ecosystems, and local fruit production in Kyrgyzstan.

In 2015, *E. amylovora* bacteria from infested orchards were first systematically isolated, phenotypically described, and employed to evaluate antagonistic *Streptomyces* bacteria as potential fire blight biocontrol agents [Doolotkeldieva and Bobushova, 2016]. Our previous studies were dedicated to molecular characterization of *E. amylovora* isolated from diseased plants of the *Rosaceae* family growing in the northern part of the country, in particular in the Issyk-Kul region [Doolotkeldieva et al., 2019]. In these studies, the assignment to this taxonomic species has been corroborated by phylogenetic reconstruction using multilocus sequence analysis, and a short-sequence repeat (SSR) marker has been employed to estimate genetic diversity across the isolates. Sequence analysis of clustered regularly interspaced short palindromic repeat (CRISPR) arrays [Rezzonico et al. 2011; McGhee and Sundin 2012] has revealed both a previously unreported CRISPR-2 array pattern and a close relationship of Kyrgyz *E. amylovora* isolates to strains present in Europe and the Middle East [Doolotkeldieva et al., 2019].

This current study presents the molecular taxonomic characterization of *E. amylovora* bacteria isolated from fruit trees grown in southern Kyrgyzstan. For this purpose, we have

performed field surveys in the apple and walnut-fruit forests of the Jalal –Abad region in Kyrgyzstan, as well as in the nearby orchards and gardens to assess the current extent and impact of fire blight on the native pomaceous plant species and the related ecosystems.

2. Methods and material

2.1. Plant Samples Collection

Expeditions were conducted to Jalal -Abad region, where located Arslonbob natural forests with wild biodiversity of fruit trees and plants, including wild apple trees (*Malus sieversii, Malus niedzwetzkyana) and* pear trees (*Pyrus korshinskyi, Pyrus asia-mediae*). In total, we visited 28 sites around of wild forests and to the forestries in the heart of the wild natural forest. Approximately 150 samples were obtained from apple, quince, pear, hawthorn and dog rose trees with fire blight symptoms and without symptoms (Supplemental Fig.1).

2.2. Isolation of pure culture of *Erwinia amylovora*.

We used King's medium B, Levan medium (peptone, 5g; glycerol, 10 ml; K_2HPO_4, 1.5 g; $MgSO_4.7H_2O$-1.5 g; agar-20 g, pe liter), and Miller-Schroth medium (Tergitol 7 (sodium heptadecyl sulfate), 0.1 mL; 2% nitrilotriacetic acid in 1.46% KOH, 10 mL; peptone, 5g; sucrose, 25 g; 0.5% bromothymol blue (Na salt)[2] , 9 mL; 0.5% neutral red, 2.5 mL, per liter) for routine culture and isolation of *E. amylovora* from primary materials (diseased parts of plants). All cultures were incubated at 27°C, and suspected colonies of *E. amylovora* (white, circular, mucoid, and curved) were further purified on Levan medium. This operation was repeated two times to be sure that pure cultures were obtained for identification tests.

2.3. Immunostrip assays

The rapid assay Ea AgriStrip for *E. amylovora* detection produced by BIOREBA (Switzerland) was used. The rapid assay enables confirmation of the presence of the fire blight pathogen on-site and within minutes in pome fruit and related ornamentals and wild plants. The rapid assay Ea AgriStrip is a lateral-flow immunographic test based on an antigen - antibody reaction that is initiated by inserting the strip into the sample extract. For immunostrip assays

of composite plant samples, 150 µl of leaf and branch extracts were transferred to sterile microfuge racks, strips were added to each well and reactions read after 10-15 min. For maceration of plant tissues, we used the extraction buffer of Llop et al (1999).

2.3. Molecular identification.

Levan-positive, non-fluorescent culture at a concentration of 10^6 cells / ml in sterile distilled water suspension was prepared and used immediately or stored at -18 °C until PCR product was observed. For genetic characterization, genomic DNA was extracted from bacterial log phase liquid cultures grown over night at 27 °C in LB medium using the DNeasy Blood and Tissue kit (Qiagen) according to the standard protocol as provided by the manufacturer. Specific diagnostic PCR with primer pairs PEANT-1/PEANT- 2, G1-F/G2-R, and FER1-F/rgER2R for *E. amylovora* were developed previously[Llop et al., 2000; Taylor et al.,2001]. DNA extraction was conducted according to a published protocol [Stoger et al.,2006], and PCR analysis according to [Taylor et al., 2001]. An alternative protocol with DNeasy Plant mini Kit – QIAGEN was also used.

Specific primers targeting previously identified CRISPR genotypes were used for different spacer regions [Rezzonico et al., 2011] (Table 1). A single colony was picked from an agar plate, resuspended in 10 mL LB medium and incubated over night at 27° C. The well grown suspension was then diluted 1:20 in ddH2O and lysated at 95° C for 10 min. The lysates can then be used directly in the PCR. The total reaction mix for the PCR is listed in Table 2. The Hot-Start Polymerase was integrated in a ready to use reaction mix, for faster procedure (Ready Mix™ Taq PCR Reaction Mix, Sigma Aldrich, St.Louis, USA). The samples were then incubated in a Thermocycler (Labnet MultiGene, New York, USA). Thermocycler running protocol is presented in Table 3. PCR products, mixed with 3 µL Loading Dye, were put on a 1.5% agarose gel and run at 60 V for 2 h. The fragment sizes of the amplified isolates were then compared visually to already sequenced and genotyped samples. Therefore, the samples were only checked on similar size of the amplified regions and not the exact spacer composition. That's why the

samples are tagged as genotype XY- derived. The identified genotypes were mapped using ArcGIS Pro (Version 2.4.0) with existing GPS data of each sample point.

2.4. Hypersensitivity reaction and pathogenicity of *Erwinia amylovora isolates.*

To test the ability to induce a hypersensitivity reaction test was used [Sedlak, 2015;]. The suspension of *E. amylovora* cells in physiological saline were prepared containing 10^9 cells / ml. Immature pear fruits were inoculated with a suspension of *Erwinia amylovora* cells in RF (10^9 cells / ml) using a syringe. Pear fruit was placed in a humid chamber for 5 days at 25 ° C. The test results were considered positive when the symptoms of plant tissue necrosis developed and milky-white exudate was secreted in the inoculation area.

3. Results

3.1. Fruit plant characterization

Jalal-Abad region is located in the south-west of the country. The main mountainous terrain of the region is the northeastern part of the Fergana Valley and the mountains of the western Tian Shan. This region borders with Uzbekistan. Nut bearing forests are the center of origin of cultivated plants, a depository of biodiversity and genetic resources of flora and fauna, and form part of Western Tien Shan UNESCO World Heritage Site.

Phytopathological studies have been conducted on wild fruit plants such as *Malus niedzwetzkiana* Dieck, *Malus sievérsii*, *Purus korshinskyi* Litv, *Crataegus turkestanica* A. Pojark, *Crataegus pontica C. Koch,* as well as on domestic friut plants such as *Malus domestica* of Borkh - Golden Delicious, Aidared, Semerenko, Red Chief varieties ; Pear *P. communis* of Talgarskaya krasavitsa and Lesnaya krasavitsa varieties .

Malus niedzwetzkiana **Dieck** - Nedzwiecki apple tree is one of endangered and rare species that listed in the Red Book. In Kyrgyzstan, the species are distributed mainly in the Chatkal Range, the Fergana Range within of Arslanbob walnut forest. Niedzwiecki's apple is often growing as an isolated tree with red-fleshed, red-skinned fruit and red flowers.

Malus sievérsii - Sivers' is also endangered and rare species that listed in the Red Book. This apple-tree grows singly or in small groups at an altitude of 900 to 2500 m above sea level. Differs in weak growth, rarely reaching 8 m in height. In the mountainous and high mountain conditions, *Malus sievérsii* apple has developed, a good adaptability to the harsh climate.It has a deep root system, resistant to diseases and low temperatures. With overall life expectancy up to 150 years, it begins to bear fruit at the age of 12. It blooms in April and May, with large white and pink flowers. Apples are often not inferior in taste to garden varieties.

Purus korshinskyi **Litv -** The *Korzhinsky* pear is common in the Chatkal and Fergana Ridge. Endangered and rare species listed in the Red Book. The tree is up to 6-12 m high with white-letted, sometimes bare young shoots and lancets, 3-8 cm long leaves with a rounded and broadly wedge-shaped base. In natural places of growth, it can be found solitary in the walnut-fruit forests with Turkestan maple.

Crataegus turkestanica A. Pojark - Turkestan hawthorn widely distributed in the natural environment of Kyrgyzstan. Small, up to 7–8 m in height, trees with single spines or without reddish annual shoots. It is widespread in the Fergana, Alai, Zaalai, Turkestan and Gissar ridges at altitudes from 800 to 2400 m, but conditions are most optimal for its growth at altitudes of 1000-1700 m.

Crataegus pontica C. Koch - Pontic hawthorn, it grows very slowly, reaches a height of 60-80 cm in 10 years, lives up to 100-150 years of age. It is distributed in the Fergana, Alai, Zaalai, Turkestan and Gissar ridges at altitudes from 800 to 2000 m. The fruits can also find the widest application in the confectionery industry for the manufacture of jams, drought-resistant stock for apple trees.

3.2. SYMPTOMATOLOGY

Symptoms of fire blight on the most common hosts such as *P. communis* (pear), *M. domestica,* (apple), *Cydonia* spp. (quince), *Eriobotrya japonica* (loquat), *Cotoneaster* spp.

(cotoneaster), *Pyracantha* spp. (pyracantha) and *Crataegus* spp. (hawthorn) were similar and easily recognized (Table 4).

3.3. **Immunostrip assays of selected samples.**

In the first stage for isolation and detection of *E.amylovora bacteria* from plant samples with characteristic symptoms and without symptoms, immunostrip assay was used, which allowed to select samples in advance of the presence of pathogens. As assay results have shown, some samples were needed to check several times, because of the samples with positive reaction after first screening from plant tissue, have no positive results in the second screening from bacterial colonies (Fig.1). Therefore, to select samples with more accurate diagnoses for the presence of a pathogen, it is necessary to screen not only from plant tissue but also re-screen from the obtained primary bacterial colonies. The samples from wild apple trees (*Malus niedzwetzkiana Dieck and Malus sievérsii*) have shown negative immunostrip results in both cases, from the plant tissue, and from pure cultures. From samples of wild pear tree (*Purus korshinskyi* Litv) we have observed the symptoms of a bacterial fire blight, then *E. amylovora*- like colonies were grown on Levan media, first screening by immunostrip was a positive result, but then second screening from extracted samples of *Purus korshinskyi* Litv has shown a negative result. While, *E. amylovora* bacteria were isolated from the samples of nearly grown *Crataegus turkestanica* A. population, that were identified by all used methods. Immunostrip test has allowed obtaining the samples with bacterial fire blight pathogen in quince and hawthorn trees grown in Nooken District, Shaidan village, Fruit Nursery, and to isolate the pathogen. Where hawthorn was grown near quince trees, it was the obvious a fire blight foci (Supplement.mater, Fig.2, 3 and 4). After second screening using the immunostrip test, 19 plant samples have exhibited a clear positive result. Further, immediately from fresh extracts of positive samples were plated on semi-selective and further on differential mediums for successful isolation of bacteria.

3.4. Isolation of pure culture of *Erwinia amylovora*.

All isolated and suspected *E. amylovora* isolates gave clear colonies on Levan medium and Miller-Schroth medium. As shown on the Fig.2. *E. amylovora* colonies were grown after 3 days of incubation at 28^0C. From one single colony was isolated on the new Levan medium for cleaning extraneous microorganisms.

3.5. DNA Extraction, Amplification, and Sequencing.

Erwinia amylovora isolates were identified by using specific diagnostic primer pairs PEANT-1/PEANT-2 [Taylor et al.,2001] and Ea CR1 – F1/Ea CR1-sp 18R. In Supplementary section, Fig.5 and Fig.6 have shown electrophoresis profiles of obtained DNA fragments from plant diluted materials and bacterial culture. The dilutions from 10^8 up to 10^4 were provided more clear and visible gel profiles . This step was used for obtaining the optimal density of bacterial culture and updated for further DNA extraction and amplification of *E.amylovora.*
The subsequent results have presented in the Table 5 with primer Ea CR1 – F1/Ea CR1-sp 18R. From 12 obtained *E.amylovora* isolates, selected from different sites and different host plants, it was possible to get the sequence (Suppl.mat. Fig. 6).

The results with internal PEANT-1/PEANT-2 primers have presented in the Table 6. The electrophoretic profiles of amplified genes of obtained *Erwinia amylovora isolates* by using PEANT-1/PEANT-2 primers have shown in Figure 7 (Supplement. mat.).

3.6. Analysis and genotyping of *E. amylovora* CRISPR regions.

In total, the CRRs1 and CRRs2 of 23 different Kyrgyz *E. amylovora* isolates collected in 2018 and 2019 were analyzed (Table 7). The samples were screened for the presence or absence of different spacer deletions that were previously detected in Kyrgyz strains using whole-genome sequencing in those two CRR using the PCR technique.

In comparison to isolates from 2018, were only the genotype At was found, it could be shown that a new genotype Aa is active inside Kyrgyzstan. Of all analyzed

samples from 2019, 13 isolates had the genotype Aa (Table 8). The only isolates from 2019 which could be genotyped as At-derived were IS-ZH-19 and JA-19-19. These results show that around the region Jalal-Abad two different *E. amylovora* genotypes can be found (Figure 3). The analysis of only the CRR 1 displayed a very homogenous picture. None of the samples from Kyrgyzstan showed spacer deletions typical for the genotype Z or D. All tested isolates from 2018 and 2019 were genotyped as A-derived.

The amplification of the whole CRR 1 showed similar fragment sizes in all tested isolates from 2019. The estimated sizes are between 2.8 and 3 kb. K29 was the only sample, which showed two separate fragments after gel electrophoresis. Two isolates, JA-Korzh-19 and JA-46-19, showed after several tries no PCR products. It is possible that the isolates are not *E. amylovora* strains. Therefore, the 16S-rRNA genes of both samples were amplified and sent in for sequencing to determine the species.

3.7. Pathogenicity test.

Pathogenicity is the ability to produce disease in a host organism. Microbes express their pathogenicity by means of their virulence, a term that refers to the degree of pathogenicity of the microbe. Hence, the determinants of virulence of a pathogen are any of its genetic or biochemical or structural features that enable it to produce disease in a host. As well as we checked the degree of isolated *E.amylovora* isolates' pathogenicity**,** using a simple method. The apple or pear immature fruits and leaves were inoculated with pure culture of *E.amylovora* isolates obtained from different host plants (Fig.4 and Fig.5). The relationship between a host and a pathogen is dynamic, since each modifies the activities and functions of the other. The outcome of such a relationship depends on the virulence of the pathogen and the relative degree of resistance or susceptibility of the host, due mainly to the effectiveness of the host defense mechanisms.

About 30% of all obtained isolates have expressed high virulence, which was manifested by the appearance of ooze exudate on the surface of unripe fruits of pear and apple trees in short

time or 48 h. 60% of isolates have shown a moderate degree of pathogenicity, which was expressed in a later appearance exudate than highly virulent isolates, in 72 h and later.

Discussion

In this study, we conducted a targeted search and detection of fire blight outbreaks in the heart of wild fruit and nut forests in farms and orchards around the natural forest. Using various methods, starting with simple immunostrip assays, the classical and molecular technique approaches were able to detect the causative agent of bacterial fire blight *E. amylovora* bacteria in 13 from 28 examined sites.

E. amylovora bacteria as pathogen have not been previously detected in such wild forms of apple and pear as *Malus niedzwetzkiana Dieck, Malus sievérsii,* and Pyrus *korshinskyi Litv,* which have been growing for centuries in these forests. This fact, on the one hand, calms us those wild local apple and pear varieties of are not susceptible to this bacteria, they show their natural resistance because this pathogen is an alien, invasive of other continents of the world. The process of co-evolution of these local wild varieties with the pathogen of a bacterial fire blight was impossible because the pathogen was introduced only in the last 10 years. On the other hand, our results proved the existence of a threat of a possible outbreak of this disease in the wild forests in the near future. Only 1 km from the heart (Oogan talaa village,GIPS : 41^0 15’ 53’’ N , 73^0 01’ 14’’ E, Elevation 1057 m, a.s.l) of wild forest ecosystems, a defeat of cultivated *P.communis* pear varieties with a bacterial blight was detected and *E. amylovora* was isolated and identified from them.

Of the 13 *E.amylovora bacteria* obtained in 2019, six isolates were from quince (*Cydonia spp)*; 3 isolates from cultivated apples (*M.domestica*); 2 isolates from cultivated pears (*P. communis);* and 2 isolates from hawthorn (*Crataegus turkestanica)*. Local quince varieties were the most sensitive to a bacterial blight compared to other pome fruit trees, a significant number of isolates were also isolated from introduced apple varieties. Entire arrays of semi-dwarf apple

trees and this disease affected pears, planting material of which were imported from Serbia and Kazakhstan, and they were frequent sources for isolation of *E. amylovora* bacteria.

Our results indicate that economic trade is the main path of the spread of this disease through planting material imported from the European continent, via through Russia or Kazakhstan. In farms and orchards engaged in the cultivation of newly introduced varieties of apple and pear, this disease thanks to quick contagiousness spreads and expands its area, and almost next to the natural forests with endangered and rare pome species.

Further, the obtained new local bacterial isolates were subjected to molecular analyses to identify specific genes characteristic of these bacteria. For this, genes were amplified stepwise using the developed specific primers (Ea CR1 – F1/Ea CR1-sp 18R and PEANT-1/PEANT-2). Stretches of clustered regularly inter spaced short palindromic repeat sequences, termed CRISPR arrays, have been identified in approximately 85% of archaeal and about 50% of eubacterial genomes analyzed [Shariat and Dudley, 2014].The arrays consist of a variable number of direct repeat (DR) elements with neighboring DRs being separated by spacer sequences. The spacers are thought to constitute the molecular memory of a bacterial adaptive immune system targeting invasive DNAs as of bacteriophages or plasmids. The directionality of spacer acquisition in CRISPR arrays has been exploited in evolutionary and subtyping studies of several bacteria (Shariat and Dudley, 2014), including in *E. amylovora* [Rezzonico et al., 2011; McGhee and Sundin, 2012; Forster et al, 2015; Tancos and Cox]. The genome of *E. amylovora* typically contains three CRISPR arrays, named CRR1, CRR2, and CRR4, with spacer elements comprising, with few exceptions, 32 bp in length (Rezzonico et al., 2011). With respect to the spacer organization within these CRR loci, several CRISPR array “patterns” (McGhee and Sundin, 2012) or *E. amylovora* “genotypes“ (Rezzonico et al., 2011) have been distinguished.

This approach provided a comparative characterization of Kyrgyz strains with the previous strains isolated in 2018 in the Northern part of the country, also with *E. amylovora* bacteria isolated from other countries of the world. Complete CRR1 and CRR2 arrays from 23

E.amylovora isolates from Kyrgyzstan indicated that to be identical in spacer organization for these isolates. More exactly, the organization of CRISPR-1 arrays was identical to CRISPR-1 pattern 4 (McGhee and Sundin, 2012) or CRR1 genotype "A" (Rezzonico et al., 2011) as previously described for *E. amylovora* isolates from Europe and the Middle East. None of isolated *E. amylovora* bacteria showed spacer deletions typical for the genotype Z or D. All tested isolates from 2018 and 2019 were genotyped as A-derived. In analyzed Kyrgyz *E.amylovora* strains, the mutated position is generally conserved (5´taaatggttgtccgttcttggcgcaGacggct) in CRR1 elements were not found, that usually across the European and Middle Eastern *E. amylovora* strains. Taken together, the spacer organization of the CRISPR-2 arrays sequenced was found to be unique to the Kyrgyz isolates. Among the strains isolated in 2019, two strains (IS -ZH-19; JA-19-19) showed the "t" genotype on CRISPR-2 array , the rest strain "a" genotype, while the strains from 2018 isolated from northern Kyrgyzstan on CRISPR-2 array had the "t" genotype.

As our studies [Doolotkeldieva et al, 2019] have shown, that moreover, CRISPR-1 and CRISPR-2 array organization and nucleotide sequences were most closely related to repeat regions reported from *E. amylovora* strains isolated from across Europe (UK, France, Spain, Germany, Serbia-Montenegro, Belarus) as well as from the Middle East (Lebanon, Israel) and NewZealand, where as more distantly related CRISPR-2 genotypes and patterns were almost exclusively found in North American and East Asian *E. amylovora* strains.

Thus, as shown in the maps (Fig. 6), foci of fire blight were found around natural forests at 13 sites. We consider the closest and most threatening outbreak detected in the site (Oogan talaa village, GIPS : 41^0 15' 53'' N , 73^0 01' 14'' E, Elevation 1057 m) , where the outbreak is only 1 km from the center of wild forests. Our only hope is that the natural resistance of wild forms of apples and pears, representing a unique biodiversity and gene pool for the conservation of endangered species, could protect them from an invasion of a pathogen. In our cases, the samples from wild pear *P. korshinskyi Litv* (Kisil Unkur forestry, Chon Ak-kulak village, Garden

of pioneer camp) according to the external symptoms had very characteristic for bacterial blight symptoms, even primary immune-strip screenings showed a positive result. However, sequential PCR analyzes proved that these bacteria were from the genus Pseudomonas. However, all trees were old, sick with other diseases, and have attacks of an abiotic and biotic nature. Therefore, we consider the threat of developing a bacterial fire blight and a possible attack on natural varieties in the near future not excluded. Therefore, we believe the threat of fire blight development and a possible attack on the natural variety has not been ruled out in the near future.

Despite the fact that obtaining resistant varieties of apple trees to bacterial fire blight requires a long period, wild apples remain the main source of resistance. This has been proven in the reports [Peil et al., 2007; Peil et al., 2008; Emeriewen et al., 2014; Durel et al., 2009]. In the next generation, 20-25% of the received seedlings were free from this disease when using such wild apple trees as *Malus × robusta 5 (MR-5), Malus fusca, Malus floribunda* 821 and the ornamental cultivar 'Evereste'. Wild species offer a range of fire blight resistance loci that can be introgressed into a commercial apple genetic background. Currently, two approaches are followed at Agroscope in Wädenswil and in some other breeding programs, to shorten the long juvenile period in apple (usually 4 to 5 years): 'fast track breeding' and 'early flowering' [Kellerhals et al., 2017].

Therefore, the conservation of wild forms of apple and pear from invasion of bacterial fire blight remains a priority in theoretical and practical terms. To preserve the natural species of apples and pears and other types of pome fruits susceptible to this bacterium, it is necessary to look for alternative measures to chemicals, among them the search for natural antagonists of this bacterium that suppress its growth and development in natural conditions. Such studies have begun, and preliminary encouraging results have been found on effective natural antagonists to suppress the pathogen spread among the wild genetic pool.

Acknowledgments

The authors gratefully acknowledge receipt of financial support from the Swiss National Science Foundation (SNSF) for the research project "Preservation of Central Asian fruit tree forest ecosystems, pome fruit varieties and germplasm from the recent epidemics caused by the invasive bacterial pathogen *Erwinia amylovora (fire blight)"* (referred to as "Project No. IZ08Z0_177515/1).

Funding

This study was funded by the Swiss National Science Foundation (SNSF) (GRANT NUMBER IZ08Z0_177515/1).

Conflict of interest

Each of the authors, namely Tinatin Doolotkeldieva, Saikal Bobushova, Simon Carnal, Fabio Rezzonico declares that he/she has no conflict of interest.

References

Acimovic S.G., Zeng Q., McGhee G.C., Sundin G. W., & Wise J.C.,2015. Control of fire blight (Erwinia amylovora) on apple trees with trunk-injected plant resistance inducers and antibiotics and assessment of induction of pathogenesis related protein genes. *Frontiers in Plant Science* **6:** 16.

Adeolu M., Alnajar S., Naushad S., & Gupta R., 2016. Genome-based phylogeny and taxonomy of the 'Enterobacteriales': Proposal for Enterobacterales Ord. Nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. Nov., Pectobacteriaceae fam. Nov., Yersiniaceae fam. Nov., Hafniaceae fam. Nov., Morganellaceae fam. Nov., and Budviciaceae fam. Nov. International *Journal of Systematic and Evolutionary Microbiology* **66**: 5575–5599. https://doi.org/10.1099 /ijsem.0.001485.

Agrios G.N., 2005. Plant diseases caused by prokaryotes: bacteria and mollicutes. *In Plant Pathology* pp. 615–623. Fifth edition, Elsevier Academic Press.

Bradbury J. F.,1986. Guide to plant pathogenic bacteria pp. 332.

Dootkulova G.M., Soldatov I.V., Chakaev J. Sh., 2017. Stability of apple introducers to bacterial burn in the conditions of the Gareev Botanical Garden of the Natl. Acad. Sci. of

Kyrgyzstan. *Proceedings of the International Scientific Conference: Modern Conservation of the Biodiversity of the Plant World, Bishkek,* 05.10.2017: 85–91 (in Russian).

Doolotkeldieva T., Bobushova S., 2016. Fire blight disease caused by Erwinia amylovora on Rosaceae plants in Kyrgyzstan and biological agents to control this disease. *Advances in Microbiology* **6**: 831 –851.

Doolotkeldieva T., Bobushova S., Schuster Ch., Konurbaeva M., Leclerque A., 2019. Isolation and genetic characterization of *Erwinia amylovora* bacteria from Kyrgyzstan. *Eur J Plant Pathol.* https://doi.org/10.1007/s10658-019-01790-3.

Durel C.E., Denancé C., Brisset M.N., 2009. Two distinct major QTL for resistance to fire blight colocalize on linkage group 12 in apple genotypes 'Evereste' and Malus floribunda clone 821. *Genome* **52:** 1-9.

Emeriewen O., Richter K., Kilian A., Zini E., Hanke M.V., Malnoy M., Peil A., 2014. Identification of a major quantitative trait locus for resistance to fire blight in the wild apple species Malus fusca. *Molecular Breeding* 34: 407-419.

Forster, H., McGhee, G.C., Sundin, G.W., Adaskaveg, J.E. 2015. Characterization of streptomycin resistance in isolates of *Erwinia amylovora* in California. Phytopathology 105:1302-1310.

Kellerhals M., Schütz S., A. Patocchi A., 2017. BREEDING FOR HOST RESISTANCE TO FIRE BLIGHT. *Journal of Plant Pathology*, 99 (Special issue): 37-43.

Llop P., Caruso P., Cubero J., Morente C, López M., 1999. A Simple Extraction Procedure for Efficient Routine Detection of Pathogenic Bacteria in Plant Material by Polymerase Chain Reaction. *J Microbiol Methods* 1:23-31. doi: 10.1016/s0167-7012(99)00033-0.

Llop P., Bonaterra A., Peñalver J., López M. M., 2000. Development of a highly sensitive nested-PCR procedure using a single closed tube for detection of *Erwinia amylovora* in asymptomatic plant material. *Applied and Environmental Microbiology* 66: 2071 –2078.

Mayhew B., 2007. Central Asia: Kazakhstan, Tajikistan, Uzbekistan, Kyrgyzstan, Turkmenistan. *Lonely Planet* pp. 330–331. ISBN 978-1-74104-614-4.

McGhee G.C., Sundin, G. W., 2012. *Erwinia amylovora* CRISPR elements provide new tools for evaluating strain diversity and for microbial source tracking. PLoS One, 7, e41706. https://doi.org/10.1371/journal.pone.0041706.

Miller T. D., Schroth M. N., 1972. Monitoring the epiphytic population of *Erwinia amylovora* on pear with a selective medium. *Phytopathology* 62: 1175–1182.

Mitchell L., 2008. The Bradt Travel Guide: Kyrgyzstan. *Bradt Travel Guides* pp. 233-237. ISBN 978-1-84162-221-7.

Peil A., Hanke M.V., Flachowsky H., Richter K., Garcia-Libreros T., Celton J.M., Gardiner S., Horner M., Bus V., 2008. Confirmation of the fire blight QTL of Malus× robusta 5 on linkage group 3. *Acta Horticulturae* 793: 297-303.

Peil A., Garcia-Libreros T., Richter K., Trognitz F.C., Trognitz B., Hanke M.V., Flachowsky H., 2007. Strong evidence or a fire blight resistance gene of Malus robusta located on link- age group 3. *Plant Breeding* 126: 470-475.

Powney R., Beer S. V., Plummer K., Luck J., Rodoni B., 2011. The specificity of PCR-based protocols for detection of *Erwinia amylovora*. *Australasian Plant Pathology* 40: 87–97.

Rezzonico F., Smits T. H., Duffy B., 2011. Diversity, evolution ,and functionality of clustered regularly inter spaced short palindromic repeat (CRISPR) regions in the fire blight

pathogen *Erwinia amylovora. Applied and Environmental Microbiology* 77:3819–3829. https://doi.org/10.1128 /AEM.00177-11.

Rousseau C., Gonnet M., LeRomancer M., Nicolas J., 2009.CRISPI: ACRISPR interactive database. *Bioinformatics* 25:3317–3318. https://doi.org/10.1093/ bioinformatics /btp586.

Sedlak J., Paprstein FF., Korba J., Sillerova J., 2015. Development of a System for Testing Apple Resistance to *Erwinia amylovora* using In Vitro Culture Techniques. *Plant Protection Science* 51(1):1-1; DOI: 10.17221/94/2013-PPS

Shariat N., Dudley E. G., 2014. CRISPRs: Molecular signatures used for pathogen subtyping. *Applied and Environmental Microbiology* 80: 430 –439. https://doi. org/10.1128/AEM.02790-13.

Slack S. M., Zeng Q., Outwater C.A., Sundin G. W., 2017. Microbiological examination of *Erwinia amylovora* exopolysaccharide ooze. *Phytopathology* 107: 403 –411. https://doi.org/10.1094/PHYTO-09-16-0352-R.

Stöger A., Schaffer J., Ruppitsch W., 2006. A rapid and sensitive method for direct detection of Erwinia amylovora in symptomatic and asymptomatic plant tissues by polymerase chain reaction. *Journal of Phytopathology* 154: 469–473.

Tancos, K.A., K.D. Cox. 2016. Exploring diversity and origins of streptomycin-resistant *Erwinia amylovora* isolates in New York through CRISPR spacer arrays. Plant Dis. 100:1307-1313.

Taylor R. K., Guilford P., Clark R. G., Hal C. N., Forster R. L. S.,2001. Detection of Erwinia amylovora in plant material using novel polymerase chain reaction (PCR) primers. *New Zealand Journal of Crop and Horticultural Science* 29: 35–43.

Thompson S., 2000. Epidemiology of fire blight. In: Fire blight, the Disease and its Causative Agent, *Erwinia amylovora (*Ed. Vanneste, J). *CAB International, Wallingford (GB).*

Van der Zwet T., Beer S. V., 1995. Fire blight – its nature, prevention and control. A Practical Guide to Integrated Disease Management. *USDA Agricultural Information Bulletin* No, 631, Washington (US).

Vlˇckova L., Usupbaev A., Shigaeva J., Dietrich D., Sulaiman N., Verner V., Polesny Z., 2019. Ethnobotany of Wild Plants and Crop Wild Relatives in the Walnut-Fruit Forests of Kyrgyzstan. "*Filling gaps and removing traps for sustainable resource management"Conference Paper. Tropentag, September 18-20, 2019, Kassel.*

Table 1: Used primers, nucleotide sequence and their melting point Tm

Name	Target region	Sequence	Genotypes discriminated
C1f01	CRR1, complete region	5’ - TG AGT AGC AAA TCC GTG CGT GCT - 3’	Total CCR1 length
C1r01	CRR1, complete region	5’ - AA TCA GTC CCC CCA TGC TGT GAC - 3’	Total CCR1 length
C1f04	CRR1, Spacer 1030	5’ - CGATCAACCTGTTTTTCAGTAGGT - 3’	A/D
C1r09	CRR1, Spacer 1027	5’ - CCGCCGAGACAACCGGCTATCC - 3’	A/D
C1f03	CRR1, Spacer 1023	5’ - GAGACTTAAAGATCGTCTGCTAGT - 3’	A/Z
C1r11	CRR1, Spacer 1002	5’ - ATGCCCTCACCGTTGTGTGTG - 3’	A/Z
C2f03	CRR2, Spacer 2020	5’ - GATGGTGGCGCTGGTTGCGCTGGC - 3’	a/t
C2r02	CRR2, Spacer 2010	5’ - CTGAGTCTGGAATGTACACACTGG - 3’	a/t

Table 2: PCR Reaction Mix for 10µL reaction volumes

Substance	Stock	Volume [µL]	Endconc.
ReadyMix™ Taq PCR Reaction Mix	2x	5	**1x**
Primer Forward	10 µM	0.5	**0.5 µM**
Primer Reverse	10 µM	0.5	**0.5 µM**
ddH2O	-	3,5	**-**
Lysate DNA	**n.A.**	**0,5**	**n.A**

Table 3: Thermo cycler running protocol

Step	Temp. [°C]	Time	Cycle
Denaturation	94	1 min	**1x**
Denaturation	94	30 s	**5x**
Elongation	65	1 min	
Denaturation	94	30 s	
Annealing	62	30 s	**30x**
Elongation	65	1 min	
Elongation	65	5 min	**1x**
End	**12**	∞	∞

Table 4**.** The list of sites and taken plant samples during the expeditions in Jalal -Abad region (May 2019).

# samlpes	Place, Location, GIPS coordination	Plant species	Symptoms	Immuno-strip assays results
1	Jalal- Abad region, Nooken Dictrict, Shaidan village, Fruit tree nursery GIPS : 41^0 04’ 06’’ N 72^0 42’ 20’’ E, Elevation 755 m, a.s.l,	*Cydonia* spp. *(*quince tree), 35 years ago were planted,	For last years, the trees were affected in the blossom time. Drops of exudate were visible from the affected fruiting shoots	Positive
2	Jalal- Abad region, Nooken Dictrict, Shaidan village, Fruit tree nursery GIPS : 41^0 04’ 06’’ N 72^0 42’ 20’’ E, Elevation 755 m, a.s.l,	*Crataegus turkestanica* A. Pojark - Turkestan hawthorn tree	The most symptoms on leaves.	Positive
3	Jalal- Abad region, Nooken Dictrict , Birdik village, Ak Jol place GIPS : 41^0 03’ 86’’ N 72^0 40’ 84’’ E, Elevation 735 m, a.s.l,	*Crataegus pontica C.* Koch - Pontic hawthorn	The leaves were affected by other fungal diseases.	No positive
4	Jalal- Abad region, Nooken Dictrict, Birdik village, Ak Jol place, Private garden of Booronbay GIPS : 41^0 06’ 60’’ N 72^0 41’ 99’’ E, Elevation 870 m, a.s.l,	*P. communis* (pear), “Granit” variety. 2-3 ago were planted	The most symptoms on leaves and branches.	No positive
5	Jalal- Abad region, Nooken Dictrict, Birdik village, Ak Jol place, Private ocrhade of Ashimov Kushnazar GIPS : 410 06’ 67’’ N 720 41’ 87’’ E, Elevation 870 m, a.s.l,	Cydonia spp. (quince tree), 3-4 years ago were planted,	For last years, the trees were affected in the blossom time. Drops of exudate were visible from the affected fruiting shoots	Positive

6	Jalal- Abad region, Nooken Dictrict, Tockool-Ata forestry Ermendi gorge GIPS : 41^0 13’ 23’’ N 72^0 40’ 45’’ E, Elevation 1274 m, a.s.l,	1. Local variety of pear *Purus korshinskyi* Litv. about 70 years old, 2. Crataegus turkestanica A.	Tall tree, the leaves were damaged severely by fire blight and other pathogen’s symptoms	Positive from samples of *Crataegus turkestanica A.*
7	Jalal- Abad region, Nooken Dictrict, Tockool-Ata forestry Ermendi gorge GIPS : 41^0 12’ 52’’ N 72^0 41’ 36’’ E, Elevation 1292 m, a.s.l,orge,	*M. domestica,* (apple)local and introduced varieties of apple (Limon, Jonathan, Rosemary), which were planted 50 years ago, during the Soviet Union time	They were not affected by fire blight although only 50 meters of local pears were affected by this disease. Apple tree plantations are located a height on the slopes of small mountains. .	Negative
8	Aral village, private orchard GIPS : 40^0 59’ 48’’ N 72^0 38’ 26’’ E, Elevation 614 m, a.s.l,	*M. domestica,* (apple). Semi-dwarf apple varieties of Semerenko, 8 years of planting,	Samples of damaged fruits, leaves and branches were taken for analysis	Positive
9	Aral village, plantation 1.5 hectares of pear, privately owned, GIPS : 41^0 00’ 12’’ N 72^0 36’ 55’’ E, Elevation 614 m, a.s.l,	*P. communis,* Talgarka semi-dwarf pears. Seedlings were brought from Kazakhstan; perhaps the plant material was infected. 3 years of planting,	Almost every bush was severely affected by this disease.Samples were from leaves, shoots and fruits.	Positive
10	Basar- Korgon region, Kyzyl-ay village. Private orchard of Omaykan GIPS : 41^0 00’ 57’’ N 72^0 42’ 46’’ E, Elevation 658 m, a.s.l,orge,	*M. domestica,* Apple tree of 30 years old	The trees were affected by mix infection	Negative
11	Basar- Korgon region, Seydicum village, Aygir-Jol place. Private orchard. GIPS : 40^0 58’ 18’’ N 72^0 42’ 32’’ E,	*M. domestica,* (apple). Rainfed land without irrigation.	Apple tree of 7 years old, trees were without any symptoms of diseases.	Negative

	Elevation 668 m, a.s.l,orge,			
12	Basar- Korgon region, Seydicum village, Xadgi Rabat place, Private orchade, GIPS : 40^0 56’ 44’’ N 72^0 37’ 30’’ E, Elevation 587 m, a.s.l,orge,,	1. Cydonia spp. (quince tree), local variety, 7 years old. 2. *M. domestica,* (apple), Local variety Tash -Alma. 4 years old.	Symptoms of diseases are appeared this year on leaves, on flowers	Negative
13	Basar- Korgon region, Gorge of Ken- Koo adir. GIPS : 41^0 06’ 17’’ N 72^0 45’ 18’’ E, Elevation 823 m, a.s.l,	*Crataegus turkestanica* A.	Affected the flowers -40-45%, the leaves about 5-10%/	Negative
14	Basar- Korgon region, Kenesh village, Attokurov street, Japarkul, 68. GIPS : 41^0 04’ 14’’ N 72^0 19’ 23’’ E, Elevation 763 m, a.s.l,orge,	Cydonia spp. (quince tree), local variety Private ochard,	With affected leaves about 40-50% by fire blight symptoms.	Positive
15	Suzak region,Topurak Bel passage GIPS : 40^0 55’ 31’’ N 72^0 52’ 20’’ E, Elevation 895 m, a.s.l,orge,	A garden of 6 hectares M. *domestica,* (apple). Aplle trees of 10-14 old.	There is mix infection was observed on the trees	Positive
16	Suzak region, Jalgis Jangak, Komsomol, GIPS : 41^0 04’ 41’’ N 73^0 10’ 43’’ E, Elevation 1096 m, a.s.l,	State orchard about 10 hectares, there are apple and Hawthorn trees, 40-50 years ago were planted.	No symptoms of diseases.Well -kept garden	Negative
17	Suzak region, Kara alma forestry GIPS : 41^0 04’ 41’’ N 73^0 10’ 43’’ E, Elevation 1096 m, a.s.l,	Natural plantings of apple, hawthorn trees.	No symptoms of diseases.	Negative
18	Galal- abad city, GIPS : 400 58’22’’ N 720 59’ 53’’ E, Elevation 819 m, a.s.l,	10-12 old ago planted private orchard, Cydonia spp. (quince tree), local variety	With affected leaves about 50-60 % fire blight symptoms. The dog rosé bush next without any symptoms.	Positive
19	Nooken district, Aral village GIPS : 400 59’13’’ N 720 37’ 23’’ E, Elevation 606 m, a.s.l,	Private orchard, 2 years ago was planted. M. domestica, (apple). Semi-dwarf	With affected leaves, branches about 50-60 % fire blight symptoms.	Positive

		apple Ayderek varieties. Planting material from SERBIY, BOSSIEVIENI		
20	Nooken district, Tomonku Aral village GIPS : 40^0 59’57’’ N 72^0 35’ 21’’ E, Elevation 601 m, a.s.l,	Private orchard Cydonia spp. (quince tree), local variety	Strongly affected leaves, flowers and branches by fire blight symptoms.	Positive
21	Nooken district, Kusul-Oktyabr village GIPS : 41^0 05’387’’ N 72^0 46’ 59’’ E, Elevation 805 m, a.s.l,	Private orchard Cydonia spp. (quince tree), local variety	Strongly affected leaves, flowers and branches by fire blight symptoms	Positive
22	Apsrlonbob -Ata forestry Yshelie Sak Mazar GIPS : 41^0 18’24’’ N 72^0 57’ 44’’ E, Elevation 1471 m, a.s.l,	1.Wild variety of *Malus sievérsii*, 2.Wild varieties of *Crataegus turkestanica,* 3.Wild varieties of barberry, mountain ash 4. Local old-born variety - Belfilio , stock- *Malus sievérsii,*	All taken samples from wild and hybrid varieties without symptoms of fire blight.	Negative
24	Apsrlonbob Ata forestry Yshelie Sak Mazar village, GIPS : 410 18’ 19’’ N 720 57’ 41’’ E, Elevation 1494 m, a.s.l,	Old tree of Wild variety of *Malus sievérsii*	We have found a mix infection symptoms of moniliosis, scab and others	Negative
25	Apsrlonbob Ata forestry, Kisil-Suu village, GIPS : 41^0 18’ 05’’ N 72^0 59’ 11’’ E, Elevation 1197 m, a.s.l	Private orchard, Kuliev street, 18. 70 old of *P. communis* pear tree, Winter variety	The tree strongly affected by mix infection (scab and others)	Negative
26	Apsrlonbob Ata forestry,Oogan talaa village,GIPS : 41^0 15’ 53’’ N 73^0 01’ 14’’ E, Elevation 1057 m, a.s.l	Private orchard, *P. communis* pear tree.	The tree strongly affected by fire blight symptoms. This place only 1 km from wild Apsrlonbob foresty. .	Positive

27	Kisil Unkur forestry, Chon ak-kulak village, Garden of pioneer camp GIPS : 41^0 21 ' 02'' N 73^0 04' 13'' E, Elevation 1260 m, a.s.l	Young bushes of wild forms of Pear trees *(P. communis*) and dog roses (Rosa canina)	Fire blight symptoms have found on pear trees, but no symptoms on nearer growing dog roses	Positiive
28	Achi forestry, Kok alma place,GIPS : 41^0 17 ' 48'' N 73^0 04' 53'' E, Elevation 1107 m, a.s.l	1 Wild forms of Pear trees (P. communis), 2.Hawthorn (Crataegus turkestanica), 3.Dog roses *(Rosa canina) and* 4.Introduced forms of pear trees (P. communis)	No symptoms of fire blight	Negative

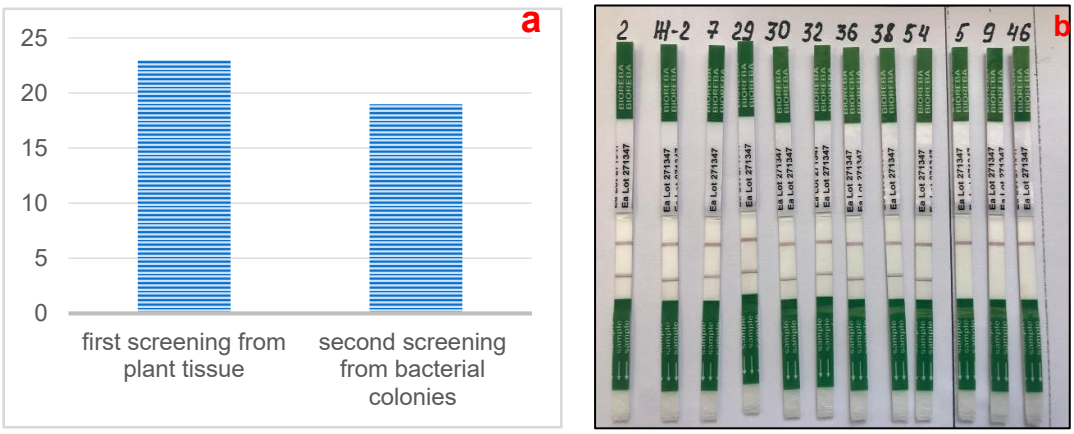

Fig.1.The results of screening of plant tissue and from colonies (a); (b) the picture shows the results after testing with a pure pathogen culture, where 2 red lines are positive

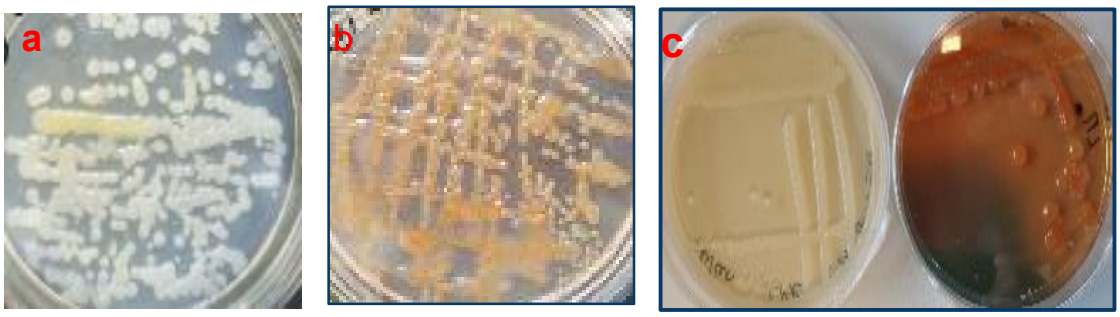

Fig. 2. The colonies of *E. amylovora* on Levan medium (a, b), and Miller- Schroth medium(c)

Table 5**.** Sequencing results with primer Ea CR1 – F1/Ea CR1-sp 18R

	Collection number of Erwinia amylovora isolates	Number of sequence	Host plant and place
1.	JA-30	4933	Winter variety of *P. communis* (pear), Oogan Talaa village, ,Apsrlonbob -Ata forestry, Private Orchard GIPS : 41^0 15’ 53’’ N 73^0 01’ 14’’ E, Elevation 1057 m, a.s.l
2.	JA- 32	4934	*Cydonia spp* (quince tree), Yshelie Sak Mazar village, Apsrlonbob -Ata forestry GIPS : 41^0 18’ 19’’ N 72^0 57’ 41’’ E, Elevation 1494 m, a.s.l,from Arslanbob
3.	JA-9	4935	*Cydonia spp* (quince tree), Nooken Dictrict, Shaidan village, Fruit tree nursery GIPS : 41^0 04’ 06’’ N 72^0 42’ 20’’ E,
4.	JA- 39	4936	*Crataegus turkestanica, (*Hawthorn tree), Basar- Korgon region, Kenesh village, Attokurov street, Japarkul, 68. GIPS : 41^0 04’ 14’’ N 72^0 19’ 23’’ E, Elevation 763 m, a.s.l.
5.	JA-29	4937	*M. domestica* (Semi-dwarf apple Ayderek varieties). Aral village, Private orchard, GIPS : 40^0 59’ 48’’ N 72^0 38’ 26’’ E, Elevation 614 m, a.s.l,
6.	JA-16	4938	*M. domestica (Apple tree)* Galal- abad city, GIPS : 40^0 58’22’’ N 72^0 59’ 53’’ E, Elevation 819 m, a.s.l,
7.	JA- 2	4939	*Crataegus turkestanica,* (Hawthorn tree),Nooken Dictrict, Tockool-Ata forestry GIPS : 410 13’ 23’’ N 720 40’ 45’’ E, Elevation 1274 m, a.s.l,
8.	JA-38	4940	Cydonia spp (quince tree), Nooken district, Tomonku aral village, GIPS : 400 59’57’’ N 720 35’ 21’’ E,

			Elevation 601 m, a.s.l,
9.	JA- H1a	4941	*Cydonia spp* (quince tree), Nooken Dictrict, Birdik village, Ak Jol place, Private ocrhade GIPS : 41^0 06' 67'' N, 72^0 41' 87'' E, Elevation 870 m, a.s.l,
10.	JA- 54	4943	*Cydonia spp* (quince tree), Quince Nooken district, Tomonku aral village GIPS : 40^0 59'57'' N 72^0 35' 21'' E, Elevation 601 m, a.s.l,
11.	JA- 6	4944	*Cydonia spp* (quince tree), Nooken district, Kusul- Oktyabr village, GIPS : 41^0 05'387'' N 72^0 46' 59'' E, Elevation 805 m, a.s.l,
12.	JA- 36	4945	*M. domestica (Apple tree),* Suzak region,Topurak Bel passage GIPS : 40^0 55' 31'' N 72^0 52' 20'' E, Elevation 895 m, a.s.l,orge,

Table 6. Sequencing results with primer PEANT-1/PEANT-2

#	Collection number of *Erwinia amylovora* isolates	Number of sequence	Host plant and place
1.	JA-30	4750	Winter variety of *P. communis* (pear), Oogan talaa village, Apsrlonbob -Ata forestry, Private Orchard GIPS : 41^0 15' 53'' N 73^0 01' 14'' E, Elevation 1057 m, a.s.l
2.	JA- 32	4752	*Cydonia spp* (quince tree), Yshelie Sak Mazar village, Apsrlonbob -Ata forestry.GIPS : 41^0 18' 19'' N 72^0 57' 41'' E, Elevation 1494 m, a.s.l,
3.	JA-9	4753	*Cydonia spp* (quince tree), Nooken Dictrict, Shaidan village, Fruit tree nursery GIPS: 41^0 04' 06'' N, 72^0 42' 20'' E.
4.	JA- 39	4754	*M. domestica* (Semi-dwarf apple Ayderek varieties). Aral village, private orchard GIPS : 40^0 59' 48'' N 72^0 38' 26'' E, Elevation 614 m, a.s.l,
5.	JA-29	4755	*M. domestica (Apple tree)* Galal- abad city, GIPS : 40^0 58'22'' N 72^0 59' 53'' E, Elevation 819 m, a.s.l,
6.	JA-16	4756	*Crataegus turkestanica,*

			(Hawthorn tree),Nooken Dictrict, Tockool-Ata forestry GIPS : 410 13’ 23’’ N 720 40’ 45’’ E, Elevation 1274 m, a.s.l,
7.	JA- 2	4757	Young bushes of wild forms of Pear trees (P. Purus korshinskyi Litv.) Kisil Unkur forestry, Chon ak-kulak village, Garden of pioneer camp GIPS : 410 21 ’ 02’’ N 730 04’ 13’’ E, Elevation 1260 m, a.s.l
8.	JA-38	4758	*Cydonia spp* (quince tree), Quince Nooken district, Tomonku Aral village,GIPS : 40^0 59’57’’ N 72^0 35’ 21’’ E, Elevation 601 m, a.s.l,
9.	JA- H1a	4759	*Cydonia spp* (quince tree), Nooken Dictrict, Birdik village, Ak Jol place, Private ocrhade GIPS : 41^0 06’ 67’’ N 72^0 41’ 87’’ E, Elevation 870 m, a.s.l,
10.	JA- 54	4761	*M. domestica (Apple tree),* Suzak region,Topurak Bel passage GIPS : 40^0 55’ 31’’ N 72^0 52’ 20’’ E, Elevation 895 m, a.s.l,orge,
11.	JA- 6	4762	*Cydonia spp* (quince tree), Nooken district, Kusul -Oktyabr village, GIPS : 41^0 05’387’’ N 72^0 46’ 59’’ E, Elevation 805 m, a.s.l,
12.	JA- 36	4763	*M. domestica (Apple tree),* Suzak region,Topurak Bel passage GIPS : 40^0 55’ 31’’ N ,72^0 52’ 20’’ E, Elevation 895 m, a.s.l,orge,

Table 7: Analysed E. amylovora samples from 2018 and 2019.

Isolate	Geographic Origin	Host	Year
JA-30-19	Oogan Talaa village, Arslanbob -Ata forestry, private orchard	*P. communis*	2019
JA-Korzh-19	Kisil Unkur forestry, Chon ak-kulak village, Garden of pioneer camp	*P. korshinskyi Litv.*	2019
JA-6-19	Nooken district, Kizil- Oktyabr village	*Cydonia spp*	2019
JA-32-19	Yshelie Sak Mazar village, Arslanbob -Ata forestry	*Cydonia spp*	2019
JA-38-19	Nooken district, Tomonku Aral village	*Cydonia spp*	2019
JA-2-19	Jalal- Abad region, Nooken Dictrict, Shaidan village, Fruit tree nursery	*Crataegus turkestanica*	2019
JA-16-19	Suzak region, Topurak Bel passage	*M.domestica*	2019
IS-ZH-19	Jeti Oguz	*Pyrus communis var. Talgarskaya Krasavitsa*	2019
JA-54-19	Nooken district,Tomonku Aral village	*Cydonia spp*	2019
JA-46-19	Kara alma plot, Achi forestry	*M. domestica*	2019

JA-19-19	Nooken district,Tomonku Aral village	*Pyrus communis*	2019
JA-39-19	Attokurov street, Japarkul, 68 Basar- Korgon region, Kenesh village,	*Crataegus turkestanica,*	2019
JA-28-19	Nooken district,Tomonku Aral village	*M. domestica*	2019
JA-9-19	Nooken Dictrict, Shaidan village, Fruit tree nursery	*Cydonia spp*	2019
JA-N1a-19	Nooken Dictrict, Birdik village, Ak Jol place, private orchade	*Cydonia spp*	2019
JA-36-19	Suzak region, Topurak bel passage	*M. domestica*	2019
JA-29-19	Aral village, private Orchade	*M. domestica*	2019
KTMU-15-18	Jeti Oguz	*Pyrus communis var. Talgarskaya Krasavitsa*	2018
KTMU-16-18	Jeti Oguz	*Pyrus communis var. Talgarskaya Krasavitsa*	2018
KTMU-17-18	Jeti Oguz	*Pyrus communis var. Talgarskaya Krasavitsa*	2018
KTMU-21-18	Saruu	*Pyrus communis var. Talgarskaya Krasavitsa*	2018
KTMU-24-18	Jeti Oguz	*Pyrus communis var. Talgarskaya Krasavitsa*	2018

Naming structure for isolates: First two letters are the place of isolation (JA= Jalal-Abad, IS= Issykul, KTMU= Samples already named). After that comes the internal number of each isolation year (30 = Sample 30 of 2019) and the last two numbers represent the year of isolation

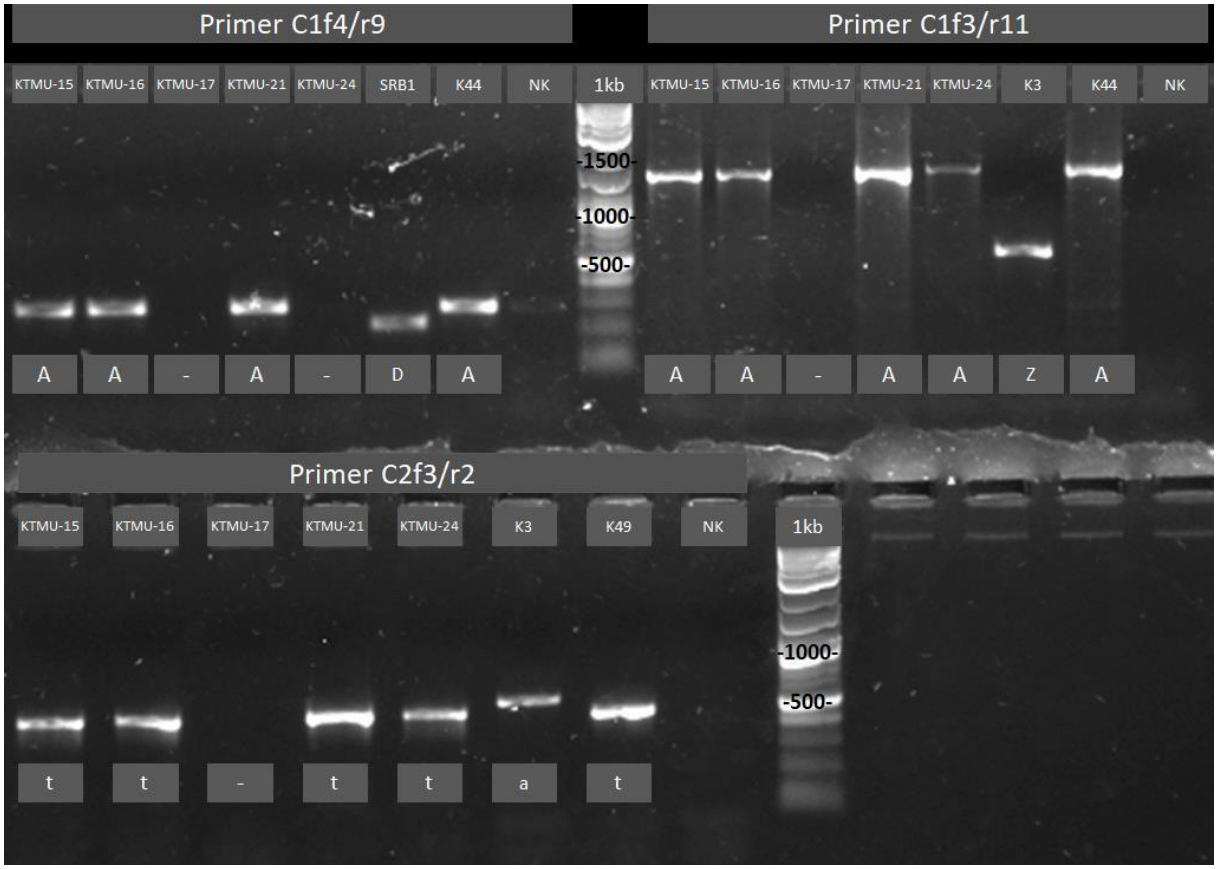

Figure 3: Products of CRR1 and CRR2 amplification from all analyzed KTMU-X-2018 samples.SRB01, K44, K49 and K3 were samples already analyzed in Switzerland and functioned as positive controls.

Table 8. Analyzed isolates and their genotypes. NA = No fragments for any genotype visible after amplification (isolate most likely not E. amylovora)

		Genotype derived - CRR1			Genotype derived - CRR2	
Isolate	**Year**	**A**	**D**	**Z**	**t**	**a**
JA-30-19	2019	A	-	-	-	a
JA-Korzh-19	2019	NA	NA	NA	NA	NA
JA-6-19	2019	A	-	-	-	a
JA-32-19	2019	A	-	-	-	a
JA-38-19	2019	A	-	-	-	a
JA-2-19	2019	A	-	-	-	a
JA-16-19	2019	A	-	-	-	a
IS-ZH-19	2019	A	-	-	t	-
JA-54-19	2019	A	-	-	-	a
JA-46-19	2019	NA	NA	NA	NA	NA
JA-19-19	2019	A	-	-	t	-
JA-39-19	2019	A	-	-	-	a
JA-28-19	2019	A	-	-	-	a
JA-9-19	2019	A	-	-	-	a
JA-N1a-19	2019	A	-	-	-	a
JA-36-19	2019	A	-	-	-	a
JA-29-19	2019	A	-	-	-	a
KTMU-15-18	2018	A	-	-	t	-
KTMU-16-18	2018	A	-	-	t	-
KTMU-17-18	2018	A	-	-	t	-
KTMU-21-18	2018	A	-	-	t	-
KTMU-24-18	2018	A	-	-	t	-

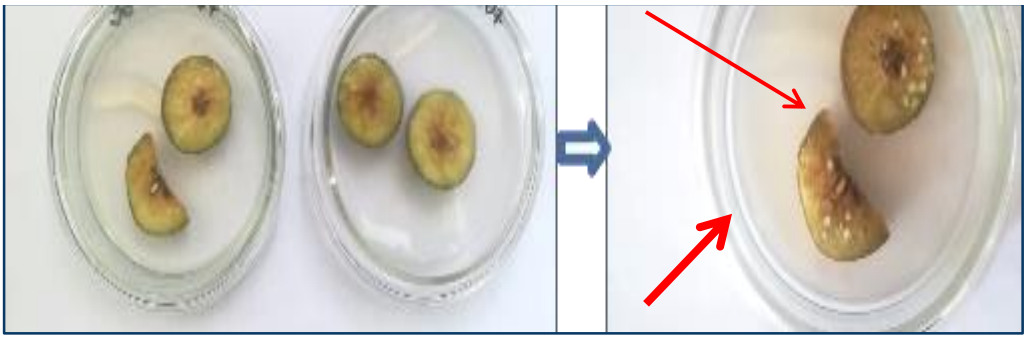

Fig 4. Isolated from apple trees (location : Suzak region,Topurak Bel place GIPS : 40^0 55’ 31’’ N 72^0 52’ 20’’ E, Elevation 895 m, E.amylovora isolates have caused a rot of fruit tissue with exudate formation

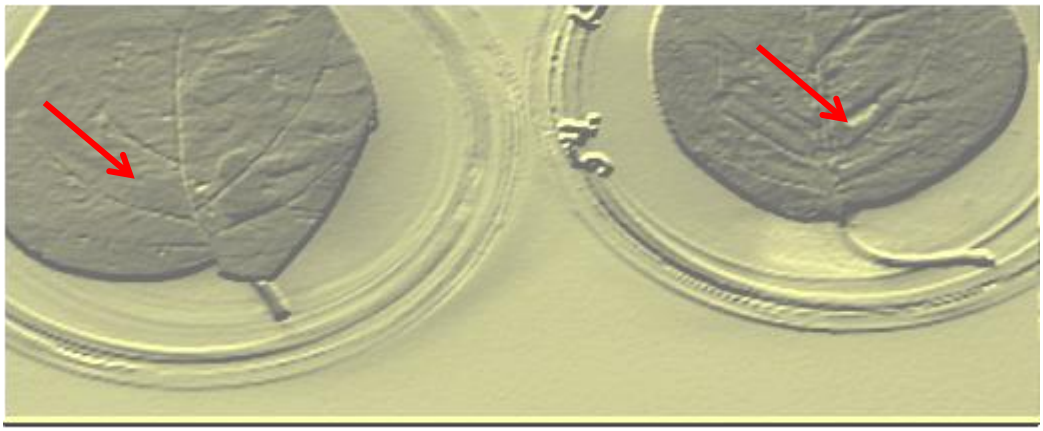

Fig.5. The examples emerging of necrosis on the surface of young pear leaves after artificial infection.

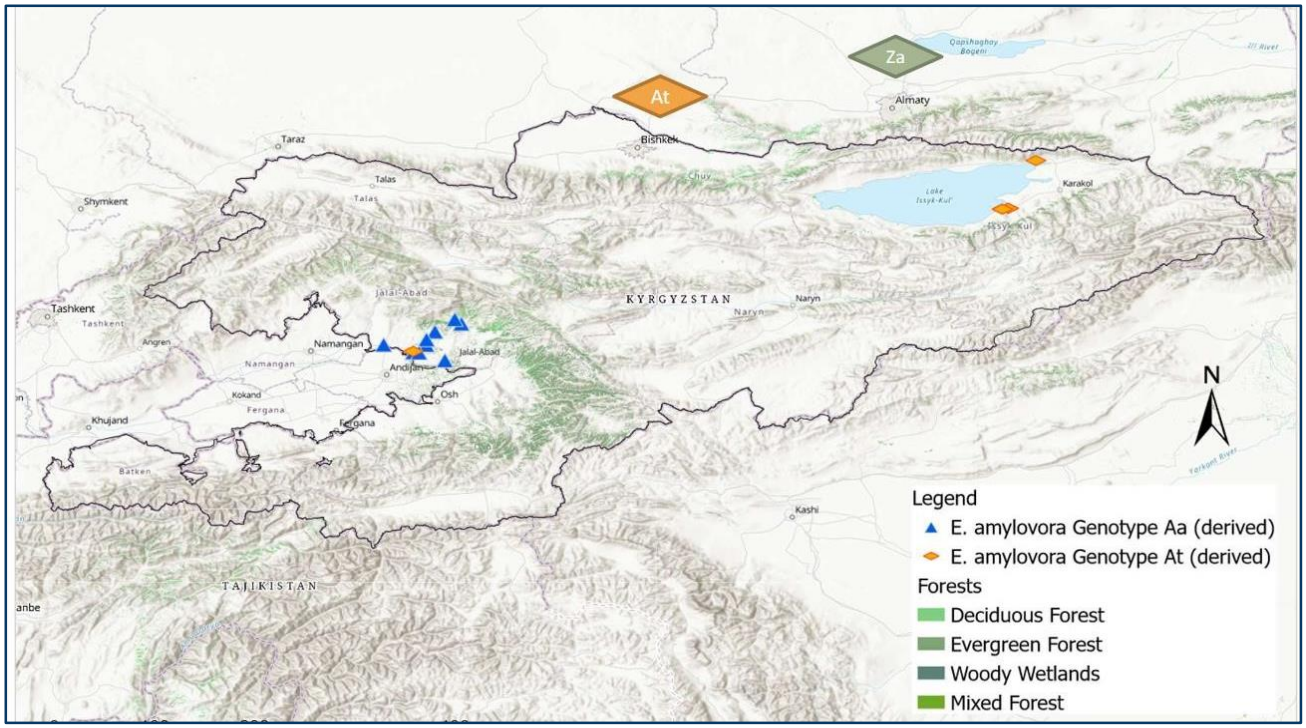

Fig.6. The map of Kyrgyzstan is indicating the sites (blue delta), where E.amylovora, as pathogen of fire blight were found in fruit and ornamental trees from *Rosaceae*