Annals of Agrarian Science 16 (2018) 12–16

Contents lists available at ScienceDirect

Annals of Agrarian Science

journal homepage: http://www.journals.elsevier.com/annals-of-agrarian-science

Fire blight in Georgia

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ARTICLE INFO

Article history:
Received 15 August 2017
Accepted 19 November 2017
Available online 5 February 2018

Keywords:
Fire blight
Erwinia amylovora
Conventional PCR
Real time PCR
DNA
Bacterium

ABSTRACT

Fire blight is distinguished among the fruit tree diseases by harmfulness. Fire blight damages about 180 cultural and wild plants belonging to the *Rosaceae* family. Quince, apple and pear are the most susceptible to the disease. At present, the disease occurs in over 40 countries of Europe and Asia. Economic damage caused by fire blight is expressed not only in crop losses, but also, it poses threat of eradication to entire fruit tree gardens. *Erwinia amylovora*, causative bacteria of fire blight in fruit trees, is included in the A2 list of quarantine organisms. In 2016, the employees of the Plant Pest Diagnostic Department of the Laboratory of the Georgian Ministry of Agriculture have detected *Erwinia amylovora* in apple seedlings from Mtskheta district. National Food Agency, Ministry of Agriculture of Georgia informed FAO on pathogen detection. The aim of the study is detection of the bacterium *Erwinia amylovora* by molecular method (PCR) in the samples of fruit trees, suspicious on fire blight collected in the regions of Eastern (Kvemo Kartli, Shida Kartli and Kakheti) and Western Georgia (Imereti).

The bacterium *Erwinia amylovora* was detected by real time and conventional PCR methods using specific primers and thus the fire blight disease confirmed in 23 samples of plant material from Shida Kartli (11 apples, 6 pear and 6 quince samples), in 5 samples from Kvemo Kartli (1 quince and 4 apple samples), in 2 samples of apples from Kakheti region and 1 sample of pear collected in Imereti (Zestafoni).

Introduction

Fire blight is distinguished among the fruit tree diseases by harmfulness. Differently from other bacterial diseases [1] fire blight damages wide spectra of plants, in particular, about 180 cultural and wild plants belonging to the *Rosaceae* family [2]. Apple, pear and quince are the most susceptible to the disease. The disease occurs rarely or weakly in cherry, plum, apricot, strawberry and hawthorn [3]. The disease was first observed in New York State, from which it spreads very rapidly throughout the North America. The loss caused by the disease in the United States consists in 100 million dollars annually [4]. In Washington DC and North Oregon, damages amounted to 68 million [5].

At present, the disease occurs in over 40 countries of Europe and Asia. According to the European Organization for International Plant Protection, in 1971–1972, 18,000 plants were destructed in Germany, and 175 thousand bushes – in Holland. In 1989, thousands of pear and quince trees were destructed in Armenia [3,6]. Fire blight has been reported in 11 regions of the European part of Russia. The first sporadic outbreaks of fire blight were seen in Slovenia in 2001 and 2002 [7,8]. Fire blight was discovered for the first time in Southwest Austria in 1993 on *Cotoneaster salicifolius* in Vorarlberg and later in the north of Austria [9]; it then spread to other regions. Recent outbreaks were detected in Carinthia (southeast Austria).

The symptoms of the disease are blackening/browning of buds, young shoots, leaves and flowers, the plant becomes as if burned by fire [10].

Fire blight is caused by the Gram-negative bacterium *Erwinia amylovora* and affects apple, pear, quince and other rosaceous plant. *Erwinia amylovora*, is included in the A2 list of quarantine organisms (EPPO 2007) by the European and Mediterranean Plant Protection International Organization (EPPO). It is also registered in the list of Australian [11] and South African quarantine organisms [12].

The source of spreading bacteria is transmission of milky

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Peer review under responsibility of Journal Annals of Agrarian Science.

https://doi.org/10.1016/j.aasci.2018.02.001

exudates from the damaged tissues of the plant by rain, wind, insects, and birds, including agricultural tools. The bacteria are distributed through agricultural techniques, vehicles, infected fruits [13]. High air humidity and temperature promote its distribution [14]. The bacterium penetrates into the plant through the stomata or injuries caused by insects and via other natural wounds [15]. The life cycle of the bacterium is not fully understood; it is known that it can survive as endophyte or epiphyte for variable periods depending on environmental factors [16].

Economic damage caused by fire blight is expressed not only in crop losses, but also, it poses threat of eradication to entire fruit tree garden [15].

The difficulty in fire blight control is conditioned by lack of preparations effecting directly on pathogen, and the resistance of the bacterium *Erwinia amylovora* to antibiotic streptomycin [17,18].

E. amylovora can be identified by several methods but PCR assays is reliable in most cases. Thus, the goal of our investigation was detection of *E. amylovora* and diagnostic of the fire blight in plant material by PCR assays.

Objectives and methods

The aim of the study is the detection of the bacterium *Erwinia amylovora*, the causal agent of fire blight in fruit trees, by using the molecular method (PCR) in the samples of fruit trees collected in the regions of Eastern Georgia and Western Georgia.

The samples have been provided by the National Food Agency, Ministry of Agriculture of Georgia. The samples were collected in the regions of Eastern Georgia (Kvemo Kartli, Shida Kartli and Kakheti) and Western Georgia (Imereti region). The samples of plant materials for the analysis have been represented by leaves, branches and fruits of apples, quinces and pears.

Based on the visual observation the samples were divided into two groups. The samples from symptomatic material have been combined in the first group, and the samples from the asymptomatic material - in the second.

Parts of the plant materials combined in the both groups (fruits, leaves, and branches) were cut into the small pieces, weighed and macerated by PBS buffer by the ratio 1:50, after 30 min of incubation at 25 °C.

For DNA extraction [19], one ml of macerate was centrifuged at 10 000 g for 5 min at room temperature. The supernatant was discarded and the pellet was resuspended in 500 µl extraction buffer (Tris-HCl pH 7.5, 24.2 g; NaCl, 14.6 g; ethylenediaminetetraacetic acid (EDTA), 9.3 g; sodium dodecyl sulphate (SDS), 5 g; PVP (20 g)/ 1 L distilled water sterilized by filtration) and incubated for 1 h at room temperature. After the lysis a deproteinization with chloroform:isoamyl alcohol (24:1) was conducted. DNA was precipitated by isopropanol, washed by ethanol and dissolved in TE buffer (10 mM Tris.HCl, 1 mM EDTA, pH 8.0). Agarose gels (0.8 or 1.2% in 1XTAE buffer) were used for resolving DNA according to the standard procedure described by Sambrook [20]. Real Time PCR and conventional PCR were used for detection *Erwinia amylovora* in plant material.

The Real-time PCR was carried out on the 7500 FAST Real Time PCR system. The specific primers Ams116F: 5′-TCC CAC ATA CTG TGA ATC ATC CA-3′, Ams189R: 5′-GGG TAT TTG CGC TAA TTT TAT TCG-3′, Ams141T of *Erwinia amylovora ams* region region is responsible for the synthesis of the main component of bacterial biofilm, polysaccharide amylovoran [21] and the probe FAM-CCA GAA TCT GGC CCG CGT ATA CCG-TAMRA, labeled by the fluorescent dye [22] were used for the analyses. The reaction was carried out in a final volume of 25 µl. The PCR mixture was composed: ultrapure water 2.5 µl; 2× TaqMan Fast Universal PCR Master Mix (Applied Biosystems) 12.5 µl; Ams116F (10 pmol/µl) 2.25 µl; Ams189R (10 pmol/µl) 2.25 µl; FAM-labelled Ams141T (pmol/µ)

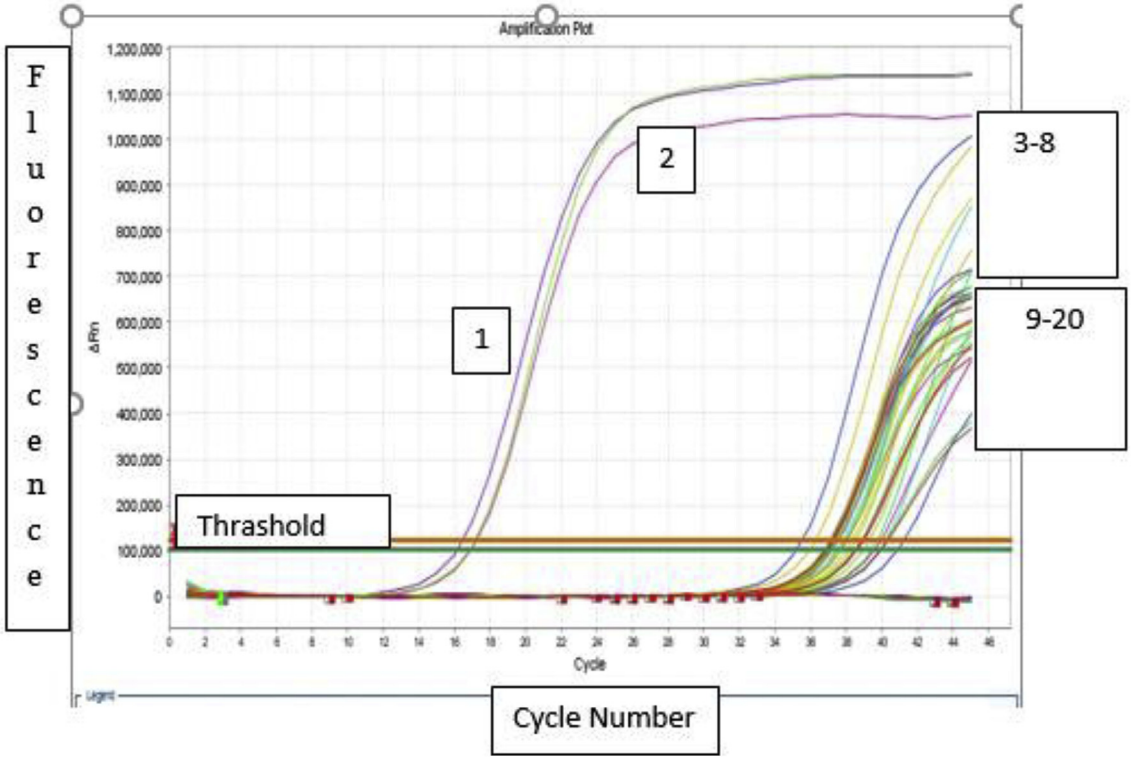

Fig. 1. Real Time PCR assays of 17 samples from Shida Kartli: 1 -Positive control NCPPB 683 (*Erwinia amylovora*); 2–Positive control Erwinia amylovora Georgian strain isolated in 2016; 3–8 - Samples of pears from Shida Kartli, 9–20 - Samples of apples from Shida Kartli.