XIII International Symposium on Agricultural Sciences, AgroRes 2024

PRESENT STATUS OF *ACIDOVORAX CITRULLI*- CAUSING BACTERIAL FRUIT BLOTCH OF WATERMELON FRUITS IN NORTH MACEDONIA

Emilija Arsov[1*], Saša Mitrev[1]

emilija.arsov@ugd.edu.mk

[1]*Goce Delcev University, Stip, Faculty of Agriculture, Deparment for Plant and Enviormental Proteticon, Stip 2000, North Macedonia*

AGRORES 2024

Introduction

Acidovorax citrulli **is the causal agent of Bacterial Fruit Blotch (BFB) of cucurbit plants. So far, *A. citrulli* has been added to the EPPO A1 List version 2023-09.**

Despite the economic importance of the disease, little is known about the basic aspects of *A. citrulli* pathogenesis. In the late spring months of 2020 to 2022, the appearance of fruit blotch symptoms on fully ripened watermelon crops (observation regions Kavadarci and Strumitsa), attributed to *A. citrulli* was documented. In our research, we conducted a comprehensive analysis utilizing various methods including observation of fruit symptoms, pathogenicity assessment on young watermelon fruit and seedlings, as well as biochemical tests, ELISA test and Real-time PCR analyses, all compared with positive controls (KBF 0520). As a result of this thorough examination done for the first time, we have conclusively identified the pathogen responsible for the observed symptoms as *A. citrulli*, the causal agent of BFB in watermelon fruits in the Republic of North Macedonia.

Because of the costly lawsuits and lack of efficient management, BFB represents a serious threat to the cucurbit industry. As this pathogen is on the EPPO A1 List, we already informed our Ministry of Agriculture, and all the farmers that grow watermelon, about *A. citrulli* that it is very important to implement eradication measures to prevent the further spread of this bacterium across the region.

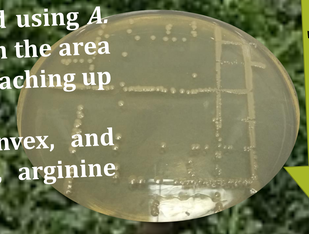
Fig. 1 Field symptoms on watermelon fruits

Material and methods

Samples from symptomatic watermelon fruits, should be preferably processed within 24–48 h to allow maximum recovery of the pathogen and to avoid heavy multiplication of saprophytes. The bacterium was isolated from fruit tissue and colonies were visible after a few days incubation at 27°C, such as wet mount formed smooth, round, small cream-coloured, non-fluorescent colonies on King's B medium with basic characteristics such as gram-negative, rod-shaped, aerobic, and oxidase-positive.

A total of 20 isolates were subjected to characterization through physiological, biochemical, and pathogenicity tests. For identification and comparison, PCR analyses were conducted using *A. citrulli*-specific primer pairs (Bulletin OEPP/EPPO Bulletin (2016) 46 (3), 444–462). In the area under investigation, it was determined that there were substantial economic losses, reaching up to 70%.

The colonies were gram-negative, cream-colored with smooth margins, and convex, and individual cells were rod-shaped. Isolates were oxidase positive, gram-negative, arginine dihydrolase negative and aerobic.



Conclusion

Because of the costly lawsuits filed by growers against seed companies and the lack of efficient management methods, BFB represents a serious threat to the cucurbit industry, primarily to watermelons and melons. Despite the economic importance of the disease, little is known about the basic aspects of *A. citrulli* pathogenesis. In our research, for the first time, on the basis of the fruit symptoms, pathogenicity on watermelon young fruit and seedlings, results of biochemical tests, ELISA test, and Real-time PCR analyses compared with positive controls, the pathogen was identified as *Acidovorax citrulli*.

Acidovorax citrulli **is biotrophic Gram negative, economically important seed-borne pathogen, showing typical symptom on watermelon and melon worldwide, with the ability to affect other cucurbits, such as cucumber, squash and pumpkin. Seed disinfestation treatments, seed health testing and chemical control in the field are limited in their ability to reduce the yield losses associated with BFB (Burdman and Walcott, 2012). Seeds represent the most important source of primary inoculum for BFB outbreaks.**

This confirms what has been observed in field, that expanded leaves and stems are the main inoculum sources for melon blossoms and fruit (Alves et al., 2010). Conventional and real-time PCR are used for identification our positive isolates. A single unique band of 450 bp was amplified for all isolates tested and compared with two positive controls: KFB 0250 (collection from A. Obradovic, University of Belgrade) and *A. avenae* subsp. *citrulii* No. 08154PC (commercial control from LOEWE). On the basis of the fruit symptoms, pathogenicity on watermelon young fruit and seedlings, results of biochemical tests, and PCR analyses using known positive controls, the pathogen was identified as *Acidovorax citrulli (Fig. 2 & Fig. 3)*.

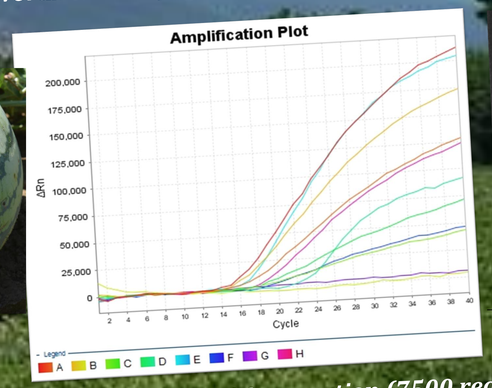


Fig. 2 Real-time PCR positive reaction (7500 real time PCR) **Taq-Man® technology**

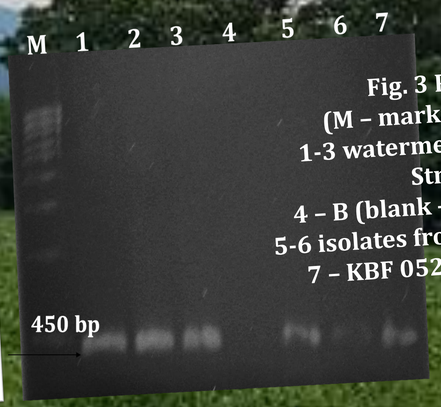


Fig. 3 PCR profile
(M – marker 1 Kb ladder;
1-3 watermelon isolates from Strumitsa;
4 – B (blank – negative control)
5-6 isolates from Sopot, Kavadarci;
7 – KBF 0520 positive control

Results and discussion

In the investigation field, from where watermelon fruits were collected, significant economic losses (up to 70%) were diagnosed. Late summer 2020 and 2022, *A. citrulli* on watermelon fruits, appear also in Strumitsa region in Eastern part of N. Macedonia. In recent years, the disease has spread to many parts of the world, mainly via the inadvertent distribution of contaminated commercial seeds. Because of the costly lawsuits filed by growers against seed companies and the lack of efficient management methods, BFB represents a serious threat to the cucurbit industry, and primarily to watermelons and melons.