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ACIDOVORAX CITRULLI- CAUSING BACTERIAL FRUIT BLOTCH OF WATERMELON FRUITS IN STRUMICA REGION

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Abstract

During August 2019/20, fruit blotch symptoms on mature watermelon fruits caused by *Acidovorax citrulli*, for the first time were observed in the village of Mokrievo, Strumica region, on the Bibo and Olakala watermelon varieties. *Acidovorax citrulli* is the causal agent of bacterial fruit blotch (BFB) of cucurbit plants. In recent years, the disease has been spread to many parts of the world, mainly via the inadvertent distribution of contaminated commercial seeds. So far, *A. citrulli* has already been reported in the Europe EPPO region, such as Turkey, Israel, Greece, Hungary, Italy, and Serbia, but in these reported countries, *A. citrulli* is under very careful control, and hygiene measures have already been taken. *A. citrulli* has been added to the EPPO A1 List version 2022-09 (https://www.eppo.int/ACTIVITIES/plant_quarantine/A1_list).

Material and methods

Fruit samples were collected from the field, washed with running water, and the bacterium was isolated from the margin of lesions from within the mesocarp, within 24-48 h to allow maximum recovery of the pathogen and to avoid multiplication of saprophytes. The solution of macerated tissue was plated onto King's B medium and non-fluorescent colonies were visible after a few days. 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.

Fig. 1 Symptoms on watermelon fruits

Fig. 2 Isolation A. citrulli on NaS media

Fig. 3 Artificial inoculation on young watermelon fruit

Fig. 4 Watermelon variety Bibo with BFB symptoms

Fig. 5 Olakala seedling production

Introduction

***Acidovorax citrulli* is the causal agent of bacterial seedling blight and fruit blotch of cucurbits (Webb and Goth, 1965; Schaad et al., 1988). It has become one of the most serious disease threatening watermelon production since its first outbreak in the late 1988's in United States (Wall and VM, 1988; 1990; Latin and Hopkins, 1995; Schaad et al., 2003).**

This disease has been reported as epidemic disease in the last 20 years, particularly on watermelon and on severe genotypes of melon (Zhang and Rhodes, 1990; Somodi et al., 1991; Burdman et al., 2005; Holeva et al., 2010, Popovic and Ivanovic, 2015; Zlatkovic et al., 2015).

***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).**

Results and discussion

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*.

						1 hour	2 hour			
		Measurement count: 1		Filter: 405		A. citrulli				
		1	2	3	4	1	2	3	4
OLAKALA	A	0,456	0,535	0,467	0,636	0,879	1,234	1,054	1,427
BIBO	B	0,234	0,241	0,247	0,361	0,422	0,491	0,493	0,828
KAORI	C	0,19	0,201	0,22	0,264	0,338	0,372	0,393	0,529
OLAKALA	D	0,226	0,202	0,211	0,267	0,513	0,365	0,377	0,569
BIBO	E	0,181	0,172	0,159	0,257	0,375	0,28	0,279	0,524
KAORI	F	0,193	0,181	0,186	0,291	0,387	0,316	0,341	0,624
NEGATIV CONT.	G	0,154	0,17			0,267	0,289		
POSITIV CONT.	H	0,276	0,428			0,633	0,958		

Fig. 6 ELISA test results – Bibo and Olakala watermelon fruit samples

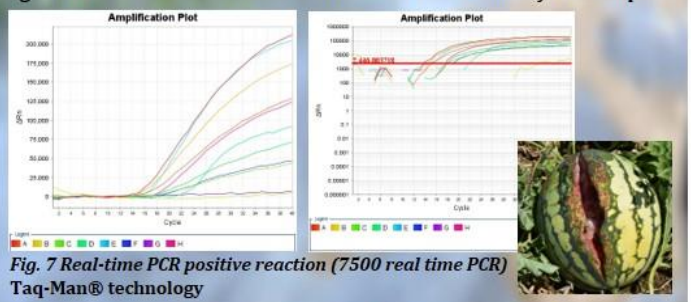

Fig. 7 Real-time PCR positive reaction (7500 real time PCR) Taq-Man® technology

Conclusion

In the investigation field, from where watermelon fruits were collected, significant economic losses (up to 70%) were diagnosed. Late summer 2019 and 2020, *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.