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STUDY OF QUARANTINE PLANT DISEASES IN GEORGIA

Our Organization has been conducting plant diseases monitoring and diagnostics of their causal agents. We found the following quarantine pathogens: Dickeya sp., Ralstonia solanacearum, Erwinia amylovora, Pseudomonas syringae pv. actinidiae, Synchytrium endobioticum. To fix diseases on time and study their causing pathogens, is fundamental for the country safety and makes it possible not to infect a huge territory.

plant quarantine, phytopathogenic microorganisms

The history of plant diseases is as old as agriculture itself. Plant diseases have great impact on the lives of human being and animals. Sometimes they effect the standard living for human communities and, consequently, affect the economy of an entire country. Big losses of crop yields were the results of severe outbreaks of plant diseases due to the introduction of pathogens from outside of the country. In recent years some of plant pathogens were introduced in Georgia through seed and planting materials. This is shortcoming of the system of plant quarantine in the country.

The scientists of Laboratory for Plant Disease Monitoring, Diagnostics and Molecular Biology are engaged in the identification and study of quarantine diseases of plants. The results of these research are present in this article.

In June of 2010 a wilt disease affecting tomato seedlings was reported by farmers in Chkhorotsku region, Western Georgia, causing up to 100% plant loss. Unconfirmed reports suggested that eggplant and sweet pepper plants on farms in the same region were also infected. Similar symptoms, indicative of bacterial wilt caused by *Ralstonia solanacearum* which included wilting and vascular discoloration were also observed in 2010 by farmers in Kutaisi region, and the disease was observed in tomato plants examined in

a plant health clinic in Kutaisi in July 2011. Initial diagnosis was carried out at our laboratory by amplifying a DNA extract obtained from infected material with primers OLI1 and Y2 (Seal *et al.*, 1993), which indicated the presence of *R. solanacearum*. The result of diagnostics was confirmed in FERA(UK), where in same disease samples *R. solanacearum* was identified by real-time PCR amplification as described by Weller *et al.*, [1]. Analysis of whole cell fatty acids was also undertaken [2] which identified the organism as *R. solanacearum* with a similarity index of 0.856. Our investigations have therefore confirmed that *R. solanacearum* is present and causing wilt disease of tomato in Georgia. This was the first report of bacterial wilt caused by *R. solanacearum* in Georgia [3]. Since 2010, bacterial wilt in tomato presumed to be caused by *R. solanacearum* has been an important disease in Georgia as this is a valuable crop for farmers in many regions across Georgia.

Since first report of tomato bacterial wilt, several cases of this disease have been documented on potato in home gardens in the Kobuleti region. During 2011–2013 a survey of potato plantations and storage facilities in different potato-producing regions of Georgia, samples were collected of tubers with brown rot symptoms and wilted potato leaves and stems. In 2012–2013, brown rot symptoms were detected in several commercial potato cultivars. i.e. Jelly, Marfona, Picasso, Finca, Agria, Alliance, Marabely in Khulo (west Georgia) and Akhaltsikhe (south Georgia). Bacteria isolated from wilted potato plants and rotten tubers produced typical pearly white, flat, irregular, fluidal colonies on CPG and fluidal, irregular in shape and milky white colonies with pink centers on SMSA media, respectively. These colonies were presumed to belong to *R. solanacearum*, thus their identity was sought and confirmed by specific PCR using the primer pair OLI/Y2 [4]. All tested isolates and positive control DNA extracted from *R. solanacearum* type strain NCPPB 325 produced the expected 288 bp product, confirming their identity as *Ralstonia solanacearum*. According to conducted research, this was the first report on the presence of potato brown rot in Georgia [5].

In the framework of PhD dissertation the distribution area of potato brown rot were determined. Namely, the disease was occurred in four geographical zones of Georgia: Samtskhe (Akhaltsikhe region), Javakheti (Akhalkalaki region), Shida Kartli and Colchis lowland (Khulo, Keda, Kobuleti regions). The highest intensity of distribution of potato brown rot was observed in the Samtskhe-Javakheti region comprised 63.6% and the lowest — in the Shida Kartli region (23%).

As a result of our research in 2014, *R. solanacearum* from the list of A 1 (quarantine objects not registered in the territory of the country) was transferred to the list A 2 (quarantine objects are limited in the territory of the country).

In accordance with PhD work races and biovars of *R. solanacearum*

population were identified. According to the Endoglucanase (Egl) sequencing the most strains isolated in potato industrial region in South Georgia belong to a most important single phylogenetic group of the race 3 biovar 2 (Phylotype IIB/Sequevar 1 (IIB-1) designation), which is supposed to be spread with the worldwide trade of seed potato [6].

It is obvious that Georgian isolates belongs to phylotype IIB and should be originated in South America. Common ancestor of phylotype II appeared on the American continent and divided into two sub-groups phylotype IIA and IIB. The Georgian isolates could be transferred from America to Europe by contaminated potato seeds. The comparison of genome of Georgian isolates to strain from phylotype II shows: i) besides high similarity of Georgian isolates to UY031 strain deficient of UY031 genes is found on chromosome and megaplasmid of Georgian isolates. ii) sequence similarity of some consecutive genes (that do not exist in UY031 strain) of Georgian isolates to strains CFBP2957, Po82, IBSBF1503 and UW163. Thus, Georgian isolates should be considered as new strains of *R. solanacearum* [7].

Because bacteriophages lytic to *R. solanacearum* are considered as a potential effective tool for prevention of infection spread in the environment and for treatment of seed plant material within of the project funded by the Georgian Science Foundation, studies were begun on the use of bacteriophages isolated from various substrates in combating brown rot of potatoes, the causative agent of which is *R. solanacearum* race 3 biovar 2 [8]. Twenty five individual Rs phages were isolated from different soil and water samples in Georgia. From them the mixture of 4 selected phages with overlapping spectrum was composed for *in vivo* challenge experiments.

In 2009, in Akhalkalaki region (southern Georgia, Samtskhe-Javakheti zone) has first been detected potato blackleg disease, the causing by bacterium *Dickeya* sp [9]. Fortunately, this disease has not been widely spread in Georgia.

Since its introduction in the mid 20th century, Kiwifruit (*Actinidia deliciosa* and *A. chinensis*) has been an important crop in Georgia. Several fungal diseases were found on the plant. In the fall of 2013, symptoms of a disease previously unrecognized in Georgia were observed on kiwifruit (*A. deliciosa* cv. Hayward) plants growing on a 30-ha plantation in the Lanchkhuti Municipality of western Georgia. Disease incidence was approximately 10%. Seven bacterial cultures isolated from leaves and stems of symptomatic plants on King's B medium were identified as *Pseudomonas syringae* pv. *actinidiae* [10]. To identify *P. syringae* pv. *actinidiae* the pathogenicity tests using Kokh's postulates and PCR with primer pairs KN-F/KN-R [11] and Psaf1/PsaR2 [12] specific for 16S-23S rDNA ITS region was performed. The obtained amplicons were 492 bp and 280 bp in size, respectively. *P. syringae* pv. *actinidiae* reference strain NCPPB 3738 was used as a positive control for the PCR. This study confirmed for the first time the

presence of *P. syringae* pv. *actinidiae* associated with kiwifruit bacterial canker in Georgia. Fortunately, the disease has not been progressed in Georgia [13].

In 2012 during investigation of the apple-tree saplings imported from Turkey the fire blight caused by *Erwinia amylovora* was first detected by field express methods. In 2016 the fire blight was registered already in private gardens. Late the presence of fire blight was confirmed by specific PCR (unpublished data). At the moment, the pathogen is isolated from *Malus domestica*, *Cydonia oblonga*, *Prunus domestica*, *Prunus armeniaca*. Studies are under way.

Potato wart disease (PWD), are considered to be major constraints to potato production. PWD has spread throughout the world through the movement of warted tubers. The National Agency of Food Safety of Georgia lists *Synchytrium endobioticum* as an A2 quarantine pest. Observations of potato plantations undertaken in different agro-ecological zones of Georgia during 2009–2016 showed that potato wart occurred only in the Khulo area, which is the most mountainous region in the Autonomous Republic of Adjara, Western Georgia. The region is considered as economically important for potato production. PWD symptoms — galls of various colours (from yellow-green to brown) — were observed on the tubers for the first time in Tabakhmela and Didajara villages in June 2009. However, the growers from these villages informed us that disease symptoms had been noted in 2006 and 2007. The disease symptoms were observed on the tubers, stolons and root neck. In 2013, by the end of potato growing season, the disease was also found in other villages of Khulo region [14, 15].

All samples obtained were identified as *S. endobioticum* based on the morphological descriptions (OEPP/EPPO, 2004) and a specific PCR test using primers F49 and R502. Samples were also sent to the Netherlands Plant Protection Service, where the identification was confirmed. The research of PWD is continuing under a PhD programme.

Since 2009, within the framework of the BGRI program, the Institute has been included in the network for the global monitoring of rust diseases of cereals in order to identify the highly virulent race Ug99, as Georgia is in a zone of high risk.

Annually we conduct the Plant Health Clinics, which started in 2010 and we also advise farmers on plant protection issues. It should be noted that interest in this knowledge has significantly increased in recent years. Plant diseases diagnostics is very important as timely detection of diseases and the study their causal agents is the base of food safety of the country and allows to prevent large-scale contamination of the territory.

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Мепарішвілі С.У., Мурадашвілі М.Т., Гогоберидзе С.
Вивчення карантинних хвороб рослин в Грузії**

Виявлено наступні карантинні патогени: Dickeya sp., Ralstonia solanacearum, Erwinia amylovora, Pseudomonas syringae pv. *actinidiae*, *Synchytrium endobioticum*. Своєчасне виявлення хвороб і вивчення патогенів, що їх викликають, є основою біобезпеки країни і дає змогу не допустити масштабного зараження території.

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Мепаришвили С.У., Мурадашвили М.Т., Гогоберидзе С.
Изучение карантинных болезней растений в Грузии**

Обнаружены следующие карантинные патогены: Dickeya sp., Ralstonia solanacearum, Erwinia amylovora, Pseudomonas syringae pv. *actinidiae*, *Synchytrium endobioticum*. Своевременное выявление болезней и изучение вызывающих их патогенов является основой биобезопасности страны и позволяет не допустить масштабного заражения территории.