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Research Article

PREVALENCE OF POTATO BLACKLEG AND SOFT ROT IN GEORGIA

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ABSTRACT. Potato is an important crop in Georgia, but the average yields are considerably low (12 t/ha). One of the significant reasons for this is bacterial diseases. During 2020-2021, 73 samples of diseased potato tubers and stems were collected from fields and storage facilities, in different locations in Georgia. Following microbiological and biochemical tests, 54 strains (70%) were gram-negative, facultative anaerobic, oxidase negative, and catalase-positive, they developed at 37°C and in 5% NaCl, non-fluorescent on King´s B medium, and produced cavities on Crystal Violet Pectate (CVP) media, thus indicating they were either *Dickeya* or *Pectobacterium* spp. 27 out of selected 33 strains were identified as *Dickeya* spp. using conventional PCR and 21 of them were further characterized as *D. solani* (using TaqMan RT-PCR). According to the present research, *D. solani* is the major pathogen associated with potato soft rot and black leg in different regions of Georgia. The most prevalent species in Europe, *P. brasiliense*, as well as *D. dianthicola* have not been detected in the current research.

Keywords: *Potato, bacterial disease, soft rot, Dickeya solani.*

INTRODUCTION

Potato (*Solanum tuberosum*) is known to be one of the most important sources of food in the majority of the world including Georgia. This crop has special significance for developing countries due to the high production potential per unit of area and time with high nutritional value. It has a main agricultural value, especially for farmers in mountainous regions (Akhalkalaki, Akhaltsikhe, Ninotsminda, Adigeni, Tsalka, Dusheti, Tianeti, Marneuli, Bolnisi, Dmanisi, Khulo, Mestia, Oni) of Georgia. Potato yields vary greatly across the country due to the wide range of agroecological zones and farming practices, but average yields ranged from 8.6 to 12.6t/ha in the cropping season of 2015-

2020, which is significantly lower than the average rate of the developed countries - 50-60 tons/ha [1].

One important limiting factor in potato production in Georgia is potato disease. Particularly, the decline in potato yields is related to the use of seed tubers latently infected with bacterial pathogens, which cause potato blackleg and soft rot [2]. The diseases are caused by bacterial pathogens belonging to two different genera *Pectobacterium* and *Dickeya*. Pectinolytic bacteria, under favorable climatic conditions, can cause significant yield loss in potato fields. Some of these bacteria may survive in the soil and may decrease yield in subsequent productions of potatoes in the same field [3]. According to the previous reports [1] yield losses, caused by blackleg were estimated at 10% annually. In accordance with the farmers' stories, the yield losses of potatoes with the soft rot disease symptoms in the different potato-producing zones were high every year.

The potato soft rot caused by the *Dickeya* species was first found in Holland in 2005, after which it spread rapidly in many countries of the world, and the damage caused by it was 25-30 million Euros annually [4]. It is noteworthy that potato soft rot was first revealed in 2008 in Georgia, Akhalkalaki region, by scientists from Georgia and Israel during the studying of 100 ha potato plantations on the varieties such as Picasso, Milva, and Jelly. As a result of genetic analysis of the diseased samples conducted in Israel was established that the disease causal agent distributed in the Akhalkalaki area was biovar 3 of *Dickeya chrysanthemi* [5].

Other species or biovar may be prevalent in other potato-producing zones depending on the climatic conditions. Accordingly, the detection and study of these diseases are very relevant. The current research aimed to reveal the distribution area of potato soft rot diseases and identify the species diversity in different regions of Georgia.

MATERIALS AND METHODS

Sample collection

The surveys were conducted in 2020-2021 and covered regions with different climatic conditions and included Kobuleti (10 m above sea level, 20^0C), Keda (200 m above sea level, 20^0C), Khulo (900 m above sea level, 20°C), Akhaltsikhe (1200 m above sea level, 20°C), Akhalkalaki (1718 m above sea level, 16°C), Goderdzi pass (2025 m above sea level, 12°C). Potato stems and tubers were individually collected from symptomatic plants (blackleg or aerial stem rot) and placed in separate labeled bags to avoid cross-contamination.

Isolation and identification of bacterial strains

The disease samples were transferred to the laboratory of plant disease monitoring and diagnostics. Plant material was washed to remove excess soil and surface-sterilized in 0.3% (v/v) hypochlorite for 3 min, washed in sterile water and dried in a laminar flow cabinet for 2 h. Ten segments (5-10-mm long) from each stem or tuber stolon end, were homogenized in 10 mL sterile distilled water for 60 to 90 seconds at 4°C [6]. Sample homogenates (100µL) were plated on Nutrient Agar and incubated for 24 h at 28°C. Single colonies were purified on Crystal Violet Pectate (CVP) medium [7].

Bacteria that form pits on Crystal Violet Pectate (CVP) medium were transferred to nutrient agar and identified based on colony morphology and biochemical tests such as catalase and oxidase activity and oxidation/fermentation tests [8, 9].

Pathogenicity tests

To examine the pathogenicity of the collected strains, an analysis for maceration of healthy tubers of the Impala variety was carried out in three replications. Bacterial suspensions for inoculation were prepared by culturing isolation LB media at 27ºC for 24h. Tubers were washed in running tap water, surface-sterilized with hypochlorite for 10 min, and air-dried in a laminar chamber. The bacterial suspension (10µl) at a 10^8 CFU/ml concentration was injected into the potato slice (4 slices per strain). Controls were injected with sterile distilled water. Tuber slices were placed in a moist chamber (Petri dishes with wet paper) and incubated at 27-30°C. After 24-72, h observed softness was assessed as a positive result [10]. Only visual observation was done to determine if any maceration occurred.

PCR analyses for molecular identification

Genomic DNA of bacterial strains was extracted using QLAamp DNA mini Kit (QIAGEN, Germany) or GF-1 Nucleic Acid Extraction Kits (VIVANTIS, Malaysia), according to the respective manufacturers' protocols. Approximately 100 ng of DNA was used for PCR assays [6]. Primers Y1/Y2 [11] and BR1f/ L1r [12] were used to detect *Pectobacterium* spp., and *P. brasiliense*, respectively; ADE1/ADE2 primers [13] to detect *Dickeya* spp. The reference strains Pbr #97 and Ds #4 were used as positive controls (provided by the Agricultural Research Organization, Gilat Research Center, Israel). TaqMan RT-PCR analyses were performed with a Corbett Research Rotor-Gene (Sydney, Australia) using specific primers and probes for *D. solani* and *D. dianthicola* (using sequences of recA and dnaX, respectively [14, 15]. Molecular identification and characterization were implemented on thirty-three selected strains (Table 1).

RESULTS AND DISCUSSION

The observations of potatoes in small home gardens and farm fields were taken place in early August for five days in regions with different climatic conditions. Over the same observation period at the harvest phase of potato, the disease incidence and severity were high on the fields located in Kobuleti, Khulo, and Akhaltsikhe regions where high average air temperatures (20^0C) was indicated besides different altitude of (900-1200 m above sea level), In contrast, at Goderdzi pass (2025 m above sea level), where the temperature was sharply lower (12^0C), and the crop was in the flowering phase, no signs of disease were observed at this time. As for the Akhalkalaki, different findings were observed. In fields where the potato was in the flowering phase, disease symptoms have not shown and in fields where the crop was in the tuberization phase, diseased specimens were infected with *Dickeya* spp.

As a result of observations of potato fields, a total of 73 samples were collected. 34 samples were from potato depositaries and 43 samples were from fields. No signs of disease were observed in the potato fields near the Goderdzi Pass, where the temperature was sharply lower and plants were in the potato flowering stage. 6,16, 18, 21, and 12

samples were collected from Marneuli, Akhalkalaki, Akhaltsikhe, Khulo, and Kobuleti, respectively.

A total of 54 cultures of the bacteria isolated from the samples were identified as *Dickeya* and *Pectobacterium* spp. using microbiological and biochemical methods. All of the bacterial cultures were gram-negative, facultative anaerobic, grew at 37ºC, in 5% NaCl, were oxidase negative, catalase-positive, and non-fluorescent on King´s B (KB) medium, and cavities production on CVP. According to the test evaluating the blue pigmentation on YDC and NGM 70% of the strains were positive, thus assigned as *Dickeya* spp. and the remaining 30% of the strains negative were assigned to *Pectobacterium* spp. However, some strains of *Dickeya* spp. do not produce the blue pigment on this medium and results need to be confirmed by another method [16]. In maceration bioassays using potato slices, all strains were producing tissue maceration. In several samples, *Ralstonia solanacearum*, *a* causal agent of potato brown rot was identified. The incidence and diversity of *Ralstonia solanacearum* were studied in previous years [17].

The study showed that the majority of cases of the disease were caused by *Dickeya spp*., which is characterized by a high diversity of species. Thirty-three selected isolates (Table 1) were subjected to conventional PCR analyses to detect *Dickeya* spp, *Pectobacterium* spp., *Pectobacterium brasiliense*, *Dickeya solani*, and *Dickeya dianthicola.*

Table 1. *Bacterial strains isolated from different locations in Georgia, which were used in this molecular study*

Strain no.	Origin of isolates		Specific PCR			Real-time PCR identification		
	Location	Variety	*Pectobacterium* spp.	*P. carotovorum subsp. carotovorum*	*Dickeya* spp.	*P. carotovorum subsp. brasiliense*	*D. solani*	*D. dianticola*
Akh.2.20	Akhalkalaki	Potato tuber, Unknown	nd	nd	Positive	nd	Negative	Negative
Ac. 4.20	Akhaltsikhe, V. Tsnisi,	Potato stem, Sofia	nd	nd	Positive	nd	Negative	Negative
Kob.5.20	Kobuleti,V. Gvara	Potato stem, Impala	nd	nd	Positive	nd	Positive	Negative
Kh.6.20	Khulo	Potato tuber, Picasso	Negative	Negative	Negative	Negative	Negative	Negative
Akh.8.20	Akhalkalaki	Potato stem, Unknown	Negative	Negative	Positive	Negative	Positive	Negative
Akh.12.20	Akhalkalaki,	Potato stem, Jelly	nd	nd	Positive	nd	Positive	Negative
Kh.13.20	Khulo, Dioknise	Potato stem Unknown	nd	nd	Positive	nd	Positive	Negative
Ac.14.20	Akhaltsikhe	Potato tuber, Unknown	nd	nd	nd	nd	nd	Negative

- nd – not detected

Table 1. (continues)

Strain no.	Origin of isolates		Specific PCR			Real-time PCR identification		
	Location	**Variety**	***Pectobacterium* spp.**	***P. carotovorum subsp. carotovorum***	***Dickeya* spp.**	***P. carotovorum subsp. brasiliense***	***D. solani***	***D. dianticola***
Kh.15.20	Khulo, Dioknise	Potato tuber, Unknown	Negative	Negative	Positive	Negative	Negative	Negative
Ac.1.21	Akhaltsikhe	Potato tuber, Sofia	nd	nd	Positive	nd	Positive	nd
AKh.2.21	Akhalkalaki	Potato tuber, Picasso	nd	nd	Positive	nd	Positive	nd
Ac. 3.21	Akhaltsikhe	Potato tuber, Jelly	nd	nd	Positive	nd	Positive	Negative
Kob.4.21	Kobuleti	Potato tuber,	nd	nd	Positive	nd	Positive	Negative
Akh.5.21	Akhalkalaki	Potato tuber, Queen Anna	nd	nd	Positive	nd	Positive	Negative
Akh.6.21	Akhalkalaki	Potato tuber	nd	nd	Positive	nd	Positive	Negative
Akh.7.21	Akhalkalaki	Potato tuber, Queen Anna	nd	nd	Positive	nd	Negative	Negative
Ac. 8.21	Akhaltsikhe	Potato tuber	nd	nd	Positive	nd	Negative	Negative
Ac.9.21	Akhaltsikhe	Potato tuber, Sofia	Negative	Negative	Positive	Negative	Positive	Negative
Ac.10.21	Akhaltsikhe	Potato tuber	Negative	Negative	Positive	Negative	Positive	Negative
Kh.11.21	Khulo	Potato tuber	nd	nd	Positive	nd	Positive	Negative
Kh.12.21	Khulo	Potato tuber	nd	nd	nd	nd	Negative	Negative
Mar.13.21	Marneuli	Potato tuber	nd	nd	nd	nd	Negative	Negative
Mar.14.21	Marneuli	Potato tuber	Negative	Negative	Positive	Negative	Negative	Negative
Mar.15.21	Marneuli	Potato tuber	Negative	Negative	Positive	Negative	Positive	Negative
Mar.16.21	Marneuli	Potato tuber	Negative	Negative	Positive	Negative	Positive	Negative
Mar.17.21	Marneuli	Potato tuber	nd	nd	Positive	nd	Positive	Negative
Mar.18.21	Marneuli	Potato tuber	nd	nd	Positive	nd	Positive	Negative
Akh.19.21	Akhalkalaki	Potato tuber	nd	nd	nd	nd	Negative	nd
Kob.20.21	Kobuleti	Potato tuber, Impala	Negative	Negative	Positive	Negative	Positive	nd

- nd – not detected

Table 1. (continues)

Strain no.	Origin of isolates		Specific PCR			Real-time PCR identification		
	Location	**Variety**	***Pectobacterium* spp.**	***P. carotovorum subsp. carotovorum***	***Dickeya* spp.**	***P. carotovorum subsp. brasiliense***	***D. solani***	***D. dianticola***
Kob.21.21	Kobuleti	Potato tuber, Impala	Negative	Negative	Negative	Negative	Negative	nd
Akh.22.21	Akhalkalaki	Potato tuber	nd	nd	Positive	nd	Positive	nd
Kh.23.21	Khulo	Potato tuber, Sylvana	nd	nd	Positive	nd	Positive	Negative
Kh.24.21	Khulo	Potato tuber, Sylvana	nd	nd	Positive	nd	Positive	Negative

- nd – not detected

According to the results of specific PCR conducted in Gilat Research Center (Israel) in the frame of the grant project, 27 out of the 33 tested isolates - Akh.2.20, Ac.4.20, Kob.5.20, Akh.8.20, Akh.12.20, Kh.13.20, Kh.15.20, Ac.1.21, Akh.2.21, Ac.3.21, Kob.4.21, Akh.5.21, Akh.6.21, Akh.7.21, Akh.8.21, Ac.9.21, Ac.10.21, Kh.11.21, Mar.14.21, Mar.15.21, Mar.16.21, Mar.17.21, Mar.18.21, Kob.20.21, Akh.22.21, Kh.23.21, Kh.24.21 successfully amplified with pel gene-specific primers pair ADE1 / ADE2 and produce the expected 430 bp product, which was revealed to belong to the genus of *Dickey spp.* According to TaqMan Real time-PCR assay, 21 isolates were identified as *Dickeya solani.* The results of the molecular study have not confirmed the presence of *Pectobacterium* spp., *P. Brasiliense,* and *Dickeya dianthicola* in the tested isolates Fig1.

Fig.1. *Agarose gel electrophoresis for PCR. Amplified 430 bp product. Lane 1,14 - 100bp DNA ladder, Lane 2,3, positive control, 4- negative control, Lane 5,6,7,8,9,10,11,12,13 –bacterial strain*

CONCLUSION

The current study has shown that potato soft rot and blackleg caused by *Dickeya solani* are especially prevalent in investigated regions of Georgia and cause severe yield losses

in Khulo, Akhaltsikhe, Akhalkalaki, and Kobuleti regions. Although *P. Brasiliense* is currently considered to be the most common and harmful species in Europe and Israel, any of the tested strains from Georgia were not identified as *P. brasiliense*. From 2006 to 2010, *D. solani* has been spreading across potato-producing countries in Europe (via the trade of seed tubers) and has become the most important blackleg agent [4]. However, since 2014, the incidence of blackleg caused by *D. solani* in Western Europe has been gradually decreasing, while the prevalence of *P. brasiliense*as the causal agent of blackleg disease in some countries like Switzerland, Belgium, the Netherlands, and Israel has risen quickly [15,18]. Currently, *P. brasiliense* is one of the most commonly isolated pectinolytic bacteria on potatoes in Europe, has spread rapidly since its first appearance, and has largely replaced *D. solani* in many areas [19]. Both, *D. solani* and *P. brasiliense* are more virulent in potatoes than other species under high-temperature conditions.

Thus, according to our research potato soft rot prevails in Georgia. Currently the most common pathogen in Europe, *P. brasiliense* has not been identified in the strains under study from Georgia. The prevalence of potato blackleg and soft rot has caused significant yield losses in Georgia. Accordingly, continuing the research on these diseases is necessary.

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