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Extracellular protease activity and glucose production in isolates of net blotch pathogens differing in virulence

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Abstract

Pyrenophora teres f. *maculata* (*Ptm*) and *Pyrenophora teres* f. *teres* (*Ptt*) incite net and spot forms of net blotch disease of barley, respectively. In this study, *Ptm* and *Ptt* isolates differing in virulence were compared in terms of protease activity and glucose production. Protease production by *Ptm* isolates ranged between 75.33 ± 4.10 and 127.33 ± 7.54 unit (U) protease activity mg^{-1} protein. Virulent *Ptm* isolates produced significantly more protease than the less virulent isolates. Similarly, glucose production by *Ptm* isolates ranged between 0.55 ± 0.03 and 0.84 ± 0.05 mg glucose g^{-1} mycelial dry weight (DW). Virulent isolates also produced significantly more glucose than the less virulent isolates. Similar results were obtained with *Ptt* isolates. Protease production by *Ptt* isolates ranged between 69.00 ± 3.79 and 123.33 ± 7.69 U protease activity mg^{-1} protein. Virulent *Ptt* isolate produced significantly more protease than the less virulent isolates. Glucose production by *Ptt* isolates ranged between 0.70 ± 0.03 and 0.75 ± 0.04 mg glucose g^{-1} mycelial DW. Differences in the glucose production of *Ptt* isolates were not significant. These findings corresponded to the severity of symptoms in the host tissues. It appeared that virulence mechanism in *P. teres* was closely associated with both protease and glucose production. The protease enzyme secreted by the fungi could be the determining factor for aggressiveness and symptom expression, and, therefore, it could be used as a marker enzyme in *P. teres* isolates.

Key words: glucose, net blotch, protease, *Pyrenophora teres* f. *maculata*, *Pyrenophora teres* f. *teres*, virulence.

Introduction

Net blotch disease is caused by the fungus *Pyrenophora teres* (Died.) Drechsler which belongs to *Ascomycota* phylum. The anamorphic stage of the fungus is referred to *Drechslera teres* (Sacc.) Shoemaker. *Pyrenophora teres* is an important pathogen of barley (*Hordeum vulgare* L.) in the world lowering the quality and quantity of barley (Mathre, 1982; Liu et al., 2011). Crop losses due to the net blotch disease can be 10–40%; however, in susceptible cultivars they could be much higher (Mathre, 1982). The causal agent of the disease has two forms. *Pyrenophora teres* f. *maculata* (*Ptm*) causes the spot form, while *Pyrenophora teres* f. *teres* (*Ptt*) causes the net form of the disease (Liu et al., 2011). *Ptm* behaves like a hemibiotroph; however, *Ptt* acts like a necrotroph in most of its life span (Able, 2003; Lightfoot, Able, 2010; Liu et al., 2011). Necrotrophic fungi use large amount of cell wall-degrading enzymes (Hammond-Kosack, Rudd, 2008).

P. teres, in general, infects and causes disease on leaves, stems, leaf sheaths and kernels of barley plants. Germinating conidia or ascospores land on the surface of leaves and infection starts. After this step, *P. teres* penetrates the host into epidermal cells within 24 h (Jørgensen et al., 1998). Although the penetration is possible through stomata, the main penetration is done by the enzymatic hydrolysis of cuticle and cell wall along with the pressure generated from the appressoria. After successful penetration, the fungal hypha form a large intracellular vesicle. Later on, within the epidermal cell, a secondary intracellular vesicle develops (Keon, Hargreaves, 1983). Then, hyphae from the secondary intracellular vesicle grow through the lower epidermal cell wall and reach the mesophyll. It has been shown that low molecular-weight compounds produced by the fungal cells facilitated the movement of intercellular hyphae during infection. After the infection process, small lesions are seen at the penetration site and dark-brown areas develop along with the leaf vein (Hargreaves, Keon, 1983).

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Lightfoot and Able (2010) investigated the infection process between the two biotypes, *Ptt* and *Ptm*. *Ptm* often forms haustoria-like intracellular vesicles and tends to grow closer to the epidermal cells and is not able to penetrate the cells which are not in contact. With this characteristic it resembles a biotroph; however, it quickly switches into necrotroph. *Ptm* is also slower in terms of germination and growth rate than *Ptt*. On the other hand, *Ptt* rarely forms intracellular vesicles, but rather infects and feeds as a necrotroph throughout the infection process. *Ptt* grows only intercellularly and is able to affect cells not immediately associated with the mycelium. *Ptt* would be classified as a necrotroph and *Ptm* as a hemibiotroph (Liu et al., 2011).

Defence reactions by the host initially encounter with the cell wall defence system. However, during pathogenicity, the fungus is able to exert some extracellular enzymes to breakdown the host resistance. The level of enzymes, however, might differ among isolates and among species. Although the genetic background of these fungi becomes identical, there might be differences in secreted enzymes which eventually result in differences in pathogenicity due to their different virulence habits. The level of secreted enzymes could be affected by environmental factors as well as genetic factors. One of the most important characteristics of proteases is their involvement in the initiation and development of the pathogenic processes (Dunaevskii et al., 2008). It was reported that pathogenic fungi produced a trypsin-like protease activity that was absent in saprophytes (Girard, Michaud, 2002; Dunaevskii et al., 2008). Proteolytic enzymes are of vital importance in the regulation of various biological processes at the molecular, cellular, tissue and organ levels and catalyze the hydrolysis of proteins. The importance of this enzyme has increased in recent years due to impairment of ecological conditions. The increased number of people with the weakened immune system was negatively correlated with the decrease of protease activity in human beings (Quirós et al., 2015).

Involvement of this enzyme in the pathogenic process facilitates the attack of the fungus on host tissues. The enzyme also protects the infecting fungus from the deleterious effects of substances produced by the host (Rao et al., 2006; Dunaevskii et al., 2008). Differences in the virulence levels of pathogenic fungi were reported. Pathogenic variation in *P. teres* was reported previously (Steffenson, Webster, 1992; McLean et al., 2014; Çelik Oğuz, Karakaya, 2015). Many reports have been published on highly, moderate and low virulent isolates (Usta et al., 2014; Yazıcı et al., 2015). However, no statements were made on the characteristics of virulence and secreted metabolites in *P. teres*. In this study, we evaluated 5 isolates of *Pyrenophora teres* f. *maculata* and 4 isolates of *Pyrenophora teres* f. *teres* differing in virulence in terms of protease and glucose production abilities.

Materials and methods

Isolation, inoculation and disease assessment procedures. Infected leaves showing symptoms of both spot and net forms of the disease were collected from 7 different provinces (Kahramanmaraş, Gaziantep, Kilis, Kırıkkale, Ankara, Eskişehir and Siirt) of Turkey during 2015 and 2016. Pathogenicity experiments were carried out in 2016, in a greenhouse of Department of Plant Protection, Faculty of Agriculture, Ankara University, Turkey. In order to obtain single spores, infected leaves were cut in 2–3 cm lengths and after surface sterilization, with 1% NaOCl for 1–2 minutes, they were placed into sterile Petri dishes containing wet filter paper. Petri dishes were incubated under room conditions. Three to four days later single spores were taken under a stereomicroscope and spores were placed onto water agar plates. After hyphal development, under a stereomicroscope, hyphal tips were cut and placed in Petri dishes containing potato dextrose agar (PDA). Net and spot forms of the isolates were verified by inoculating the isolates to susceptible cultivar 'Bülbül 89' (Usta et al., 2014; Yazıcı et al., 2015).

Seedling pathogenicity tests were conducted using cultivars 'Kombar', 'Tifang', 'Bülbül 89' and 'Avcı 2002'. Fifteen seeds of each cultivar were seeded into 10 cm in diameter clay pots containing a mix of top soil, sand and organic matter (60, 20, 20, v:v:v). Plants were maintained in a greenhouse located at Department of Plant Protection, Faculty of Agriculture, Ankara University, Turkey. Greenhouse conditions ranged between 17–24 ± 2°C (night/day) with a 14 hour light period. For inoculum production, mycelia from 10-day old cultures grown in PDA plates were used. Mycelia were scraped from Petri dishes using a sterile paintbrush. Inoculum concentration was adjusted using a hemocytometer to 15–20 × 10^4 mycelial parts per ml (Usta et al., 2014; Yazıcı et al., 2015). For each 100 ml of the inoculum, one drop of Tween 20 was added (Usta et al., 2014). Barley seedlings were inoculated during 12–13 development stages (Zadoks et al., 1974). Inoculation was accomplished with a hand sprayer. Following inoculation, plants were covered with plastic bags for 3 days. Plants were evaluated using scales developed for spot and net forms of the disease by Tekauz (1985) seven days after inoculation. Experiments were repeated three times. In spot form of net blotch scale, 7 numerical classes were recognized: 1 R – resistant, 2 R – resistant-MR: moderately resistant, 3 MR – moderately resistant, 5 MR – moderately resistant-MS: moderately susceptible, 7 MS – moderately susceptible, 8 MS – moderately susceptible-S: susceptible, 9 S – susceptible. In net form of net blotch scale, 10 numerical classes were recognized: 1 R – resistant, 2 R – resistant-MR: moderately resistant, 3 MR – moderately resistant, 4 MR – moderately resistant-MS: moderately susceptible, 5 MR – moderately resistant-MS: moderately susceptible, 6 MR – moderately resistant-MS: moderately susceptible, 7 MS – moderately susceptible, 8 MS – moderately susceptible-S: susceptible, 9 S – susceptible, 10 VS – very susceptible.

Cultivars 'Kombar' and 'Tifang' were included in *P. teres* differential set of barley (Wu et al., 2003). Cultivar 'Kombar' showed susceptible reaction, and cultivar 'Tifang' showed resistant reaction previously (Steffenson, Webster, 1992; McLean et al., 2014). In previous studies, barley cultivars 'Bülbül 89' and 'Avcı 2002' also showed susceptible and resistant reactions to *P. teres*, respectively (Usta et al., 2014; Yazıcı et al., 2015).

Protease activity. The fungal isolates were grown at room temperature in PDA medium in the dark until the mycelium covered the whole plate. These fungus-coated plates were kept at 4°C and used as a source material for protease assays. Protease activity was determined according to the method of Girard and Michaud (2002) with slight modifications by measuring the release of azocasein-derived amino acids and small peptides in the fungal culture medium. A 3-mm plug of mycelium-coated agar was removed with a cork-borer from the

outer edge of the plate and the agar plug was placed into a 1.5 ml Eppendorf tube containing 120 µl of 1 % (w/v) azocasein in 50 mmol l^{-1} Tris-HCl, pH 8.8. Azocasein hydrolysis was initiated by incubating the tubes 2 hrs at room temperature. Proteolysis was stopped by adding 300 µl of 10% (w/v) cold trichloroacetic acid (TCA) to the fungal culture. After centrifugation for 10 min at 15000 g, 350 µl of the supernatant was collected and mixed with of 300 µl of 1 N NaOH. Protease activity was then determined by reading optical density of the resulting solution at 440 nm using a microtitre plate SpectraMax M5 (Molecular Devices, USA). A change of 0.01 units per minute in absorbance was considered to be equal to one unit protease activity, which was expressed as U (unit) mg^{-1} protein. There were three replications. Data were subjected to analysis of variance and means were separated by LSD test.

Protein determination. Protein concentration was determined at 595 nm by the method of Bradford (1976) using Coomassie Brilliant Blue G-250 (Sigma-Aldrich, Germany). A standard curve was prepared using Bovine Serum Albumin Fraction V (Sigma-Aldrich). The response was linear over the range 10 to 100 µg protein.

Carbohydrate content of mycelia. The carbohydrate content was determined using the phenol sulphuric acid method described by Hodge and Hofreiter (1962). A standard curve was prepared using D-glucose over the range of 10–100 µg and distilled water was used as the control. The results were expressed as mg glucose g^{-1} dry weight (DW) mycelium. There were three replications. Data were subjected to analysis of variance and means were separated by LSD test.

Determination of protease activity, protein determination and carbohydrate content of mycelia were carried out in the laboratories of Harran University, Şanlıurfa, Turkey.

Results and discussion

Net and spot forms of the isolates were verified by inoculating the isolates to susceptible barley cultivar 'Bülbül 89'. Respective isolates produced typical net and spot forms of the symptoms on susceptible cultivar 'Bülbül 89'. Cultivar differences to *P. teres* isolates were observed. Cultivars 'Kombar' and 'Bülbül 89' mainly showed susceptible group reactions, and 'Tifang' and 'Avcı 2002' mainly showed resistant group reactions, respectively. Differences in virulence among the *Ptm* and *Ptt* isolates were evident (Tables 1 and 2). The *Ptm*1 isolate was the least virulent of *Ptm*, while isolates *Ptm*4 and *Ptm*5 were the most virulent isolates of *Ptm* (Table 1).

Protease activities and glucose contents of isolates of *Ptt* and *Ptm* were determined. Virulent *Ptm* isolates produced more protease enzyme (*Ptm*4 produced 111.67 ± 6.74, *Ptm*5 produced 127.33 ± 7.54 U protease activity mg^{-1} mycelial protein), and they were statistically significant ($P < 0.01$) (Table 1). The low or moderate virulent isolates of *Ptm* (*Ptm*1–3) were able to produce protease enzyme between 75.33 ± 4.10 and 88.00 ± 4.73 U mg^{-1} protein.

Table 1. Median scale values of barley cultivars according to Tekauz (1985) after inoculation with *Pyrenophora teres* f. *maculata* isolates and the activity of protease enzyme and glucose production by isolates

P. teres f. *maculata* isolate	Cultivar				Protease activity U (unit) mg^{-1} protein	Glucose concentration mg g^{-1} dry weight (DW)
	Bülbül 89	Avcı 2002	Kombar	Tifang		
*Ptm*1	2	2	2	2	85.67 ± 4.98 a	0.55 ± 0.03 a
*Ptm*2	5	3	5	2	88.00 ± 4.73 a	0.56 ± 0.05 a
*Ptm*3	5	3	5	2	75.33 ± 4.10 a	0.64 ± 0.03 a
*Ptm*4	8	5	7	7	127.33 ± 7.54 b	0.84 ± 0.05 b
*Ptm*5	9	5	8	7	111.67 ± 6.74 b	0.81 ± 0.04 b

Note. Protease and glucose data are presented as means and standard error; numbers followed by different letters are statistically significant ($P < 0.01$).

The same pattern was also observed with those of *Ptt* isolates (Table 2). The most virulent isolate (*Ptt*4) produced the highest protease activity (123.33 ± 7.69 U mg^{-1} protein) and it was statistically significant ($P < 0.01$). The least virulent isolate *Ptt*1 produced the least amount of protease enzyme (69.00 ± 3.79 U mg^{-1} protein). Protease activities in moderately virulent isolates (*Ptt*2 and *Ptt*3) coincided with the moderate production of protease activity (70.33 ± 3.76 and 81.33 ± 4.10 U mg^{-1} protein) as compared to those of virulent isolates.

Similar findings were also obtained for glucose accumulation in which the *Ptm*4 and *Ptm*5 isolates accumulated 0.81 ± 0.04 and 0.84 ± 0.05 mg glucose g^{-1} mycelial DW, respectively, while *Ptm*1 accumulated 0.55 ± 0.03 mg glucose g^{-1} mycelial dry weight. Virulent *Ptm* isolates produced more glucose and they were statistically significant ($P < 0.01$). Again, the most aggressive isolate

Table 2. Median scale values of barley cultivars according to Tekauz (1985) after inoculation with *Pyrenophora teres* f. *teres* isolates and the activity of protease enzyme and glucose production by isolates

P. teres f. *teres* isolate	Cultivar				Protease activity U (unit) mg^{-1} protein	Glucose concentration mg g^{-1} dry weight (DW)
	Bülbül 89	Avcı 2002	Kombar	Tifang		
*Ptt*1	4	3	4	1	69.00 ± 3.79 a	0.70 ± 0.03 ns
*Ptt*2	5	3	5	3	70.33 ± 3.76 a	0.70 ± 0.03 ns
*Ptt*3	5	4	5	2	81.33 ± 4.10 a	0.75 ± 0.03 ns
*Ptt*4	7	3	7	5	123.33 ± 7.69 b	0.75 ± 0.04 ns

Note. Protease and glucose data are presented as means and standard error; numbers followed by different letters are statistically significant ($P < 0.01$); ns – not significant.

*Ptt*4 accumulated 0.75 ± 0.04 mg glucose g^{-1} mycelial dry weight, while *Ptt*1 accumulated 0.70 ± 0.03 mg glucose g^{-1} mycelial dry weight. However, these differences were not statistically significant. These findings corresponded to the severity of symptoms. More glucose accumulation with the increase in virulence of the isolates was more evident with the *Ptm* isolates.

Microorganisms attack the host to obtain nutrients for growth. They must first overcome the physical barrier presented by the cell wall. Both the plant cell wall and the protoplasm contain nutrients that can be used by the fungi. If the fungi access the minerals, amino acids, nucleotides and simple sugars in the cytosol, the infection starts. Further hydrolysis of these compounds could be achieved by fungal enzymes. It was observed that the more aggressive isolates were able to produce more metabolites. For example, Chand et al. (2014) previously reported that extracellular enzymes differed in wild and clonal type populations of *Bipolaris sorokiniana*. The cellulase production was significantly higher in wild type (11.9 U mg^{-1} protein) than clonal type (8.15 U mg^{-1} protein). Similarly, the production of pectinase, amylase, and proteinase were higher in the wild type. The increase of enzymes in *B. sorokiniana* wild type also coincided with lesion size in barley. They suggested that aggressiveness was directly related to the production of extracellular enzymes in the *B. sorokiniana* subpopulation. Ismail et al. (2014) stated that the more virulent isolate of *Ptt* had higher rates of conidial germination and fungal development *in planta*. Virulent isolate produced longer hyphae and formed appressoria. They stated that higher number of conidial germination resulted in higher rates of penetration.

In general, more virulent isolates may establish themselves faster by producing certain types of enzymes and toxins for attachment to the cell wall (Tan et al., 2010; Ismail et al., 2014). On the other hand, the less virulent isolates might take a longer time to produce toxins and pathogen-related metabolites. For example, Nguyen et al. (2011) reported that endoxylanase mutant *Magnaporthe oryzae* invaded the rice cells more slowly providing the host cells more time to prepare an effective defence by strengthening their cell wall. Ismail et al. (2014) suggested that the virulence of *Ptt* was associated with fungal growth and toxins that can cause symptoms of the disease.

Redman and Rodriguez (2002) reported a direct relationship between extracellular activity and the induction of *Colletotrichum coccodes*. They suggested that extracellular protease activity was required for pathogenicity and virulence of *C. coccodes*. They also reported that the elimination of protease activity transformed the virulent pathogen into a non-pathogen. Bueno et al. (2012) supported the involvement of protease enzyme in pathogenicity by showing the increased expression of the acid protease and aspartyl protease genes upon invasion of *Phaseolus vulgaris* plants with *Sclerotinia sclerotiorum*.

On the other hand, glucose accumulation also plays important roles in the pathogenicity of the infecting fungi. For example, Mishra and Malik (2013) reported that glucose concentration showed the almost linear relationship with biomass yield of the fungus *Beauveria bassiana*. It is suggested that highly virulent races of fungus contain the high amount of glucose compared to those of non-virulent races of the same fungus. For example, *Cryptococcus* (a yeast-like fungus) has an efficient mechanism to move sugar molecules from a substrate to its own cells (Tchakouteu et al., 2015). They stated that this pathogenic yeast had more inositol transporters and genes regulating inositol transport than those of any fungi in the fungal kingdom. They found that a connection between the high concentration of free inositol and fungal infection was evident in the human brain. They also stated that these fungi were initially adapted to grow on plants in the wild.

In fungal pathogen-plant systems, a high level of sugars in plant tissues enhances plant resistance. Several hypotheses have been proposed to explain the mechanism of this phenomenon. Sugars provide energy and structural material for defence responses in plants. Sugars may also act as signal molecules interacting with the hormonal signalling network regulating the plant immune system. Sugars enhance oxidative burst at early stages of infection and stimulate the synthesis of flavonoids and pathogen related proteins. However, high-sugar level does not always boost the immune system in plants; in fact, it is able to stimulate the development of the pathogenic fungi. The glucose sink at the site of fungal pathogen invasion contributes to the development of sporulation and mycelial growth as well as creates a suitable substrate for fungal enzymes. For example, Barboráková et al. (2012) have documented that an increased concentration of glucose had the positive impact on biomass growth of *Penicillium scabrosum*. Similarly, Costa and Nahas (2012) reported that the addition of glucose in the culture medium increased the growth rate of *Aspergillus flavus* and *Penicillium* sp. They also stated that the amylase activity in the soil significantly increased due to the addition of glucose. Similar conclusions were also made by Boyette and Hoagland (2012) who noticed that the addition of amino acids and/or 10 mM glucose stimulated the germination of *Colletotrichum truncatum*, a potential bioherbicide for hemp Sesbania plant.

Calmes et al. (2013) highlighted the importance of mannitol metabolism with respect to the ability of *Alternaria brassicicola* to efficiently accomplish key steps of its pathogenic life cycle. Mannitol has been proposed to act as a potent protective metabolite against oxidative stress and as an antioxidant agent and protect fungal cells by quenching reactive oxygen species (ROS) produced by hosts in response to pathogen attack. For example, a mannitol low-producing mutant type of *Cryptococcus neoformans*, an animal pathogen, was found hyper-susceptible to oxidative stress (Chaturvedi et al., 1996).

Accumulation of glucose is also involved in mechanism to turn the mutant strains into pathogens. For example, when 2.5% glucose was added to the inoculum of the isocitrate lyase mutant of *Leptosphaeria maculans* (blackleg disease agent of canola), lesions of sizes were similar to those caused by wild-type isolate M1 developed on *Brassica napus* (Idnurm, Howlett, 2002). It was suggested that glyoxylate pathway was essential for disease development by this fungus. The capacity of sugar accumulation in fungal cells also leads to sugar starvation in plant cells. This may cause both ultrastructural and physico-chemical changes in the host.

The results of this study showed that severe virulent and less virulent isolates of *Ptm* and *Ptt* isolates utilized the glucose differently. It is possible that glucose affects the growth and development of fungi by activating enzymes and other related metabolic components. Clearly, further studies are necessary to explore the interaction between pathogenic fungi and various concentrations of glucose in growth media and to see if the correlation exists between melanin accumulation and

glucose/protease level. It is possible that accumulation of glucose not only results in an increase in pathogenicity of virulent pathogens but also increases the virulence of pathogens by decreasing the pH level upon secretion of gluconic acid when glucose is used as the substrate. For example, colonization of *Penicillium expansum* and severity of mould disease in the infection site increased where the gluconic acid accumulation was high (Hadas et al., 2007). This acidification increased the expression of pectolytic enzymes and the establishment of conditions for the necrotrophic development of *P. expansum*.

Variations in virulence of fungi depend on the wide range of mechanisms such as gene flow, recombination, sexual cycle, mutation and migration (Burdon, Silk, 1997). As a result of this, resistant crops could become susceptible to newly arisen pathotypes. New pathotypes of fungi could be more virulent than the current pathotypes. One of the main proposals is that they could still produce pathogenic enzymes against resistant cultivars or could be active under harsh environmental conditions (Dikilitas, Karakas, 2014). Therefore, the stability of enzymes under stress might show the ability of adaptation of fungi to adverse conditions.

Conclusion

It appears that virulence mechanism in *Pyrenophora teres* was closely associated with both protease and glucose production. This is the first study on the relationship between virulence and protease enzyme activity in *P. teres* f. *teres* (*Ptt*) and *P. teres* f. *maculata* (*Ptm*) isolates. We suggest that protease enzyme could be used as a marker in determining the virulence status of *P. teres* isolates *in vitro*.

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Ekstraląstelinės proteazės aktyvumas ir gliukozės gamyba skirtingo virulentiškumo tinkliškosios dryžligės patogenų izoliatuose

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Santrauka

Pyrenophora teres f. *maculata* (*Ptm*) ir *Pyrenophora teres* f. *teres* (*Ptt*) sukelia miežių tinkliškosios dryžligės dėmėtąją ir tinkliškąją formas. Tyrimo metu buvo palyginti skirtingo virulentiškumo *Ptm* ir *Ptt* izoliatų proteazės aktyvumas ir gliukozės gamyba. *Ptm* izoliatų proteazės gamyba svyravo tarp 75,33 ± 4,10 ir 127,33 ± 7,54 vienetų (U) proteazės aktyvumo mg^{-1} baltymų. Virulentiški *Ptm* izoliatai pagamino žymiai daugiau proteazės nei mažiau virulentiški. Buvo gauti panašūs ir gliukozės kaupimosi rezultatai. *Ptm* izoliatų gliukozės gamyba svyravo tarp 0,55 ± 0,03 ir 0,84 ± 0,05 mg gliukozės g^{-1} micelio (grybienos) sausojo svorio. Virulentiški izoliatai pagamino žymiai daugiau gliukozės nei mažiau virulentiški.
Panašūs rezultatai buvo gauti ištyrus *Ptt* izoliatus. *Ptt* izoliatų proteazės gamyba svyravo tarp 69,00 ± 3,79 ir 123,33 ± 7,69 U proteazės aktyvumo mg^{-1} baltymų. Virulentiškas *Ptt* izoliatas pagamino žymiai daugiau proteazės nei mažiau virulentiški. *Ptt* izoliatų gliukozės gamyba svyravo tarp 0,70 ± 0,03 ir 0,75 ± 0,04 mg gliukozės g^{-1} micelio (grybienos) sausojo svorio. *Ptt* izoliatai esmingai nesiskyrė gliukozės gamyba. Šie rezultatai atitiko simptomus šeimininko audiniuose.
Tyrimo metu pirmą kartą nustatytas ryšys tarp *Ptt* ir *Ptm* izoliatų virulentiškumo ir proteazės fermento aktyvumo. Nustatyta, kad *P. teres* virulentiškumo mechanizmas glaudžiai susijęs ir su proteazės, ir su gliukozės gamyba. Grybų išskiriamas proteazės fermentas gali būti agresyvumą ir simptomų išraišką lemiantis veiksnys, todėl jis gali būti naudojamas kaip fermentas žymuo, padedantis nustatyti *P. teres* izoliatų virulentiškumą.

Reikšminiai žodžiai: gliukozė, proteazė, *Pyrenophora teres* f. *maculata*, *Pyrenophora teres* f. *teres*, tinkliškoji dryžligė, virulentiškumas.