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In vitro Characteristics of Different *Pyrenophora Tritici-Repentis* Isolates

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Abstract

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Eight ascosporic isolates of different origin were cultivated on PDA, V-8 and Oat agar in controlled conditions (temperature of 22°C±2°C), at photoperiod of 12 h light vs. 12 h darkness, 24 h light vs. 24 h darkness). A morphological characteristics *in vitro* was performed to identify their radial growth, coloring and compactness of the mycelium formed as well as nutrient medium coloring. Statistically significant differences between the isolates grown on the same nutrient medium and same conditions were found, proving phenotypical and genetic differences between them.

Key words: Pyrenophora tritici-repentis, ascosporic isolates, radial growth, culture characteristics

Introduction

Drechslera tritici-repentis (Died.) Shoem. is a leaf pathogen causing wheat tan spot disease. Even though wheat is the main host of this disease, it was first isolated in 1902 by Diedicke from *Cynodon Dactylon* as *Pleospora tritici-repentis* with *Helminthosporium gramineum f.sp. tritici-repentis* as anamorph (Diedicke, 1902). He made the first description of the disease as well. In 1923, Drechsler called it *Pyrenophora tritici-repentis* with anamorph *Drechslera tritici-repentis* for the fist time (Drechsler, 1923) because he assigned it to *Pyrenophora* genus due to the formation of bristles on the fruiting bodies. In 1959, Shoemaker gave it the same name independently from Drechsler (Shoemaker, 1959) and in 1962 gave a detailed description of its asexual form based on his own morphological studies of the spores.

The economic importance of this disease strongly varies in different countries because of different climatic conditions and methods of wheat growing but the leaf pathogen *P. tritici-repentis* is found all over the world. This fungal disease spreads seriously in favorable climatic conditions, wheat monoculture and minimum soil cultivation without subsoiling (Hartleb, 1999;

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Schmitz and Grossmann, 1987). An epidemic condition is the result of accumulated infection on last year non-incorporated plant debris (straw) (Hosford, 1971; Watkins et al., 1978; Rees and Platz, 1980).

Wheat tan spot disease was identified for the first time in Bulgaria in 2004 (Todorova, 2006). Specialized literature contains a number of publications on this pathogen and the disease it causes. A number of authors have studied monospore isolates of *P. tritici-repentis* – their biology, morphology and culture peculiarities as well as their cultivation on different nutrient media (Mielke, 1999; Wolf, 1991; Friesen et al., 2003; Ali and Francel, 1998; Wolf and Hoffman, 1993; Hunger and Broun, 1987).

The objective of the present study was to characterize and compare eight monospore isolates of *P. tritici-repentis* of different origin and find the culture differences between them, if any. This would be a prerequisite for a further population analysis of pathogen virulence and aggression as well as an evaluation of resistance type and rate of wheat cultivars, grown in Bulgaria. Given the comparatively recent detection of the pathogen in our country as well as its growing importance and spreading in Europe in the recent years, the study is extremely significant for our science and practice.

Materials and Methods

The trials were carried out at the Laboratory of Phytopathology of the University of Forestry in 2005-2007. Each trial was carried out fourfold in eight replications.

Isolates: Eight ascosporic isolates of ***P. tritici-repentis,*** isolated from winter wheat straw debris, were studied for the purpose of this trial. The straw with pseudothecia with asci and ascospores of the pathogen was collected from different fields in Sofia district. Two pairs were isolated from the same ascus. Isolation was carried out by the method of Hunger and Broun (1987). Data is presented on Table 1.

Nutrient media: The possibilities for isolate cultivation were studied in controlled temperature and photoperiod on artificial nutrient media (Mielke, 1999) (Vegetable agar – **V8,** Potato Dextrose Agar **– PDA** and **Oat agar).**

Table 1

Name and origin of Pyrenophora tritici-repentis isolates

Isolate	Origin
BPTR 0105	1. Yossifovo village
*BPTR 0405	2. Leskovets village
BPTR 0605	3. Cherna Gora village
*BPTR 0705	4. Leskovets village
BPTR 0107	5. Kazichane village
BPTR 0207	6. Town of Dragoman
^BPTR 0307	7. Petarch village
^BPTR 0507	8. Petarch village

Isolates marked * and ^ were isolated from one ascus

Experiments were conducted by standard methods (Hunger and Broun, 1987).

Cultivation conditions: Isolates were cultivated and studied at a temperature of 22°C ±2°C and photoperiod 12 h light – 12 h darkness; 24 h darkness; 24 h light.

Research parameters: The following parameters were used to characterize the isolates: mycelium coloring, nutrient media coloring and mycelium compactness, recorded on a daily basis until plates (Ø =

9 mm) were full, which happened on the eighth day for most isolates. We also recorded mycelium radial growth of each isolate in cm (on the eighth day) in all three nutrient media as mentioned above. Cross measurements were made due to the incomplete symmetry of mycelium radial growth.

Reporting: Final results were reported on the eighth day after the transfer of isolates on the three nutrient media under the respective conditions. Isolates were placed at the same light conditions on white paper for mycelium coloring estimates. Mycelium compactness was evaluated under stereomicroscope.

Data processing: Data were processed statistically by ANOVA method and grouped by means of Duncan's Multiple Range Test.

Results and Discussion

The results of the studies on characteristic parameters of mycelium isolates are presented on Table 2. Isolates had differences in terms of coloring and mycelium compactness as well as nutrient medium coloring. Three of the isolates formed dark to light grey mycelium, four of them – white to grayish-white and one of the studied isolates formed grey-green colored mycelium. Nutrient medium coloring under the colony was orange in two of the isolates, grey in other two, black in one of them and no coloring in three of the isolates. Those results confirmed the studies of Wolf (1991). He found that different monospore isolates were differentiated by above morphological characteristics on different nutrient media in controlled conditions. Wolf studied pathogen variability by means of the diverse behavior of 32 monospore isolates grown on artificial medium (V-8 and PDA) thus proving the existence of different mycelium coloring.

The most distinct differences were found in two couples of isolates, isolated from the same ascus each. In both cases, one of the isolates did not color the nutrient medium, contrary to the other. They had different mycelium compactness; therefore, in both cases one of the isolates was male and the other – female (Kema, 1996; Kema et al., 1996). Even isolates, coming from fields, located close to each other, demonstrated large phenotypical, therefore, genotypic, variation, statistically significant (Kema, 1996).

Tables 3, 4 and 5 present the results on

Table 2

Morphological characteristics of isolates on all tested nutrient media

Isolate	Micelia coloring	Media coloring	Mycelia compactness
BPTR 0105	dark grey	black	high compactness
BPTR 0405	white	no coloring	loose
BPTR 0605	light grey	bright orange	medium compactness
BPTR 0705	greyish-white	grey	low looseness
BPTR 0107	white	no coloring	high looseness
BPTR 0207	grey-green	pale orange	medium compactness
BPTR 0307	grey	pale grey	high compactness
BPTR 0507	white	no coloring	non-uniform looseness

Table 3

Radial growth of mycelium (cm) of ascosporic isolates of *Pyrenophora tritici-repentis* on V-8 at different photoperiod

Photoperiod	24h dark	24h light	12h dark/12h light
BPTR 0405	0a	0a	0a
BPTR 0207	2.35b	2.1b	2.2b
BPTR 0605	2.61bc	2.11b	2.23b
BPTR 0307	5.2c	4.88c	5c
BPTR 0105	5.6cd	5.38d	5.42cd
BPTR 0507	6.97e	6.88e	6.91e
BPTR 0705	6.98e	6.66e	6.78e
BPTR 0107	8.25f	8.02f	8.1f

* Data with at least one common letter do not differ significantly

P<0.05

Table 4

Radial growth of mycelium (cm) of ascosporic isolates of *Pyrenophora tritici-repentis* on PDA at different photoperiod

Photoperiod	24h dark	24h light	12h dark/12h light
BPTR 0207	2.5a	2.15a	2.35a
BPTR 0605	2.51a	2.25ab	2.34a
BPTR 0105	5.16b	4.91c	5.03b
BPTR 0705	5.4b	5c	5.29bc
BPTR 0307	5.78bc	5.25d	5.66d
BPTR 0507	6.69d	6.55e	6.61e
BPTR 0105	7.6de	7.45f	7.5f
BPTR 0405	8.95f	8.45g	8.67g

* Data with at least one common letter do not differ significantly

P<0.05

radial growth of all isolate colonies on different nutrient media and photoperiod. Those studies showed that mycelium growth depended on nutrient medium and the presence or absence of light. The development of isolates was the slowest during permanent light. Isolate growth was comparatively good in 12 h light / 12 h darkness. The fastest growth of isolates was recorded in the absence of light. Those results supported the research of Mielke (1999), who found that isolates developed fastest in the absence of light.

The growth velocity of isolate BPTR 0107 on V-8 was the greatest. Isolates BPTR 0705 and BPTR 0507 were in the

Table 5

Radial growth of mycelium (cm) of ascosporic isolates of *Pyrenophora tritici-repentis* on Oat agar at different photoperiod

Photoperiod	24h dark	24h light	12h dark/12h light
BPTR 0507	3.15a	2.85a	3a
BPTR 0605	3.55b	2.93ab	3.32a
BPTR 0405	5.87c	5.75c	5.8b
BPTR 0705	6c	5.83cd	5.98b
BPTR 0107	7.2d	7e	7.05c
BPTR 0307	7.87de	7.37e	7.64cd
BPTR 0105	9f	9f	9e
BPTR 0207	9f	9f	9e

* Data with at least one common letter do not differ significantly

$P<0.05$

same group with a comparatively fast growth rate. Isolates BPTR 0605, BPTR 0307 and BPTR 0105 showed no significant differences in dark condition, but isolate BPTR 0605 showed a significant difference, compared to the other two, during the light period. Isolate BPTR 0405 was significantly different from all the other ones on this nutrient medium and did not form a mycelial colony but nevertheless survived. The same isolate had the highest growth rate on PDA, statistically significant during all three photoperiods. Isolates BPTR 0207 and BPTR 0605 did not differ significantly on this medium in all three variants of growing. Isolates BPTR 0105 and BPTR 0705 formed an independent group with low to medium growth rate of mycelium.

Isolates developed comparatively faster on Oat agar compared to the other two agars, same as in the studies of Mielke (199). Isolates BPTR 0105 and BPTR0207 showed no significant differences under all three control growth conditions with the largest rate of mycelial radial growth. However, those isolates were significantly different on the other two media. Isolates BPTR 0107 and BPTR 0307 were assigned to the same statistical group with comparatively high degree of mycelium radial growth. Isolates BPTR 0507, BPTR 0605 and BPTR 0405, BPTR 0705 formed two statistically proven different groups in permanent light conditions and 12 h light / 12 h darkness with slow to medium mycelial growth rate.

Conclusion

For the objective of this study, for the first time in our country we isolated ascosporic isolates of the pathogen sexual form of *Pyrenophora tritici-repentis* and cultivated them on different nutrient media in different controlled conditions. We found that isolates showed significant differences on the same medium and growing conditions.

These were the first studies on morphology and culture peculiarities of this pathogen mycelium in Bulgaria. For the

first time, ascospores of one and the same ascus were isolated and characterized.

The studies revealed that pathogen population consisted of genetically different entities. These differences were most obvious in ascospores, isolated from the same ascus.

It was found that pathogen population in closely located fields consisted of specimens of large phenotypical and genotypic variability. It would take further research to characterize the genotype of *Pyrenophora tritici-repentis* and its relationship with the host, estimated by different types of resistance and specific correlations.

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