The comparison of the Russian and the Czech populations of *Rhynchosporium secalis* (Oud.) J.J. Davis in morphological characteristics and isoymes markers

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Abstract

Barley is the most important culture in the most regions of the world. It is the base of animal feeding and the raw material for bear production. The main factors which limited the quantity and quality of barley yield are diseases. The most widely distributed and harmful diseases of barley in the whole barley cultivation areas are leaf scald caused by *Rhynchosporium secalis* (Oudem.) J.J.Davis. Yield losses on the susceptible cultivars may reach nearly 40% under epidemic conditions. In the last few years, severe epidemics have occurred especially in the northern and central areas of Europe after a more intensive cultivation of barley and the widespread usage of foreign cultivars that are not adapted to the local scald population. The main difficulty in the genetic control of scald is variability of pathogen populations. The *R.secalis* population from each of the barley producing areas in the world comprises several unique races that differ in their ability to attack different barley cultivars.

The aim of our research was to study and comparison of the Russian and the Czech populations of *Rhynchosporium secalis* using morphological (colony colors), biochemical (α–esterase, β-esterase, superoxidedismutase, aspartataminotransferase) markers. In total 46 (one isolate from one scald lesion) isolates from Russia and 127 ones from five sampling sites of the Czech Republic were studied. Single-spore cultures were divided on color into 5 groups (from black to beige color). The majority of the single-spore cultures (89, 8%) kept their color during all period of growth. The most single-spores culture was genetically homogenous on studying sign. Four enzymes of fungus were studied - α–esterase, β-esterase, superoxidedismutase (*SOD*), aspartataminotransferase (*AAT*) by using vertical slab polyacrylamide gel electrophoresis. A minimum of three replicates, were examined for each isolate. All investigated enzymes of *R.secalis* were polymorphic. A total of nine patterns *SOD*, fifteen - α–esterase, three -β-esterase and two *AAT* were obtained. Almost every studied site had the certain major type of polymorphism on esterases and *SOD*. Unique patterns were identified in Russia and in the Czech Republic.

Key Words: barley scald, genetic diversity, population genetics, *Rhynchosporium secalis* (Oud.) J.J. Davis

Introduction

Barley scald is caused by the haploid imperfect fungus *Rhynchosporium secalis*. It is an important disease of barley (*Hordeum vulgare* L.) in the cool, humid areas of the world. Yield losses ranging from 10 to 40 % have been reported (McDonald et al., 1999). In the last few years, severe epidemics have occurred especially in the northern and central areas of Europe after a more intensive cultivation of barley and the widespread usage of foreign cultivars that are
not adapted to the local scald population (Brown 1985; McDermott et al., 1989; Jørgensen & Smedegaard-Petersen, 1995). The main difficulty in the genetic control of scald is variability of pathogen populations. The *R. secalis* population from each of the barley producing areas in the world comprises several unique races that differ in their ability to attack different barley cultivars. Pathogenicity, isozyme, colony color, ribosomal DNA and RFLP DNA markers have been used to study genetic variability in *R. secalis* populations (Goodwin et al., 1993, 1994; McDermott et al., 1989; Salamati et al., 2000).

Although the sexual stage has not been reported for *R. secalis*, the recent studies of geographically diverse collections of isolates showed that most of the alleles at isozyme loci were in gametic equilibrium (Burdon et al., 1994).

**Materials and Methods**

Infected plant material was collected from naturally infected barley fields during the summer of 2003 from one location in Russia (North-West region) and from five locations in the Czech Republic (the Moravia region). The collections was made using hierarchical sampling strategies (McDonald et al. 1999).

Leaf pieces with scald lesions were sterilized with 70% ethanol and 1% sodium hypochlorite for 30 s, rinsed in pure water and placed on potato-saccharose agar (PSA) with 1% yeastrel. Leaf sections were incubated at 18°C in darkness for two weeks, until the sporulating colonies were visible. A total of 127 isolates (one isolate from one scald lesion) from the Czech Republic and 46 ones from Russia were obtained (Table 1). Single-spore cultures were selected from each isolate and incubated at 18°C for 6 weeks.

Isozyme analyses were performed on enzyme extracts prepared from single-spore cultures grown in PSA medium. Mycelium of the fungus was ground with a little quantity of glass (Polyclar AT) and added extraction buffer (sucrose, ascorbic acid, cysteine hydrochloride in tris-HCL buffer, pH 8.0, based on the recipe of Sako, Stachmann (1972). These homogenates were transferred to tubes and centrifuged at 12 000 g at 0°C for 15 min. The resulting supernanant was used for electrophoresis.

Division of isozymes has been carried out in the device for electrophoresis with vertical slab polyacrylamide gel with tris-glyzyn buffer (pH 8.3). 7.5% polyacrylamide gel was used for *AAT* and *SOD*, 6.0% one for esterases. A constant current from 50 to 100 mA was maintained throughout electrophoresis for 2.5 h.

Gel slices were stained for enzyme activity following published protocols: *AAT* by Brown et al. (1978), *SOD* by Beauchamp & Fridovich (1971), α-esterase and β-esterase by Wehling (1986) where α and β-naphtylacetates were utilized as substrates.

A minimum of three replicates were examined for each isolate.

A relative mobility value ($R_f$) was assigned to each band of enzyme activity detected. For each isolate, presence or absence of a band of enzyme activity for each enzyme used in this study was defined as an electrophoretic phenotype. Electrophoretic phenotype similarities were inferred from a cluster analysis. The dendrogram was constructed by using UPGMA (unweighted pair-group method using arithmetic averages) procedure in Neighbor-Joining program of PHYLIP v.3.6 package. Standard and molecular diversity indices and genetic differentiation between and within isolate groups, collected from different sites were calculated by Arlequin software.
Results

A total of 173 isolates was included in this study. The colonies of *R. secalis* become visible usually only after 14 days growing on PSA with 1% yeast extract. Small, light, yeast-like, growing upward colonies were formed on media. All isolates were divided into 5 groups on the color after 21 days of cultivation (Figure 1). Dark-colored isolates as usual have mycelia manner of growth, but lightly colored isolates have yeast-like type of growth.

However, after 6 weeks of growth on PSA, the greater part of all isolates with light color darkened and changed color into black, brown and black with a white bloom. The isolates became more dense and developed mycelium. Only 2% of all isolates kept beige color and yeast-like structure. The majority of the single-spore cultures (89.8%) isolated from each group kept structure and color typical for each group. Only small part of light cultures discolored and became dark. They did not change their color and structure at each passage on medium.

All investigated enzymes of *R. secalis* were polymorphic. A total of nine patterns of SOD, fifteen -α–esterase, three -β-esterase and two AAT were obtained. Six SOD, two AAT, six α–esterase and two β-esterase bands were identified.

Genetic diversity of the Czech and Russian populations is represented by 95 different electrophoretic phenotypes. In other words, almost 50% of *R. secalis* isolates had unique genotypes. The pairwise comparison of genetic variations between isolates and within each site was tested statistically. Values of FST (population differentiation) greater than 0.25 indicate significant genetic differentiation (Hartl & Clarke, 1997). The level of differentiation among six sites ranged from 0.09 to 0.38 (Table 2). Significant levels of gene and genotype diversity were found (Table 3). Gene diversity ranged from 1.00 to 0.93 within locations. The Russian populations possessed more gene diversity over loci than the CZ population. Also, all field populations showed high degrees of genetic variability (Table 3).

Discussion

The results of this experiment show that populations of *R. secalis*, collected from naturally infected barley fields from one location in Russia (North-West region) and from five locations in the Czech Republic (the Moravia region) are significantly variable phenotypically for morphology (color of colony), biochemically by isozymes structure. Ninety-five different electrophoretic phenotypes were identified: 54 from 173 isolates were unique. Gene and genotype diversity was high in all six locations. similar genetic variability for pathogenicity, isozyme, ribosomal DNA and colony color variants in populations of *R. secalis* have been reported by other investigators (McDonald et al., 1989; Goodwin et al., 1994).

The highest level of genetic diversity in *R. secalis* in our study may be explained by several possible sources: spontaneous mutation and migration or parasexual recombination (Jackson & Webster, 1976; Newman & Owen, 1985). Although the sexual stage has not been reported for *R. secalis* till now, the study of geographically diverse collections of isolates from Australia, California, Finland and Norway using isozyme (Burdon et al., 1994), RFLP markers and DNA fingerprints (McDonald et al., 1999, Salamati et al., 2000) provided additional evidence for regular recombination in studying populations.

The presence of the isolates with different genotypes confirms higher gene flow between five sites of the Moravian region of the Czech Republic. This gene flow can be considered as a result of longer-distance dispersal of conidia by wind swirling during storms or as a result of infected
seed transmission or wind blowing of small particles of infected straw during harvest. It is also possible that the observed genetic distances are due to differences in the historical movement of barley seeds between these sites. It is known, the Czech Republic is agrarian country with movement of farm products (barley seeds and straw used for animal feed).
We can believe that low pathogen genotype diversity at this site was mainly due to the higher plasticity of the pathogen, quicker adaptation to changes in the environment, including introduction of new resistance genes. Therefore, the traditional method of introducing single major resistance genes into productive cultivars may not provide long-term control of barley scald disease.

References
### Pictures and Photos

Figure 1. Variability of *Rhynchosporium secalis* in morphological (color) markers

### Tables Legends

**Table 1. Isolates of *Rhynchosporium secalis***

<table>
<thead>
<tr>
<th>No. of group</th>
<th>Isolates</th>
<th>No. of isolates</th>
<th>Origin of isolates</th>
<th>Locations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>K-1,-2,-3,-4,-5,-6,-7,-8,-9,-10,-11,-12,-15,-16,-17; N -1,-2,-3,-5; M -1,-2,-3,-4,-6,-7,-8,-9.</td>
<td>15</td>
<td>Kompakt</td>
<td>The Czech Republic, Kroměříž</td>
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<tr>
<td></td>
<td></td>
<td>4</td>
<td>Nelly Malz</td>
<td></td>
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<td></td>
<td></td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Ak -1,-2,-5,-6,-7.</td>
<td>5</td>
<td>Akcent</td>
<td>The Czech Republic, Medlov</td>
</tr>
<tr>
<td>3</td>
<td>L -1,-2,-4,-6,-8,-9,-11,-12,-13,-14,-15,-16,-17,-18,-19,-20,-21,-22,-23,-25,-26,-27,-28,-29,-30,-31,-33,-34,-35,-36.</td>
<td>30</td>
<td>-</td>
<td>The Czech Republic, Lysice</td>
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<tr>
<td></td>
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</tr>
<tr>
<td>Location</td>
<td>Samples</td>
<td>Gene diversity</td>
<td>No. of genotypes</td>
<td>Average gene diversity over loci</td>
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<tr>
<td>----------</td>
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<td>---------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>27</td>
<td>0.935 ± 0.031</td>
<td>16</td>
<td>0.354 ± 0.195</td>
</tr>
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<td>5</td>
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<td>5</td>
<td>0.30 ± 0.20</td>
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<tr>
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<td>17</td>
<td>0.289 ± 0.162</td>
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<tr>
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<td>46</td>
<td>1.000 ± 0.005</td>
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<td>0.444 ± 0.236</td>
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