

Structure of Polish Isolates of *Bipolaris sorokiniana* and Effect of Different Pathotypes on Spot Blotch Severity of Selected Spring Barley Cultivars

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Spot blotch of barley (*Hordeum vulgare* L.), caused by *Bipolaris sorokiniana*, is worldwide and economically one of the most important diseases. The structure of the *B. sorokiniana* population is not uniform. Within isolates of this species, there are strains that differ in virulence and aggressiveness. The aim of the study was to determine the variability of virulence within Polish isolates of *B. sorokiniana* and to test selected strains of this fungus terms of their pathogenicity in relation to different spring barley cultivars. The diversity of 70 Polish isolates of *B. sorokiniana* was determined based on the reaction of three spring barley test lines – Bowman, ND5883 and NDB12 with a certain susceptibility to infection by this pathogen and compared to three isolates of *B. sorokiniana*: ND93-1 classified to pathotype 0, ND85F – pathotype 1 and ND90Pr – pathotype 2. In the population of 70 isolates of *B. sorokiniana*, two pathotypes – 0 (14 isolates) and 1 (56 isolates) were identified. The mean values of leaf infection index evaluated for lines – Bowman, ND5883 and NDB12 in the case of *B. sorokiniana* isolates of pathotype 0 ranged: 17.08, 25.42 and 18.13, respectively, and in the case of *B. sorokiniana* isolates of pathotype 1: 15.57, 59.81 and 17.98, respectively. In the second experiment, the susceptibility of 8 spring barley cultivars to leaf infection by 10 selected isolates of *B. sorokiniana* (5 of pathotype 0 and 5 of pathotype 1) was tested. The mean value of leaf infection index calculated for analyzed cultivars in experimental combination with pathotype 0 isolates of *B. sorokiniana* was 1.56, and in the case of isolates of pathotype 1 was 16.58.

Keywords: *Bipolaris sorokiniana*, pathogenicity, infection, pathotypes, disease index

Introduction

Bipolaris sorokiniana Shoemaker causes foliar spot blotch (syn. leaf blight), root rot, and black point on grains, head blight and seedling blight of barley, wheat (*Triticum* spp.) and oat (*Avena sativa* L.) (Kumar et al. 2002; Kiecana and Cegielko 2007; Knight et al. 2010; Jahani et al. 2014). Spot blotch of barley and wheat caused by *B. sorokiniana* is worldwide and economically one of the most important diseases. If weather conditions are

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conducive, i.e. continuous rain for 5–6 days followed by warmer temperatures (day average of 20–30 °C), spot blotch epidemic can develop very rapidly (Zhong and Steffenson 2001; Kumar et al. 2002; Ahmed et al. 2003; Ghazvini and Tekauz 2007). Under such conditions, yield losses from 16 to 33% can occur in susceptible barley cultivars (Zhong and Steffenson 2001). Spot blotch results in reduced photosynthetic area and can lead to premature senescence of the infected leaves as well as death of the plant under severe cases (Ghazvini and Tekauz 2004 according to Gyawali et al. 2012). *Bipolaris sorokiniana* can produce secondary metabolites with phytotoxic effects (Kumar et al. 2002; Jahani et al. 2014). They can play an important role in the etiology of spot blotch disease, affect the permeability of cell membranes, or the germination of cereal grains (Kumar et al. 2002; Jahani et al. 2014). It was found that the structure of the *B. sorokiniana* population is not uniform. Within isolates of this species, there are strains that differ in both morphological and physiological traits as well as the virulence and aggressiveness associated with the genetic variability of this fungus (Zhong and Steffenson 2001; Arabi and Jahwar 2004; Pandley et al. 2008; Poloni et al. 2009; Gyawali et al. 2012; Jahani et al. 2014; Mann et al. 2014). Physiological specialization of *B. sorokiniana* at the species level was first described by Christensen (1926 according to Kumar et al. 2002). The author showed that fungal isolates varied considerably in virulence to wheat (*T. aestivum* L.) and barley. It was observed that field populations shifted to more aggressive races with long-term continuous cultivation of one species (Kumar et al. 2002). Valjavec-Gratian and Steffenson (1997) identified three pathotypes of the fungus from 33 *B. sorokiniana* isolates of North Dakota (designed 0, 1 and 2). Recent studies based on large numbers of strains of this fungus, collected from different world regions suggest that *B. sorokiniana* includes isolates varying in virulence and aggressiveness with specific and nonspecific interactions (Kumar et al. 2002; Poloni et al. 2009). Fusions between hyphae, that stem from different spores, may result in somatic hybridization and the emergence of new fungal variants (Kumar et al. 2002). The aim of the study was to determine the variability of virulence within Polish isolates of *B. sorokiniana* and to test selected strains of this fungus belonging to the previously described pathotypes 0 and 1 in terms of their pathogenicity in relation to different spring barley cultivars.

Materials and Methods

The greenhouse experiment was conducted in 2016 in the Department of Plant Pathology, North Dakota State University (NDSU), Fargo, USA. Three barley lines: Bowman, NDB 112 and ND 5883 were tested for their reaction to spot blotch infection by seventy single spore Polish isolates of *B. sorokiniana* (Table 1) and three previously tested isolates from the USA classified to pathotypes 0, 1, 2 were used as a control: isolate ND93-1 – pathotype 0, isolate ND85F – pathotype 1 and isolate ND90Pr – pathotype 2. In the greenhouse trials, two replicates (2 seeds per replicate) of each barley line were planted in plastic cones (18.4 cm deep and 3.8 cm diameter) containing a 75% peat moss and 25% perlite. The experimental conditions were the same as described by Fetch and Steffenson (1999). Seedlings were inoculated with conidial suspensions (8000 conidia per ml) of individual

B. sorokiniana isolates at the two-leaf stage (14 days old). Inoculum (approximately 0.15 ml per plant) was applied to seedlings with an atomizer (model 15; DeVilbiss Co., Somerset, PA) pressured (55 kPa) by an air pump. After inoculation, plants were incubated in the dark for 16 h in chambers (21 °C with relative humidity near 100%) misted by ultrasonic humidifiers. Next, the plants were replaced to the greenhouse under the same conditions previously described. The investigations of the susceptibility of 8 spring barley cultivars (Table 2) to leaf infection by 10 representative isolates of *B. sorokiniana* previously tested in greenhouse experiment in the NDSU, belonging to pathotype 0 or 1 (Tables 2, 3) were conducted in the Department of Plant Protection, the University of Life Sciences in Lublin (Poland) in a growth chamber by the method described by Mańka (1989). Ten seeds per replicate of each barley cv. were planted in plastic pots (18.4 cm deep and 10 cm diameter). Each experimental treatment had three replicates, with 10 plants in each. The method of inoculum preparation and inoculation, and the period of plant growth were the same as previously described. The control combination consisted of pots in which the leaves of 8 barley cv. were sprayed with sterile distilled water. In both experiments second leaves of seedlings were assessed for their infection responses (IR) 7 days after inoculation using the 1 to 9 degree graphical rating scale. The rating scale was developed based on the type (presence of necrosis and chlorosis) and relative size of spot blotch lesions observed on the second leaves of barley seedlings. The nine IRs were classified into three general categories of low (IRs 1 to 3), intermediate (IRs 4 and 5), and high (IRs 6 to 9) host-parasite compatibility. Low IRs consisted of minute to small necrotic lesions with no or very slight diffuse marginal chlorosis. Intermediate IRs consisted of medium-sized necrotic lesions with a distinct but restricted chlorotic margin, while high IRs consisted of large necrotic lesions with distinct chlorotic margins and varying degrees of expanding diffuse chlorosis (Fetch and Steffenson 1999). The disease indexes for leaves of analyzed lines inoculated with different *B. sorokiniana* isolates were calculated using the formula of McKinney (Cegielko 2006).

The values of infection indexes for leaves of barley lines derived from the growth chamber and greenhouse experiments were statistically analyzed using Statgraphics 4.1 for Windows (Statistical Graphics Corp. 1999, Statpoint Technologies, Inc. Warrenton, Virginia, USA). The significance of mean differences was evaluated by one-way analysis of variance. The statistical hypotheses were tested with an error rate of $\alpha = 0.05$.

Results

In the population of 70 Polish isolates of *B. sorokiniana*, tested under controlled temperature and humidity conditions in the Department of Plant Pathology, NDSU, two pathotypes – 0 and 1 were found based on the reaction of leaves of three barley test cultivars with known different susceptibility to infection by this pathogen, to infection by individual isolates of this fungus (Table 1). Fourteen of these *B. sorokiniana* isolates belonged to the pathotype 0 and 56 to the pathotype 1. Pathotype 1 isolates of *B. sorokiniana* exhibited high virulence on ND5883, but low virulence on Bowman and NDB112 lines. Pathotype 0 isolates exhibited low virulence on all three barley genotypes. None of Polish

Table 1. Comparison of susceptibility of the barley lines to infection with *B. sorokiniana* isolates of pathotypes 0, 1 and 2, based on the value of leaves infection index

| <i>Bipolaris sorokiniana</i> isolates | | Host plant (year of isolation) | Barley lines | | | Mean value of infection index |
|---------------------------------------|----------------------|--------------------------------|--------------|---------|---------|-------------------------------|
| | | | Bowman | ND5883 | NDB112 | |
| Pathotype 0 | 28Co | corn-grain (2015) | 12.50 A | 25.00 C | 12.50 A | 16.67 AB |
| | 47Ba | barley-stem base (2015) | 18.75 B | 43.75 F | 25.00 A | 29.17 C |
| | 48Ba | barley-roots (2016) | 12.50 A | 25.00 C | 18.75 A | 18.75 AC |
| | 51Ba | barley-stem base (2012) | 31.25 D | 31.25 D | 25.00 A | 29.17 C |
| | 61Ba | barley-stem base (2015) | 18.75 B | 18.75 B | 18.75 A | 18.75 AC |
| | 63Ba | barley-stem base (2016) | 18.75 B | 25.00 C | 12.50 A | 18.75 AC |
| | 64Ba | barley-stem base (2016) | 12.50 A | 31.25 D | 18.75 A | 20.83 AC |
| | 65Ba | barley-stem base (2016) | 25.00 C | 31.25 D | 25.00 A | 27.08 BC |
| | 66Ba | barley-stem base (2015) | 18.75 B | 31.25 D | 12.50 A | 20.83 AC |
| | 73Ba | barley-stem base (2015) | 12.50 A | 37.50 E | 12.50 A | 20.83 AC |
| | 78Oa | oat-stem base (2016) | 25.00 C | 18.75 B | 18.75 A | 20.83 AC |
| | 80a | oat-leaves (2015) | 12.50 A | 25.00 C | 25.00 A | 20.83 AC |
| | 81Ba | barley-stem base (2015) | 12.50 A | 12.50 A | 18.75 A | 14.58 A |
| | 80Ba | barley-stem base (2016) | 12.50 A | 12.50 A | 12.50 A | 12.50 A |
| ND93-1 | barley-grain (1993) | 12.50 A | 12.50 A | 15.63 A | 13.54 A | |
| Mean value of infection index | | | 17.08 | 25.42 | 18.13 | 20.21 |
| Pathotype 1 | 3Ba | barley-leaves (2016) | 12.50 A | 12.50 A | 12.50 A | 12.50 A |
| | 58Ba | barley-roots (2013) | 12.50 A | 12.50 A | 18.75 A | 14.58 A |
| | 53Ba | barley-leaves (2013) | 12.50 A | 18.75 A | 12.50 A | 14.58 A |
| | 54Ba | barley-stem base (2015) | 12.50 A | 25.00 A | 12.50 A | 16.67 A |
| | 68Ba | barley-stem base (2015) | 12.50 A | 25.00 A | 12.50 A | 16.67 A |
| | 33Ba | barley-stem base (2016) | 12.50 A | 18.75 A | 18.75 A | 16.67 A |
| | 74Ba | barley-stem base (2015) | 12.50 A | 31.25 A | 12.50 A | 18.75 A |
| | 60Ba | barley-stem base (2015) | 12.50 A | 25.00 A | 18.75 A | 18.75 A |
| | 62Ba | barley-stem base (2016) | 12.50 A | 25.00 A | 18.75 A | 18.75 A |
| | 50Ba | barley-stem base (2016) | 12.50 A | 31.25 A | 12.50 A | 18.75 A |
| | 2Ba | barley-leaves (2015) | 12.50 A | 43.75 A | 12.50 A | 22.92 A |
| | 34Ba | barley-stem base (2016) | 12.50 A | 31.25 A | 25.00 A | 22.92 A |
| | 79Ba | barley-stem base (2016) | 12.50 A | 50.00 A | 12.50 A | 25.00 A |
| | 37Oa | oat-leaves (2014) | 12.50 A | 50.00 A | 12.50 A | 25.00 A |
| | 40Ba | barley-stem base (2015) | 18.75 B | 37.50 A | 25.00 A | 27.08 A |
| | 67Ba | barley-roots (2012) | 12.50 A | 62.50 A | 12.50 A | 29.17 A |
| 9Ba | barley-leaves (2016) | 12.50 A | 62.50 A | 12.50 A | 29.17 A | |

Table 1 (cont.)

| <i>Bipolaris sorokiniana</i> isolates | | Host plant (year of isolation) | Barley lines | | | Mean value of infection index |
|---------------------------------------|----------------------|--------------------------------|--------------|---------|---------|-------------------------------|
| | | | Bowman | ND5883 | NDB112 | |
| Pathotype 1 | 30Ba | barley-leaves (2015) | 12.50 A | 62.50 A | 12.50 A | 29.17 A |
| | 49Ba | barley-roots (2016) | 12.50 A | 68.75 A | 12.50 A | 31.25 A |
| | 45Ba | Barley-roots (2015) | 12.50 A | 62.50 A | 18.75 A | 31.25 A |
| | 72Ba | barley-roots (2016) | 18.75 B | 62.50 A | 12.50 A | 31.25 A |
| | 20Oa | oat-roots (2015) | 12.50 A | 56.25 A | 25.00 A | 31.25 A |
| | 70Ba | barley-roots (2016) | 18.75 B | 62.50 A | 12.50 A | 31.25 A |
| | 44Ba | barley-roots (2016) | 12.50 A | 68.75 A | 12.50 A | 31.25 A |
| | 36Ba | barley-stem base (2015) | 12.50 A | 68.75 A | 12.50 A | 31.25 A |
| | 7TC | barley-leaves (2015) | 12.50 A | 62.50 A | 18.75 A | 31.25 A |
| | 13Ba | barley-roots (2016) | 12.50 A | 56.25 A | 25.00 A | 31.25 A |
| | 77Ba | barley-leaves (2012) | 12.50 A | 62.50 A | 18.75 A | 31.25 A |
| | 75Ba | barley-stem base (2016) | 12.50 A | 68.75 A | 12.50 A | 31.25 A |
| | 52Ba | barley-leaves (2012) | 12.50 A | 68.75 A | 12.50 A | 31.25 A |
| | 24W | wheat-grain (2016) | 12.50 A | 68.75 A | 12.50 A | 31.25 A |
| | 22Ba | barley-leaves (2016) | 12.50 A | 75.00 A | 12.50 A | 33.33 A |
| | 42Ba | barley-roots (2015) | 12.50 A | 68.75 A | 18.75 A | 33.33 A |
| | 29W | wheat-grain (2015) | 12.50 A | 62.50 A | 25.00 A | 33.33 A |
| | 55Ba | barley-stem base (2014) | 12.50 A | 62.50 A | 25.00 A | 33.33 A |
| | 35Ba | barley-stem base (2016) | 18.75 B | 50.00 A | 31.25A | 33.33 A |
| | 43Ba | barley-roots (2016) | 12.50 A | 75.00 A | 18.75 A | 35.42 A |
| | 4Ba | barley-leaves (2016) | 12.50 A | 75.00 A | 18.75 A | 35.42 A |
| | 38Ba | barley-stem base (2016) | 18.75 B | 68.75 A | 18.75 A | 35.42 A |
| | 26Oa | oat-roots (2015) | 18.75 B | 75.00 A | 12.50 A | 35.42 A |
| | 46Ba | barley-stem base (2015) | 25.00 C | 68.75 A | 18.75 A | 37.50 A |
| | 15Ba | barley-leaves (2015) | 37.50 E | 56.25 A | 18.75 A | 37.50 A |
| | 76Ba | barley-roots (2016) | 12.50 A | 81.25 A | 18.75 A | 37.50 A |
| | 41Ba | barley-stem base (2015) | 12.50 A | 75.00 A | 25.00 A | 37.50 A |
| | 39Ba | barley-stem base (2016) | 25.00 C | 75.00 A | 18.75 A | 39.58 A |
| | 6Ba | barley-leaves (2014) | 25.00 C | 68.75 A | 18.75 A | 37.50 A |
| | 12Ba | barley-leaves (2016) | 25.00 C | 75.00 A | 18.75 A | 39.58 A |
| 18Ba | barley-leaves (2015) | 18.75 B | 87.50 A | 12.50 A | 39.58 A | |
| 31Oa | oat-leaves (2014) | 12.50 A | 81.25 A | 31.25 A | 41.67 A | |
| 57Ba | barley-leaves (2016) | 12.50 A | 87.50 A | 25.00 A | 41.67 A | |

Table 1 (cont.)

| <i>Bipolaris sorokiniana</i> isolates | | Host plant (year of isolation) | Barley lines | | | Mean value of infection index |
|--|--------|--------------------------------|--------------|---------|---------|-------------------------------|
| | | | Bowman | ND5883 | NDB112 | |
| Pathotype 1 | 56Ba | barley-leaves (2016) | 12.50 A | 87.50 A | 25.00 A | 41.67 A |
| | 25W | wheat-grain (2016) | 25.00 C | 81.25 A | 18.75 A | 41.67 A |
| | 71Ba | barley-leaves (2016) | 25.00 C | 68.75 A | 37.50 A | 43.75 A |
| | 5Ba | barley-leaves (2015) | 12.50 A | 75.00A | 12.50 A | 33.33 A |
| | 32Ba | barley-leaves (2015) | 25.00 C | 87.50 A | 25.00 A | 45.83 A |
| | 59Ba | barley-leaves (2015) | 31.25 D | 87.50A | 18.75 A | 45.83 A |
| | ND85F | barley-leaves (1985) | 12.50 A | 90.63 A | 18.75 A | 40.63 A |
| Mean value of infection index | | | 15.57 | 59.81 | 17.98 | 31.12 |
| Pathotype 2 | ND90Pr | barley-leaves (1990) | 90.63 | 21.88 | 15.63 | 42.71 |
| Mean value of infection index for all pathotypes | | | 16.91 a | 52.23 b | 17.98 a | 29.04 |

Different letters in columns next to the numbers indicate significant differences among barley lines in plant reaction to applied *B. sorokiniana* pathotype.

isolates tested exhibited high virulence on Bowman, but low virulence on ND5883 and NDB112, hence pathotype 2 was not found (Table 1). In the case of three *B. sorokiniana* isolates obtained in USA used for inoculation of leaves of barley lines tested, in addition to pathotypes 0 and 1, the pathotype 2 was found (Table 1).

In the case of *B. sorokiniana* isolates included in the pathotype 0, on leaves of seedlings of the test lines, small, necrotic spots were noted. The mean values of infection index evaluated for all three barley lines – Bowman, ND5883 and NDB112 in the case of isolates of this pathotype ranged respectively: 17.08, 25.42 and 18.13 (Table 1). In the case of *B. sorokiniana* isolates ranked into pathotype 1, mean values of leaf infection index of analyzed lines ranged: 15.57, 59.81 and 17.98 respectively (Table 1). In the case of the American isolate of *B. sorokiniana* ND90Pr 2, included in pathotype 2 the mean values of infection index ranged from 15.63 (NDB112) to 90.63 (Bowman) (Table 1).

In the growth chamber experiment conducted in the Department of Plant Protection, University of Life Sciences in Lublin, in the experimental combination with leaves of barley cultivars inoculated with *B. sorokiniana* isolates of pathotype 0, lesions on leaf blades of 8 analyzed cultivars ranged from about 0.3 to 1.3 mm in length and from 0.3 to 0.8 mm in width (1–3 degree on infection response rating scale). The mean values of disease indexes for leaves of analyzed cultivars in this experimental combination ranged from 0 to 21.67 (Table 2). In the case of plants of selected barley cultivars inoculated with isolates *B. sorokiniana* of the pathotype 1 – necrotic spots on leaf blades of analyzed cultivars were often bigger compared to those caused by pathotype 0 isolates of *B. sorokiniana*, elliptical, with chlorotic margin. They ranged from 0.5 to 2.0 mm in length and from 0.5 to 1.5 mm in width. (2–5 degree on infection response rating scale). The mean

Table 2. Comparison of the susceptibility of spring barley cultivars to infection with *B. sorokiniana* isolates of pathotype 0 based on the values of leaf infection index

| Isolates of <i>B. sorokiniana</i> | Barley cultivars | | | | | | | | Mean value of infection index |
|-----------------------------------|------------------|----------|---------|---------|----------|---------|---------|----------|-------------------------------|
| | Hajduczek | Kormoran | Promyk | Oberek | Serwal | Skald | Skarb | Stratus | |
| 48BA | 1.67 A | 5.00 ABC | 5.00 A | 12.50 B | 7.50 AB | 3.33 B | 9.17 B | 2.50 AB | 5.83 B |
| 64BA | 9.17 B | 8.33 CD | 9.17 AB | 21.67 C | 11.67 BC | 3.33 B | 12.50 B | 12.50 C | 11.04 C |
| 65BA | 2.50 A | 11.67 D | 5.83 A | 8.33 AB | 16.67 C | 2.50 AB | 3.33 A | 10.00 BC | 7.60 B |
| 61BA | 2.50 A | 6.67 BC | 15.00 B | 2.50 A | 13.33 BC | 1.67 AB | 13.33 B | 6.67 AC | 7.71 B |
| 780A | 0.00 A | 1.67 A | 5.00 A | 12.50 B | 11.67 BC | 1.67 AB | 4.17 A | 8.33 AC | 5.63 B |
| Mean value of infection index | 3.17 | 6.67 | 8.00 | 11.50 | 12.17 | 2.50 | 8.50 | 8.00 | 7.56 |
| Control | 0.00 A | 2.50 AB | 1.67 A | 0.83 A | 2.50 A | 0.83 A | 1.67 A | 0.83 A | 1.35 A |

Different letters in columns next to the numbers indicate significant differences among cultivars in reaction to *B. sorokiniana* pathotype 0 isolates.

Table 3. Comparison of the susceptibility of spring barley cultivars to infection with *B. sorokiniana* isolates of pathotype 1 based on the values of leaf infection index

| Isolates of <i>B. sorokiniana</i> | Barley cultivars | | | | | | | | Mean value of infection index |
|-----------------------------------|------------------|----------|---------|----------|----------|---------|----------|---------|-------------------------------|
| | Hajduczek | Kormoran | Promyk | Oberek | Serwal | Skald | Skarb | Stratus | |
| 72BA | 7.50 AB | 10.00 AB | 9.17 A | 35.00 C | 20.83 AB | 5.83 AB | 18.33 AB | 31.67 A | 17.29 BCD |
| 51BA | 11.67 B | 20.00 B | 23.33 A | 11.67 AB | 26.67 AB | 10.83 B | 16.67 AB | 30.00 A | 18.86 CD |
| 67BA | 10.00 AB | 10.00 AB | 9.17 A | 16.67 AC | 10.00 AB | 1.67 A | 8.33 AB | 11.67 A | 9.69 B |
| 13BA | 5.00 AB | 10.83 AB | 16.67 A | 16.67 AC | 13.33 AB | 8.33 AB | 24.17 AB | 15.00 A | 13.75 BC |
| 77BA | 4.17 AB | 15.00 AB | 27.50 A | 29.17 BC | 38.33 B | 6.67 AB | 38.33 B | 27.50 A | 23.33 D |
| Mean value of infection index | 7.67 | 13.17 | 17.17 | 21.84 | 21.83 | 6.67 | 21.17 | 23.17 | 16.58 |
| Control | 0.00 A | 2.50 A | 1.67 A | 0.83 A | 2.50 A | 0.83 A | 1.67 A | 0.83 A | 1.35 A |

Different letters in columns next to the numbers indicate significant differences among barley cultivars in reaction to *B. sorokiniana* pathotype 1 isolates.

values of disease indexes for leaves of analyzed spring barley cultivars inoculated with pathotype 1 isolates ranged from 1.67 to 38.33 (Table 3).

Among the *B. sorokiniana* isolates used in the growth chamber experiment, the highest mean value of the leaf infection index calculated for the 8 analyzed spring barley cultivars, was noted in the case of the *B. sorokiniana* isolate No. 77BA (Table 3). The mean values of disease indexes for leaves of control plants of 8 analyzed spring barley cultivars ranged from 0 to 2.50 (Table 3).

Results of pathogenicity test of *B. sorokiniana* isolates indicated diverse virulence of pathotype 0 subpopulation. Depending on the tested cultivars significant or nonsignificant differences in infection degree among isolates were observed. No significant differences in infection degree among isolates were noticed on cultivar, Skald (Table 2). All the other inoculated plant genotypes had significant differences in virulence among isolates of pathotype 0 (Table 2).

The pathogenicity test outcomes for isolates of pathotype 1 were more homogeneous. Generally no disease differences were found among isolates for almost all tested barley cultivars, exception was Oberek (Table 3). Reaction of this cultivar differentiated isolates into two groups, i.e. less and more pathogenic. Similar results were obtained for reaction of the American barley genotypes to infection with isolates of both *B. sorokiniana* pathotypes (Table 1). Barley cv. Bowman and ND5883 line differentiated the tested set of pathotype 0 isolates into a few independent pathogenicity groups. No such differentiation was noticed for pathotype 1 isolates (Table 1).

Discussion

The necrotrophic fungus *B. sorokiniana* has a high pathogenic variability (Zhong and Steffenson 2001; Kumar et al. 2002; Ghazvini and Tekauz 2007; Poloni et al. 2009; Gyawali 2010) which was confirmed by the results of our studies. The earlier investigations of Kumar et al. (2002), Cegielko (2006) and Poloni et al. (2009) showed the morphological diversity of this fungus too. A considerable level of interaction between different *B. sorokiniana* isolates and barley cultivars has been found (Valjavec-Gratian and Steffenson 1997; Zhong and Steffenson 2001; Arabi and Jawhar 2004).

In the case of Polish isolates of *B. sorokiniana* tested, two pathotypes (namely – 0 and 1) were found. Three different pathotypes of *B. sorokiniana* were previously identified by Zhong and Steffenson (2001) among the American population of the fungus in North Dakota, USA in the case of the same barley cultivars. The high value of leaf infection index in Bowman in the case of *B. sorokiniana* isolate ND90Pr (– characteristic of pathotype 2), confirmed the previous study of Zhong and Steffenson (2001). Ghazvini and Tekauz (2008) reported new virulence pathotypes of *B. sorokiniana* isolated from barley leaves in Canada, which exhibited high virulence on barley line NDB 112, considered as the major source of durable resistance to spot blotch in USA (Steffenson et al. 1996). In Canada eight virulence groups of *B. sorokiniana* were identified among 127 isolates of this fungus (Ghazvini and Tekauz 2007). The isolates of *B. sorokiniana* tested

in our study, classified as pathotypes 0 and 1, were not so different in terms of virulence in relation to the analyzed spring barley genotypes.

In the studies of harmfulness of fungi towards cereals different methods of inoculation are used (Mańka 1989; Almgren et al. 1999; Ghazini and Tekauz 2007; Kiecana and Cegielko 2007; Kiecana et al. 2012). In our studies of pathogenicity of *B. sorokiniana* to leaves of barley cultivars, the method of leaf inoculation using conidial suspension of the fungus proved to be effective under our conditions. In the case of *B. sorokiniana* isolates included in the pathotype 0, on leaves of seedlings of spring barley cultivars tested, small, necrotic spots were noted. Similar disease symptoms caused by *B. sorokiniana* on leaves of oat genotypes were observed by Cegielko (2006) in a growth chamber conditions.

The pathogenicity of *B. sorokiniana* is related to the production of non-host-specific toxins. According to Apoga et al. (2002) a correlation exists between prehelminthosporol production and the degree of virulence of *B. sorokiniana* isolates. The differentiation between leaf infection indexes in analyzed spring barley cultivars may be related to the amount of toxins produced by individual isolates.

Polish isolates of *B. sorokiniana* used to inoculate leaves of seedlings of eight spring barley cultivars, obtained from barley leaves, proved to be more damaging to these organs of plants as compared to the isolates obtained from the roots or stem base of barley and oat. This result is in agreement with Gyawali (2010) who reported that isolates *B. sorokiniana* originated from leaves with spot blotch symptoms were more virulent in causing spot blotch in barley, whereas the most isolates of this fungus from roots were more virulent in causing common root rot of barley. Gyawali et al. (2012) showed the genetic diversity among *B. sorokiniana* population originated from different host plant tissue. These results suggested that this fungus may have some level of host and tissue specificity. Virulence heterogeneity noticed on some spring barley cultivars tested may suggest that Polish population of pathotype 0 isolates do not represent uniform, fully characterized group of low pathogenicity isolates only. A further virulence analysis seems to be necessary to differentiate pathotypes and develop a better set of differentials using new host genotypes better characterize *B. sorokiniana* pathogenicity, especially the subpopulation classified as pathotype 0.

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