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The Infection of Barley at Different Growth Stages by *Bipolaris sorokiniana* and Its Effect on Plant Yield and Sowing Value

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Abstract: *Bipolaris sorokiniana*, a barley pathogen, can infect via seeds, co-growing plants, or soil, causing yield and seed value reductions. This study aimed to assess *B. sorokiniana*'s impact at various growth stages on seed yield and quality. Results showed no direct impact on yield (which ranged from 0.49 kg·m^{-2} for the naked variety Rastik to 0.77 kg·m^{-2} for the Widawa variety), but revealed significant seed quality differences. Thousand kernel weight (TKW) and germination capacity (GC) varied notably between examined varieties. The largest kernels were observed for the Bryl variety (27.33 g), which also had the best germination (82.8%). The variety Ryton had the smallest kernels (24.04 g), while the poorest germination (56.7%) was observed for naked kernels of Rastik. A seed health analysis found a relation between inoculation stage and the intensity of spontaneous infection by pathogenic fungi, ranging from 86.4% for the control to more than 95% for the kernels harvested from plants inoculated at the flowering stage. Strong correlation emerged between *B. sorokiniana* grain infection and germination capacity, highlighting the fungus's role in seed quality decline.

Keywords: barley; *Bipolaris sorokiniana*; growth phases; infection; sowing value; yield

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1. Introduction

The fungus *Bipolaris sorokiniana* is a known pathogen of barley and the causative agent of common root rot, leaf spot disease, seedling blight and black point [1]. This pathogen can be found not only in barley, but also in wheat and many grass species around the world [2–5].

This fungus is a warm climate zone pathogen, and its presence in a cooler climate should not cause serious damage to crops [6]. The symptoms of leaf blotch, which refers to the appearance of irregular, discolored patches on the leaves, are most frequent in regions with high humidity and temperatures between 22°C and 30 °C during the growing season [7,8]. However, due to the dynamic climate changes, there is an increasing loss in yield caused by *B. sorokiniana*, so it was decided to deal with this species more widely. The impact of *B. sorokiniana* on seed quality has been a subject of research in various regions. In regions with insufficient moisture, the infection causes diseases of seedlings and the development of root rot, while in areas with sufficient or excessive moisture, it can damage seeds [9]. Furthermore, *B. sorokiniana* has been associated with reducing seed quality and germination in wheat, leading to black point disease and affecting grain quality and trade. The fungus has been shown to infect wild and cultivated barley, causing spot blotch disease, which further emphasizes its significance in affecting the quality of barley and wheat seeds [10,11].

The pathogen survives as mycelium in infected seeds or as a saprotroph on dead plant tissues [12]. In cocci, mycelium may remain viable for up to four years [13]. Additionally, *B. sorokiniana* is a facultative parasite that can live saprophytically in soil. It survives as thick-walled spores, which are the main causative agent of root rot [14]. The harmfulness of *B. sorokiniana* depends not only on the weather conditions, but above all on the susceptibility

of the cultivars [15,16]. An equally important issue is the pathogenic specialization of this species, which was first described by Christensen [17]. It is well known that the populations of this pathogen are characterized by a high variability of virulence.

When barley plants are infected with *Bipolaris sorokiniana*, the fungus causes the appearance of characteristic brown lesions on the leaves, stems, and spikes of the plant. These lesions can coalesce, causing significant damage to the plant and reducing the photosynthetic capacity of the leaves. The reduction in photosynthetic capacity, combined with the loss of plant tissue due to the lesions, can lead to a decrease in barley yield. In severe cases, the disease can cause yield losses of up to 30% [15,18].

The source of infection can be both seed and soil. In the case of infected grain, infection occurs through the mycelium located between the chaff and the fruit-seed cover and through conidia contaminating the surface of the kernel [6]. The frequency of infection of grain by *B. sorokiniana* depends on the amount of infectious material in the surroundings of the plants during flowering and grain formation. Most often, they are conidia carried by air currents from infected leaves. High humidity favors the assimilation apparatus of the leaves, sporulation and grain infection in the ears [19]. Diseased seedlings grow out of the infected seed, and on the sheaths and leaf blades the fungus forms conidia causing secondary infections.

An important discovery from this research is the significant influence of the barley's development phase, during which the pathogen *B. sorokiniana* infects the plant, on the size of the harvested grains, their germination, and colonization by microscopic fungi. In addition our results reflect the significant impact of *B. sorokiniana* on seed quality, particularly in terms of germination capacity and grain infection, highlighting the importance of managing this pathogen to ensure high-quality barley and wheat production. Many studies confirm these results that seed infection by *B. sorokiniana* may result in reduced seed quality and germination ability [10,20], but no information has been found on the impact of the time during which infection occurs.

The aim of this article was to study the effect of the development stage of barley, when the infection by *B. sorokiniana* occurs, on the yield and seed quality.

2. Materials and Methods

2.1. Plant Infection at Different Development Stages

2.1.1. Isolation of Fungus Isolate and Its Aggressiveness

The fungus material used in this study was collected from different samples of barley kernels from different European breeding companies (Table 1). The presence of *B. sorokiniana* was confirmed by comparing them with the characteristic features of the fungus: the characteristic appearance of the conidial spores (Figure 1) of the fungus and the colony on PDA (Figure 2) [21]. The pathogen was isolated on potato dextrose agar (PDA) medium, purified by monoconidial isolation [22], and stored at 5 °C on PDA. Hereafter, the monoconidial cultures are referred to as the isolates. In addition, the identification was confirmed by a molecular test based on the methodology of Zhao et al. [23].

The aggressiveness of five isolates (BS1Ra, BS2Ra, BS3Ra, BS4Ra, BS5R) was tested on susceptible (cv. Widawa) and resistant (cv. Lailla) genotypes grown under controlled conditions in the greenhouse [3]. Fifteen plants from each genotype were planted in pots which were each sprayed with spore suspensions of examined isolates (2.5×10^5 spores/1 mL) on the flag leaf. Disease severity, as shown in Figure 3 (9-point scale, where 1 is 50% of the infected flag leaf area and 9—no symptoms) was recorded at three different growth stages of plant growth (GS 57: 70% of inflorescence emerged; GS69: end of flowering: all spikelets have completed flowering, but some dehydrated anthers may remain; and GW77: late milk) given by Zadoks et al. [24].

Additionally in the laboratory, the pathogenicity of these isolates was determined on a sample of 100 barley kernels that were tested. This method for determining the susceptibility of cultivars to *B. sorokiniana* consisted in assessing the infection of 7-day-old

seedlings according to a 5-point scale (Figure 4) and calculating the disease index according to the formula given by Łacicowa [25].

Table 1. Cultivar names with seed provider name and country.

Cultivar Name	Country of Origin	Breeder/Seed Provider
Antek	AUT	Probstdorfer Saatzucht GesmbH&Co. KG, Saatzuchtstraße, Austria
Barke	DEU	Saatzucht Josef Breun GmbH&Co. KG, Herzogenaurach, Germany
Bryl	POL	Hodowla Roślin Smolice Sp. z o.o Grupa IHAR, Smolice, Poland
Hanka	DEU	Lantmännen SW Seed Hadmersleben GmbH, Stockholm, Sweden
Johan	AUT	Probstdorfer Saatzucht GesmbH&Co. KG, Saatzuchtstraße, Austria
Justina	DEU	Nordsaat Saatzucht GmbH Saatzucht Langestein, Langenstein, Germany
Lailla	DEU	Lantmännen SW Seed Hadmersleben GmbH, Stockholm, Sweden
Prosa	AUT	Probstdorfer Saatzucht GesmbH&Co. KG, Saatzuchtstraße, Austria
Rastik	POL	Hodowla Roślin Smolice Sp. z o.o Grupa IHAR, Smolice, Poland
Refren	POL	Hodowla Roślin Smolice Sp. z o.o Grupa IHAR, Smolice, Poland
Ryton	POL	Hodowla Roślin Smolice Sp. z o.o Grupa IHAR, Smolice, Poland
Widawa	DEU	KWS Lochow GmbH, Bergen, Germany

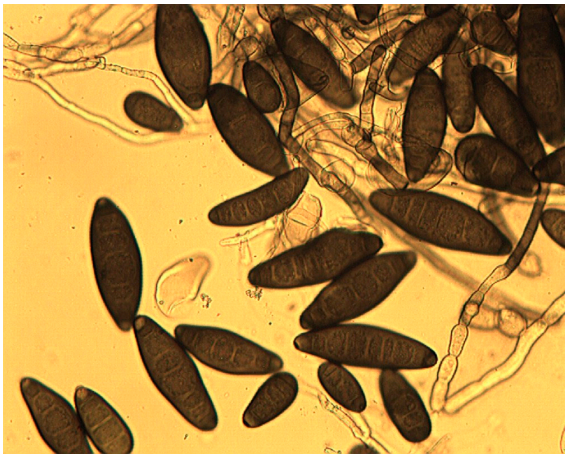


Figure 1. Conidial spores.

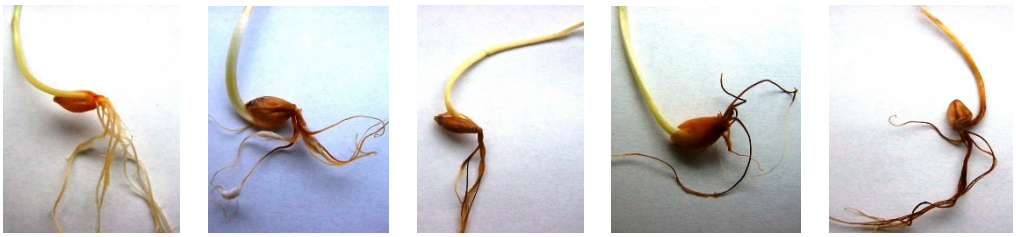


Figure 2. Colonies on PDA medium.



9 8 7 6 5 4 3 2 1

Figure 3. Leaf infection scale. Plant infection symptoms as related (in %) to the leaf area: 1—at least 50%; 2—40%; 3—30%; 4—20%; 5—15%; 6—10%; 7—5%; 8—1% (traces of disease), 9—0% (no signs of disease).



0° 1° 2° 3° 4°

Figure 4. Scale of the seedling infection.

The isolate BS2Ra, as the most pathogenic in the greenhouse experiment (mean 3.6) and in the laboratory test (52% ungerminated seed and 34% infected seedlings) was selected and used for further research.

2.1.2. Preparation of the Inoculum

The inoculum was prepared on the basis of a monospore culture of chosen *B. sorokiniana* isolate (BS2Ra), which was previously isolated from the barley kernels and incubated for 14 days at 24 °C in alternating 360 nm NUV lighting and darkness (12 h/12 h). A suspension of conidial spores with a concentration of 2.5×10^5 in 1 mL of solution was prepared and used for further research in this experiment.

2.1.3. Laboratory Experiment

The research was carried out on seeds and plants of 12 spring barley (*Hordeum vulgare* L.) cultivars, all from European breeding stations (Table 1).

The randomized design was used with 6 treatments and 4 replicates per genotype. Seeds of tested cultivars were sown in March 2019 in metal pots (4 pots per genotype, 5000 cm^3, 20 cm in diameter, with drainage hole in the bottom), filled with ca. 6.0 kg of soil mixture (compost soil, sand, peat in equal proportions). The soil mixture pH was 7.6, bulk density—1.193 $g \cdot cm^{-3}$, sand—5.6%, silt—79.2% and clay 15.2% (sandy loam soil). Total N content was 0.28%, P—0.47% and K—2.42%. Soil organic matter content was 5.5%.

The seeds were sown to ensure 10 plants per pot, and the number of plants per pot was adjusted during further growth.

The experiment was performed in an unheated glasshouse. From seed sowing up to heading phase, plants were grown under long-day conditions (16 h light/8 h dark) at a mean temperature of 15 °C (20 °C in the day and 10 °C at night) (constant temperature, air conditioning) in approximately 45% relative humidity. Light intensity was determined to be ~138 µmol/m^2/s. After the heading (ca. 50% of plants started to elongate heads), growth conditions were chosen to simulate the average outdoor conditions observed on-site (Radzików, Poland). During plant growth, water and fertilization were added in quantities ensuring adequate plant growth. The following development phases were selected for the above treatment [26] (Table 2).

Table 2. Description of plant development phases used in article with references to the BBCH scale.

BBCH Scale	Phase Description	Term Used in Article
BBCH 00	dry seed (kernels)	kernels
BBCH 21–23	beginning of tillering, from 1st to 3rd tiller visible	tillering
BBCH 31–33	stem elongation—from 1st node to 3rd node at least 2 cm above 2nd node	stem elongation
BBCH 55	heading phase: half of inflorescence emerged	heading
BBCH 65	full flowering—50% anthers mature	flowering

The control consisted of non-inoculated plants. Plant inoculation was performed in the evening hours when the air temperature dropped below 20 °C. Plants were sprinkled with water for 3–4 days after inoculation to ensure adequate humidity to germinate the pathogen spores. After the harvest, the thousand kernel weight (TKW) and yield (YPP, expressed in kg per 1 m^2) were determined. The germination capacity (GC) and health of harvested kernels (SH) were assessed in the laboratory according to the ISTA Rules [27].

For health test, kernels were disinfected with 1% NaOCl for 10 min and then washed with sterile water three times. Disinfected seeds were placed on potato dextrose agar medium (PDA) with 0.003% streptomycin sulphate. Fungal colonies were grown at 20 °C in an alternating cycle of 12 h NUV light (360 nm) and 12 h darkness. Developed colonies were transferred to potato dextrose agar plates and incubated in the above-mentioned conditions to stimulate sporulation. Fungi were identified after 15–20 days of incubation according to the descriptions given by Barnett [28], Chidambaram et al. [29], Ellis [30], Kwaśna et al. [31] and Malone and Muskett [32]. Fungal isolates (pure cultures) were maintained on potato dextrose agar (PDA) slants, respectively, at 4 °C until used for further study.

2.2. Statistical Analysis

All calculations were made with STATISTICA® ver. 12 for Windows. Significance of differences was accepted with 95% probability. Two-way analysis of variance (ANOVA) was used to determine the effect of components of variation on the performance of evaluated traits. The diversity of the studied varieties was defined in various developmental stages in terms of specific traits (columns of the tables) as well as the differences between the mean values of developmental stages for individual varieties (rows of the tables). Homogeneous groups in both cases were determined using the Duncan test.

3. Results

Cultivars applied in the experiment were significantly different according to the evaluated traits (Table 3). Plant inoculation in different development phases significantly affected all evaluated traits except YPP (Table 3).

Table 3. Results of two-way analysis of variance (ANOVA). F-values with significance of difference are given (ns—not significant, *** $p > 99\%$, ** $p > 95\%$).

Component of Variation	Traits Evaluated:			
	YPP	TKW	GC	SH
cultivars (1)	2.24 **	115.97 ***	27.88 ***	30.74 ***
different phases of plant inoculation (2)	ns	34.43 ***	60.71 ***	224.22 ***
interaction (1) × (2)	ns	3.78 ***	2.98 ***	6.70 ***

YPP—yield, TKW—thousand kernel weight, GC—germination capacity, SH—seed health.

3.1. Effect of Infection at Different Vegetation Stages of Plants on YPP, TKW and GC

There was no significant effect of *B. sorokiniana* infection at different development stages on the YPP of tested barley cultivars. Different yields were observed for different cultivars, with Widawa, Antek, Barke, Ryton and Hanka as the highest yielding entries (0.77–0.68 kg m^{-2}), and Rastik as the lower yielding entry (0.49 kg m^{-2}); however, these differences were most likely a consequence of genetic predisposition, not of the experimental conditions (Table 4).

Table 4. Results for the effect of inoculation with *B. sorokiniana* (mean values and standard deviations) on seed yield per plot (YPP, kg·m^{-2}) at different phases of plant development.

Cultivar	Inoculation in Following Development Phases:						Mean for Cultivar	F-Value (Difference between Treatments)
Name	Control (No Inoculation)	Kernels	Tillering	Stem Elongation	Heading	Flowering		
Antek	0.77 ± 0.10	0.81 ± 0.26	0.77 ± 0.19	0.74 ± 0.26	0.77 ± 0.23	0.71 ± 0.19	0.76 ± 0.08 a	ns
Barke	0.67 ± 0.15	0.76 ± 0.25	0.64 ± 0.16	0.72 ± 0.23	0.69 ± 0.15	0.75 ± 0.24	0.70 ± 0.09 ab	ns
Bryl	0.66 ± 0.25	0.69 ± 0.26	0.62 ± 0.17	0.61 ± 0.21	0.67 ± 0.19	0.69 ± 0.22	0.65 ± 0.15 b	ns
Hanka	0.61 ± 0.19	0.70 ± 0.29	0.65 ± 0.17	0.72 ± 0.20	0.69 ± 0.24	0.74 ± 0.24	0.68 ± 0.15 ab	ns
Johan	0.71 ± 0.20	0.66 ± 0.24	0.68 ± 0.21	0.57 ± 0.17	0.65 ± 0.14	0.65 ± 0.22	0.65 ± 0.12 b	ns
Justina	0.66 ± 0.27	0.71 ± 0.24	0.63 ± 0.14	0.72 ± 0.22	0.68 ± 0.20	0.67 ± 0.19	0.68 ± 0.14 ab	ns
Lailla	0.61 ± 0.21	0.63 ± 0.21	0.64 ± 0.18	0.61 ± 0.18	0.65 ± 0.23	0.59 ± 0.21	0.62 ± 0.14 c	ns
Prosa	0.66 ± 0.26	0.61 ± 0.18	0.63 ± 0.20	0.68 ± 0.24	0.65 ± 0.23	0.62 ± 0.26	0.64 ± 0.15 b	ns
Rastik	0.54 ± 0.14	0.51 ± 0.17	0.47 ± 0.12	0.48 ± 0.19	0.49 ± 0.12	0.48 ± 0.16	0.49 ± 0.11 d	ns
Refren	0.69 ± 0.24	0.68 ± 0.24	0.65 ± 0.17	0.65 ± 0.22	0.65 ± 0.21	0.66 ± 0.26	0.66 ± 0.14 b	ns
Ryton	0.65 ± 0.14	0.76 ± 0.27	0.66 ± 0.14	0.67 ± 0.21	0.69 ± 0.20	0.73 ± 0.20	0.69 ± 0.11 ab	ns
Widawa	0.82 ± 0.22	0.76 ± 0.18	0.71 ± 0.21	0.74 ± 0.25	0.80 ± 0.22	0.81 ± 0.25	0.77 ± 0.10 a	ns
Mean for treatments	0.67 A	0.69 A	0.64 A	0.66 A	0.67 A	0.67 A	0.67	
F-value (difference between cultivars)	ns	ns	ns	ns	ns	ns	3.79 ***	

Explanation: significance of difference between mean values: ns—not significant, *** $p > 99\%$; a, ab, b, c, d and A—homogeneity groups for cultivars and treatments, respectively, according to Duncan test.

While the pathogen did not impact the overall yield, notable variations in thousand kernel weight (TKW) were detected among the tested cultivars. The obtained results indicate that the infection of plants in the tillering phase significantly influenced TKW, and it was observed that the kernels harvested from these plants were smaller than those of both the control and other combinations (Table 5). However, in the case of some cultivars such as Antek, Johan, Lailla and Prosa, there was no significant difference between mean values recorded at all treatments applied.

In the laboratory tests, GC of the kernels collected in the experiment was also assessed (Table 6). It was found that kernels from plants inoculated in the tillering phase germinated the least (62.7%), and this value was significantly lower compared to the control and other tested combinations. It is worth noting that among the studied cultivars, there were two (Bryl and Widawa) for which no effect of *B. sorokiniana* inoculation on germination capacity was found.

Table 5. Results for the effect of inoculation with *B. sorokiniana* (mean values and standard deviations) on the thousand kernel weight (TKW, g) at different phases of plant development.

Cultivar Name	Treatments:						Mean for Cultivar	F-Value (Difference between Treatments)
	Control (No Inoculation)	Inoculation at Following Development Stages:						
		Kernels	Tillering	Stem Elongation	Heading	Flowering		
Antek	26.7 ± 1.25	27.0 ± 0.45	26.1 ± 0.78	27.3 ± 0.52	26.1 ± 0.61	26.8 ± 0.35	26.7 ± 0.8 b	ns
Barke	28.2 ± 0.30	25.5 ± 0.17	25.4 ± 0.67	26.2 ± 0.36	25.9 ± 0.27	26.4 ± 0.22	26.3 ± 0.6 bc	12.64 ***
Bryl	27.5 ± 0.29	27.8 ± 0.20	26.6 ± 0.50	27.0 ± 0.17	27.3 ± 0.18	27.7 ± 0.59	27.3 ± 0.9 a	33.28 ***
Hanka	26.3 ± 0.93	25.7 ± 0.29	25.2 ± 0.27	24.9 ± 0.24	24.6 ± 0.17	24.9 ± 0.63	25.3 ± 0.8 c	9.50 ***
Johan	24.9 ± 0.50	24.4 ± 0.30	23.9 ± 1.06	24.3 ± 0.26	25.0 ± 1.28	24.3 ± 0.47	24.5 ± 0.8 d	ns
Justina	26.7 ± 0.59	25.7 ± 0.19	24.7 ± 0.16	26.1 ± 0.45	26.7 ± 0.32	26.3 ± 0.59	26.1 ± 0.8 c	18.66 ***
Lailla	25.7 ± 1.25	25.6 ± 0.34	24.8 ± 0.15	25.5 ± 0.33	25.8 ± 0.69	24.8 ± 0.23	25.3 ± 0.7 c	ns
Prosa	25.2 ± 0.46	24.5 ± 0.27	24.1 ± 0.78	25.1 ± 0.77	23.8 ± 0.51	24.9 ± 0.60	24.6 ± 0.6 d	ns
Rastik	25.6 ± 0.26	24.4 ± 0.20	24.7 ± 1.28	25.6 ± 0.25	25.2 ± 0.61	24.8 ± 0.27	25.1 ± 0.8 c	4.76 **
Refren	26.0 ± 0.55	24.4 ± 0.33	23.5 ± 0.12	24.3 ± 0.72	24.3 ± 0.65	24.1 ± 0.49	24.4 ± 0.7 de	4.91 **
Ryton	25.8 ± 0.53	24.0 ± 0.34	22.6 ± 0.35	23.3 ± 0.91	24.1 ± 1.00	24.5 ± 0.30	24.0 ± 0.9 e	6.28 **
Widawa	24.5 ± 0.45	27.5 ± 0.32	25.4 ± 0.34	26.3 ± 0.76	27.5 ± 0.39	26.9 ± 0.22	26.4 ± 0.8 b	13.58 ***
Mean for treatment	26.08 A	25.55 B	24.75 C	25.50 B	25.53 B	25.54 B	25.49	
F-value (difference between cultivars)	12.18 ***	97.07 ***	15.10 ***	23.54 ***	18.62 ***	37.06 ***	6.88 ***	

Explanation: significance of difference between mean values: ns—not significant, *** $p > 99\%$, ** $p > 95\%$; a, b, bc, c, d, de, e and A, B, C—homogeneity groups for cultivars and treatments, respectively, according to Duncan test.

Table 6. Results for the effect of inoculation with *B. sorokiniana* (mean values and standard deviations) on germination capacity (GC, %) at different phases of plant development.

Cultivar Name	Treatments:						Mean for Cultivar	F-Value (Difference between Treatments)
	Control	Inoculation at Following Development Stages:						
	(No Inoculation)	Kernels	Tillering	Stem Elongation	Heading	Flowering		
Antek	65.3 ± 5.0	69.3 ± 4.2	51.3 ± 13.3	76.7 ± 8.1	84.7 ± 1.2	89.3 ± 4.6	72.8 ± 6.1	11.2 ***
Barke	75.3 ± 7.6	76.7 ± 4.2	62.0 ± 5.3	70.0 ± 9.2	86.7 ± 5.0	87.3 ± 1.2	76.3 ± 5.4	8.0 **
Bryl	82.7 ± 3.1	81.3 ± 6.4	80.7 ± 2.3	76.7 ± 1.2	88.7 ± 5.0	86.7 ± 7.0	82.8 ± 4.2	ns
Hanka	79.3 ± 3.3	78.7 ± 5.0	58.0 ± 2.0	64.0 ± 11.1	78.0 ± 5.3	73.3 ± 3.1	71.9 ± 4.9	7.1 **
Johan	76.0 ± 2.0	68.7 ± 7.6	40.0 ± 6.0	65.3 ± 9.9	81.3 ± 7.0	88.0 ± 0.8	69.9 ± 5.4	20.8 ***
Justina	84.0 ± 2.0	80.7 ± 2.3	76.0 ± 8.0	69.3 ± 6.1	91.3 ± 3.1	93.3 ± 1.2	82.4 ± 3.8	12.4 ***
Lailla	76.0 ± 1.9	70.0 ± 7.2	60.0 ± 8.7	68.7 ± 9.0	75.3 ± 7.0	82.0 ± 7.2	72.0 ± 6.9	3.3 **
Prosa	78.0 ± 5.3	84.0 ± 3.5	70.7 ± 3.1	86.7 ± 5.8	83.3 ± 10.3	88.0 ± 2.0	81.8 ± 5.0	3.9 **
Rastik	64.0 ± 5.3	47.3 ± 1.2	48.7 ± 7.0	50.0 ± 5.3	59.3 ± 4.6	70.7 ± 8.1	56.7 ± 5.2	8.4 **
Refren	82.7 ± 2.3	64.7 ± 6.1	66.7 ± 15.3	80.0 ± 0.9	84.0 ± 2.0	82.7 ± 3.1	76.8 ± 4.8	4.7 **
Ryton	76.7 ± 1.2	66.7 ± 4.2	63.3 ± 5.0	62.0 ± 4.0	74.7 ± 9.0	84.0 ± 5.3	71.2 ± 4.8	7.9 **
Widawa	84.0 ± 2.0	78.0 ± 5.3	75.3 ± 9.5	81.3 ± 3.1	82.7 ± 4.2	86.0 ± 5.3	81.2 ± 4.9	ns
Mean for treatment	77.0 B	72.2 BC	62.7 C	70.9 AB	80.8 AB	84.3 A	74.6	20.74 ***
F-value (difference between cultivars)	8.70 ***	11.76 ***	6.61 ***	6.29 ***	6.10 ***	5.66 ***	7.01 ***	

Explanation: significance of difference between mean values: ns—not significant, *** $p > 99\%$, ** $p > 95\%$; A, AB, B BC, C—homogeneity groups for cultivars and treatments, respectively, according to Duncan test.

3.2. Seed Health Analysis

The analysis of colonization of seeds obtained from plants inoculated with *B. sorokiniana* at different stages of development revealed a relationship between the dynamics of seed colonization by this fungus and the developmental stage of the inoculated plants. A total of 37 species of fungi were identified, including the inoculated one (Table 7). Colonies of bacteria were also observed, varying in intensity, as well as a small degree of non-sporulating

mycelium. The most abundant fungus in terms of seed colonization among the tested barley varieties was *Alternaria alternata*, with an average colonization of 61.6 colonies per 100 seeds. Second in line was the artificially introduced fungus *B. sorokiniana* (average of 36.9 colonies per 100 seeds).

Table 7. Results of seed infection analysis (mean values and standard deviations) performed on seeds harvested from plants inoculated with *Bipolaris sorokiniana* at different phases of development.

Species	Seed Infection Rate (No. of Colonies per 100 Seeds) from Different Treatments (i.e., Plant Infection with *Bipolaris* at Different Phases of Development):						
	Control	Kernels	Tillering	Stem Elongation	Heading	Flowering	Mean
Alternaria alternata (Fr.) Keissler	75.7 ± 7.3 a	74.1 ± 4.6 a	68.8 ± 9.9 b	45.8 ± 7.8 c	27.6 ± 7.1 d	78.2 ± 6.8 a	61.7 ± 19.9
Bipolaris sorokiniana (Sacc.) Shoem.	10.5 ± 4.7 e	17.7 ± 4.3 d	27.2 ± 9.4 c	25.4 ± 11.2 c	58.3 ± 9.6 b	81.9 ± 6.7 a	36.8 ± 26.3
Aureobasidium pullulans (dre Bary) G. Arnoud	7.2 ± 1.2 a	7.6 ± 1.9 a	11.4 ± 3.2 a	4.5 ± 1.0 c	2.3 ± 0.6 d	12.1 ± 2.1 a	7.5 ± 4.6
Fusarium equiseti (Corda) Sacc.	9.5 ± 2.7 a	4.7 ± 1.3 b	6.2 ± 1.4 ab	5.1 ± 1.9 b	4.2 ±0.9 b	6.8 ± 2.0 ab	6.1 ± 3.8
Stemphylium consortiale (Thüm.) Gr. et Skol.	7.0 ± 2.8 a	4.8 ± 1.1 b	6.5 ± 1.9 ab	5.0 ± 2.0 b	2.4 ± 0.5 c	8.5 ± 3.0 a	5.71 ± 3.1
Epicoccum nigrum Link.	6.8 ± 2.9 a	7.0 ± 3.5 a	6.3 ± 1.8 a	2.6 ± 0.9 b	1.7 ± 0.1 b	6.9 ± 2.0 a	5.2 ± 3.1
Fusarium solani (Mart.) Sacc.	1.8 ± 0.3 b	1.6 ± 0.2 b	1.0 ± 0.2 b	1.6 ± 0.1 b	1.8 ± 0.3 b	3.3 ± 1.1 a	1.8 ± 1.8
Fusarium semitectum Berkeley and Ravenel	2.6 ± 0.5	1.7 ± 1.0	1.1 ± 0.9	1.6 ± 0.5	0.9 ± 0.3	2.0 ± 1.0	1.6 ± 1.5
Phoma sp.	1.2 ± 0.4 bc	1.2 ± 0.5 c	1.8 ± 0.9 b	0.9 ± 0.4 c	0.2 ± 0.1 d	2.9 ± 0.9 a	1.4 ± 1.3
Fusarium sporotrichioides Sherb.	1.4 ± 0.6	1.2 ± 0.7	1.4 ± 0.6	1.0 ± 0.7	0.9 ± 0.4	1.8 ± 0.5	1.3 ± 1.3
Fusarium oxysporum Schltdl	2.2 ± 0.9 a	1.0 ± 0.6 a	1.3 ± 0.8 a	0.1 ± 0.04 b	1.0 ± 0.4 a	1.8 ± 0.9 a	1.2 ± 1.5
Fusarium poae (Peck) Wollenw.	2.2 ± 0.8 a	0.7 ± 0.2 b	0.7 ± 0.1 b	1.3 ± 0.9 ab	0.4 ± 0.1 b	2.0 ± 0.3 a	1.2 ± 1.6
Penicillium spp.	0.5 ± 0.2	1.4 ± 1.0	-	0.4 ± 0.2	0.7 ± 0.2	1.3 ± 0.9	0.7 ± 1.3
Cladosporium herbarum (Pers.) Link	1.2 ± 0.9	0.7 ± 0.3	0.9 ± 0.4	0.4 ± 0.2	0.3 ± 0.1	0.4 ± 0.3	0.7 ± 1.1
Fusarium culmorum (Wm.G. Sm.) Sacc.	0.2 ± 0.1	0.9 ± 0.7	0.6 ± 0.5	0.6 ± 0.3	0.2 ± 0.1	0.5 ± 0.3	0.5 ± 0.9
Stemphylium botryosum Wallr.	0.4 ± 0.3	0.6 ± 0.4	0.7 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	0.8 ± 0.7	0.5 ± 0.8
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not.	0.6 ± 0.4	0.4 ± 0.3	0.3 ± 0.2	0.7 ± 0.3	-	0.7 ± 0.2	0.5 ± 0.9
Fusarium avenaceum (Fr.) Sacc.	0.6 ± 0.2 a	0.1 ± 0.07 b	0.8 ± 0.3 a	0.3 ± 0.1 b	0.1 ± 0.06 b	0.9 ± 0.3 a	0.4 ± 0.8
Drechslera teres (Sacc.) Shoemaker	0.3 ± 0.2 a	0.7 ± 0.2 a	0.6 ± 0.2 a	0.1 ± 0.1 b	-	0.8 ± 0.3 a	0.4 ± 0.8
Fusarium verticillioides (Sacc.) Nierenberg	0.2 ± 0.3 b	0.3 ± 0.1 b	0.3 ± 0.1 b	1.0 ± 0.3 a	0.2 ± 0.2 b	0.1 ± 0.1 c	0.3 ± 0.6
Rhizoctonia solani J.G. Kuhn	0.4 ± 0.1	0.7 ± 0.9	0.3 ± 0.6	0.2 ± 0.4	-	0.2 ± 0.6	0.3 ± 0.6
Nigrospora oryzae (Berk & Br.) Petch	0.8 ± 0.6 a	0.3 ± 0.8 a	0.2 ± 0.4 b	-	0.1 ± 0.3 b	0.4 ± 0.7 a	0.3 ± 0.5
Acremonium spp.	0.7 ± 1.1	0.4 ± 0.7	0.1 ± 0.3	0.1 ± 0.3	-	0.5 ± 1.0	0.3 ± 0.4
Papularia arundinis (Corda) Fr.	0.3 ± 0.9	0.2 ± 0.6	0.4 ± 1.1	0.2 ± 0.6	0.1 ± 0.3	0.3 ± 0.5	0.2 ± 0.3
Fusarium graminearum Schwabe	0.7 ± 1.4	-	0.3 ± 0.8	0.1 ± 0.3	-	0.2 ± 0.4	0.2 ± 0.2
Fusarium tricinctum (Corda) Sacc.	0.2 ± 0.4	-	0.4 ± 0.8	-	-	0.2 ± 0.6	0.1 ± 0.2
Arthrobotrys superbus Corda	0.2 ± 0.6	0.1 ± 0.3	0.2 ± 0.6	0.1 ± 0.3	-	0.2 ± 0.6	0.1 ± 0.1
Trichothecium roseum (Pers.) Link	0.3 ± 0.8	0.1 ± 0.3	-	-	-	-	0.1 ± 0.1
Chaetomium sp.	0.2 ± 0.4	0.2 ± 0.4	-	-	-	-	0.1 ± 0.1
Botrytis cinerea Pers. ex Fr.	0.1 ± 0.2	-	0.1 ± 0.2	-	-	0.2 ± 0.4	0.1 ± 0.1
Drechslera dematioidea (Bubák & Wróbl.) Scharif	-	0.1 ± 0.3	0.2 ± 0.4	-	-	-	0.04 ± 0.01
Aspergillus spp.	-	-	-	-	0.2 ± 0.4	-	0.04 ± 0.02
Trichoderma viride Pers.	-	-	-	0.2 ± 0.3	0.1 ± 0.2	-	0.04 ± 0.01
Mucor sp.	-	-	0.2 ± 0.4	-	-	0.1 ± 0.2	0.04 ± 0.04
Ascochyta sp.	-	0.2 ± 0.4	-	-	-	-	0.03 ± 0.03
Papulaspora sp.	-	-	-	-	0.1 ± 0.2	0.1 ± 0.2	0.03 ± 0.02
Curvularia lunata (Wakker) Boedijn	0.1 ± 0.2	-	-	-	-	-	0.01 ± 0.02
non-sporulating mycelium	0.4 ± 0.9	0.1 ± 0.3	0.2 ± 0.6	0.2 ± 0.6	-	0.2 ± 0.4	0.18 ± 0.3
Bacteria (species not recognized)	29.3 ± 4.3 a	27.7 ± 7.4 a	30.1 ± 6.0 a	28.0 ± 4.5 a	20.8 ± 4.5 b	26.2 ± 4.2 a	27.0 ± 12.1
Total microorganisms	174.7 ± 4.1 a	167.7 ± 3.2 b	169.8 ± 6.2 a	160.6 ± 6.1 b	148.6 ± 7.1 c	178.2 ± a	166.6

Explanation: a, ab, b, bc, c, d, e—homogeneity groups according to Duncan test.

On average, the highest colonization of seeds was recorded after inoculation during the flowering (total number of colonies per 100 seeds—178.2) and tillering (169.8) phases, as well as in the control group, i.e., without inoculation (174.7). Conversely, the lowest colonization (average of 148.6) was observed during inoculation at the heading phase. Regarding *B. sorokiniana*, the lowest seed infection (10.5%) was observed in control plants (not inoculated); the number of this species' colonies per 100 kernels gradually increased from 17.7% (inoculated kernels) up to 81.9% in the flowering phase (Table 8).

Table 8. Results for the effect of inoculation (mean values and standard deviations) on seed infection by *B. sorokiniana* (no. colonies per 100 seeds) at different phases of plant development.

Cultivar Name	Treatments:						Mean for Cultivar	F-Value (Difference between Treatments)
	Control	Inoculation at Following Development Stages:						
		Kernels	Tillering	Stem Elongation	Heading	Flowering		
Antek	17 ± 3	24 ± 1	32 ± 2	28 ± 4	74 ± 4	81 ± 5	42.7 ± 6.1	194.8 ***
Barke	11 ± 1	14 ± 4	45 ± 3	44 ± 4	68 ± 2	93 ± 3	45.8 ± 7.0	323.8 ***
Bryl	6 ± 1	11 ± 5	24 ± 4	36 ± 4	63 ± 5	83 ± 1	37.2 ± 6.8	167.2 ***
Hanka	17 ± 3	16 ± 2	18 ± 2	48 ± 2	56 ± 6	82 ± 4	39.5 ± 6.1	181.1 ***
Johan	6 ± 0	19 ± 5	19 ± 5	21 ± 4	57 ± 3	75 ± 3	32.8 ± 6.0	87.1 ***
Justina	16 ± 0	14 ± 6	32 ± 2	18 ± 2	39 ± 1	71 ± 1	31.7 ± 4.8	183.5 ***
Lailla	9 ± 1	19 ± 5	31 ± 7	16 ± 6	48 ± 2	80 ± 4	33.8 ± 5.9	96.4 ***
Prosa	9 ± 1	21 ± 3	39 ± 5	17 ± 3	61 ± 5	85 ± 1	38.7 ± 6.5	131.4 ***
Rastik	6 ± 1	13 ± 2	19 ± 1	18 ± 1	50 ± 8	78 ± 4	30.7 ± 6.2	113.1 ***
Refren	6 ± 1	19 ± 1	33 ± 1	22 ± 4	67 ± 5	75 ± 3	37.0 ± 6.2	120.1 ***
Ryton	7 ± 2	25 ± 4	15 ± 3	20 ± 1	55 ± 3	91 ± 5	35.5 ± 7.1	145.2 ***
Widawa	16 ± 3	17 ± 5	20 ± 4	17 ± 1	62 ± 4	89 ± 3	36.8 ± 5.2	210.7 ***
Mean for treatment	10.5 E	17.7 D	27.3 C	25.4 C	58.3 B	81.9 A	36.8	
F-value (difference between cultivars)	8.46 ***	2.49 **	19.68 ***	15.68 ***	14.33 ***	11.96 ***	ns	

Explanation: significance of difference between mean values: ns—not significant, *** $p > 99\%$, ** $p > 95\%$; Explanation: A, B, C, D, E—homogeneity groups for treatments, according to Duncan homogeneity test.

Most of the fungal species observed on the seeds (22 species) were of mostly pathogenic nature. The remaining ones were of saprotrophic nature (12 species) or species of dual nature (3 species). The average infection percentage for the first mentioned group of fungi was 4.04% (excluding artificially inoculated fungus), while for saprotrophic fungi, this value was 1.12%. In the group of 'pathogens', species from the *Pleosporaceae* family dominated, including *Alernaria alternata*, *Stemphylium consortiale*, *S. botryosum*, *Dreschlera teres*, and *D. dematiodea*. The artificially inoculated fungus, *B. sorokiniana*, also belongs to this taxonomic unit. Another well-represented family of pathogenic fungi is *Nectariaceae*, which includes the genus *Fusarium*, among others. In this experiment, the presence of 11 species from this genus was observed. Among saprotrophic fungi, the dominant species was *Aureobasidium pullulans*, belonging to the *Sacchotheaceae* family. The contribution of pathogenic fungi to seed infestation was dominant, ranging from 86.4% of the total recorded fungal colonies under control conditions to 95.2% for seeds obtained from plants inoculated at the flowering stage (Figure 5). In parallel, the proportion of saprophytic fungi decreased from 12.6% of total colonies for the control to 4.6% for seeds obtained after inoculation at the flowering stage.

There was a negative and statistically significant effect of the degree of seed colonization by the inoculated fungus *B. sorokiniana* on GC of the barley varieties tested (Table 9). A similar effect was also found for several species of fungi spontaneously colonizing barley seeds. These were pathogenic fungi such as *Ascochyta* sp. and *Drechslera dematioidea*, and saprotrophs—*Chaetomium* sp. and *Cladosporium herbarum*. Interestingly, in the case of fungi of the genus *Fusarium* (the value of the sum for all species of this genus) and the species *F. solani*, a positive, significant correlation coefficient with GC was found. At the same time, statistically significant, negative correlation coefficients were found between the degree of seed colonization by artificially inoculated fungus and spontaneously colonizing fungi from more than a dozen of species. Fungi with very high correlation coefficients such as *Alternaria alternata* ($r = -0.95$, $p > 99\%$), *Epicoccum nigrum* ($r = -0.71$, $p > 99\%$), *Aureobasidium pullulans* ($r = -0.64$, $p > 99\%$) or *Stemphylium consortiale* ($r = -0.56$, $p > 99\%$) are worth mentioning here.

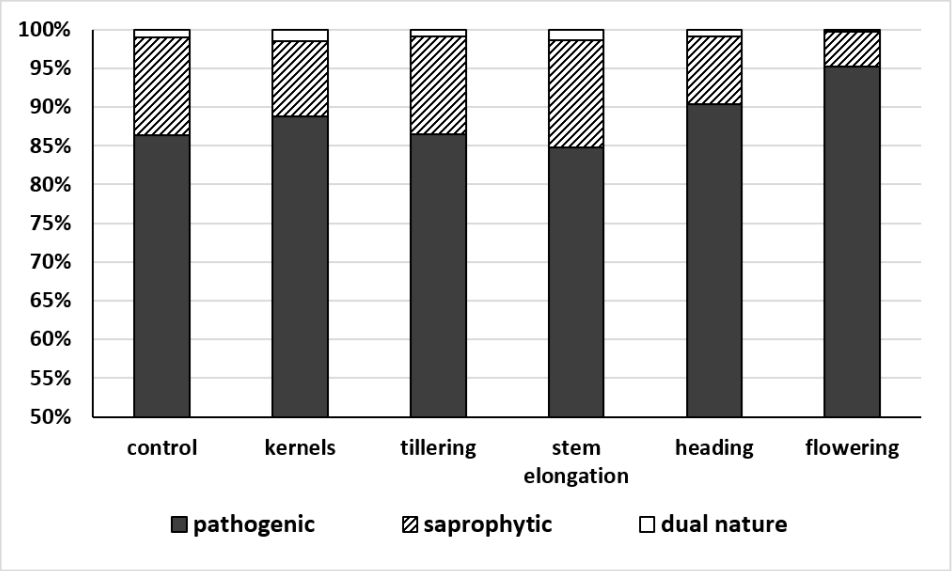


Figure 5. The contribution of fungal species of pathogenic, saprophytic and of dual nature to the total seed infestation (%), depending on time of inoculation.

Table 9. Pearson correlation coefficients between number of fungal colonies per 100 seeds and seed germination and *B. sorokiniana* seed infection rate. Fungus species with no significant correlation coefficients were not included in the above list.

Fungus Species	Germination Capacity (GC)		*B. sorokiniana* Seed Infection	
Acremonium spp.	0.12		−0.27	**
Alternaria alternata	−0.05		−0.95	***
Ascochyta sp.	−0.29	**	−0.08	
Aspergillus spp.	−0.09		0.28	**
Aureobasidium pullulans	−0.14		−0.64	***
Bipolaris sorokiniana	−0.63	***	x	
Chaetomium sp.	−0.27	**	−0.12	
Cladosporium herbarum	−0.39	***	−0.25	**
Drechslera dematioidea	−0.25	**	−0.08	
Drechslera teres	−0.15		−0.32	***
Epicoccum nigrum	−0.05		−0.71	***
Fusarium avenaceum	0.06		−0.31	***
Fusarium equiseti	0.13		−0.35	***
Fusarium graminearum	0.09		−0.26	**
Fusarium oxysporum	0.19		−0.31	***
Fusarium poae	0.16		−0.27	**
Fusarium semitectum	0.21		−0.29	**
Fusarium solani	0.36	***	−0.11	
Nigrospora oryzae	−0.06		−0.39	***
Phoma sp.	0.15		−0.50	***
Fusarium sp. (total)	0.29	**	−0.46	***
Stemphylium consortiale	−0.04		−0.56	***
Bacteria	−0.38	***	−0.37	***

Explanation: correlation coefficients significant at: *** $p > 99\%$, ** $p > 95\%$.

4. Discussion

The *B. sorokiniana* fungus attacks plants at all stages of growth, and yield losses can range from 20% to 80% [33], although under favorable environmental conditions for the development of infection, losses can be as high as 100% [34]. Barley is vulnerable to infection at all growth stages, yet there is a lack of information regarding the plant's developmental stage most susceptible to pathogen attacks and the period during which the most significant

yield decline occurs. However, there is a relationship between the occurrence of the disease, plant height and early age, as reported by Duveiller et al. [35]. Joshi et al. [36] observed that in wheat, later maturing genotypes appear to be resistant to *Bipolaris sorokiniana* because their development cycle is not synchronized with the pathogen's cycle, allowing them to avoid infection. It was also noted that *B. sorokiniana* does not exhibit host specialization [10]. However, isolates have been found to differ in their aggressiveness on wheat and barley [36,37] similarly to what was observed in *D. teres* from the same class of fungi, *Dothideomycetes* [38]. It is well known that *B. sorokiniana* is the causative agent of several cereal diseases, including black point, common root rot and spot blotch disease [39]. Li et al. [40] indicated that resistance to *B. sorokiniana*-caused black point was mainly related to counteracting oxidative stress, although the specific mechanisms involved require further investigation.

The results of this study demonstrate significant variations in the evaluated traits among the selected cultivars, highlighting the influential role of cultivar choice in shaping the outcomes of the study. These differences are likely attributed to inherent genetic variations, which are common among diverse cultivars of plants [41]. Additionally, the actual grain yield and the simulated yield potential were found to depend on the combination of the genotype, environmental conditions, and agronomic management [42,43]. For instance, a study on genetic variation in grain yield and quality traits of spring malting barley found significant differences among genotypes for grain protein content, grain retention fraction, harvest index, and yield components such as mean grain weight [44].

In our experiments, inoculation with *B. sorokiniana* did not lead to a significant reduction in yield. This observation aligns with findings from Bailey et al. [45], who determined that using barley seeds infested with *B. sorokiniana* hindered emergence, yet did not lead to a significant increase in yield per plot compared to plots where healthy seeds were used. Additionally, they noted that any emergence suppression caused by seed-borne *B. sorokiniana* was typically compensated for as the plants matured. Consequently, there was no noticeable reduction in overall yield. However, this can vary depending on the specific conditions and the balancing effect of the inoculum and the presence of antagonistic microorganisms in the soil.

A study by Cane and Hampton [46] found that the percentage of infected seedlings was linked to seedborne inoculum levels, with delayed sowing increasing the mean percentage of infected seedlings from 17% to 44%. In our results, although no significant yield reduction was observed, a significant reduction in thousand kernel weight (TKW) was observed. Similar observations were made in a study analyzing the impact of barley stripe mosaic virus (BSMV) on the yield and other characteristics of barley grains, which found a significant impact on both the yield and other tested characteristics, depending on the level of infection [47].

The infection by *B. sorokiniana* was found to have a significant impact on the quality, seed health, and germination ability of barley. The study confirmed a negative relationship between kernel infection with *B. sorokiniana* and germination capacity. The severity of the impact of this fungus on the germination rate was observed to depend on the level of seedborne inoculum and the aggressiveness of the fungal isolates [46,48,49]. Several studies have supported these findings, indicating that *B. sorokiniana* infection can result in reduced seed quality, germination, and overall plant health [20]. In a study on spot blotch disease in spring barley, the germination scores ranged from 48.5% to 71.5%, suggesting that a higher severity of infection can lead to lower germination rates. For instance, *B. sorokiniana* was reported to remain viable for several years in barley and wheat seeds, and its infection was associated with reduced germination rates and lower seed quality [10]. The decrease in germination was linked to the increase in the number of abnormal seedlings, highlighting the detrimental impact of the fungus on the germination process [50]. These findings underscore the importance of managing *B. sorokiniana* to ensure high-quality barley production and maintain optimal germination rates.

The severity of the spot blotch increases with the advancement of the growth stages [51], and the late varieties show less disease susceptibility [52]. The analysis of the results obtained indicates that, although a higher number of fungi, including *B. sorokiniana*, were isolated from plant seeds that were inoculated during the flowering phase, the most substantial impacts on seed mass, size, and germination capacity were observed when the inoculation occurred during the tillering phase. This suggests that the timing of inoculation, specifically during the tillering phase, played a more critical role in influencing these seed characteristics, despite the greater fungal presence during the flowering phase.

5. Conclusions

A highest number of fungi, including *B. sorokiniana*, were isolated from plant seeds that were inoculated during the flowering phase. The most substantial impacts on TKW and germination capacity was observed when the inoculation occurred during the tillering phase. Inoculation with *B. sorokiniana* did not lead to a significant reduction in yield, but a significant reduction in thousand kernel weight (TKW) were observed. Cultivars with lower mean thousand kernel weights were characterized by higher contamination by fungi and significantly lower germination capacity. A negative correlation was identified between germination rates and infection by *B. sorokiniana*.

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