

Mycoflora Associated with Black Point Attack on Two-row and Six-row Barley - First Report

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ABSTRACT

The research followed the signaling of the black point attack on two-row and six-row barley, the identification of the associated mycoflora and the establishment of the average seed infection rate. The biological material was represented by six-row barley from the Cardinal variety cultivated in the conventional system and during the conversion period, the Gerlach variety and Romanita barley in two-rows variety. The experiments were carried out under in vitro conditions with non-disinfected seed variants and subjected to a light disinfection with sterile water and 70% ethanol solution. The black point attack on the analyzed seeds induced a chocolate brown to black color in the embryonic area and the sector of associated fungal microorganisms was made up of species *Alternaria* spp., *Fusarium* spp., *Drechslera* spp., to which *Aspergillus* spp. was added, *Penicillium* spp. The average seed infection rate with *Alternaria* spp was 46.66 in the Cardinal and Romanita varieties and 40% with *Fusarium* spp. in the Gerlach variety. The average seed infection rate with *Drechslera* spp. was 20% in the Cardinal and Romanita varieties. When disinfecting with 70% ethanol around the seeds, colonies of *Alternaria* spp. and *Fusarium* spp. developed well.

Keywords: black point, two-row barley, six-row barley, incidenta, mycoflora.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important and suitable crops for arid and semi-arid regions due to having a high diversity of genetic resources that can adapt to abiotic and biotic stress (Vasilescu et al., 2022a, b). The attack of black point has been reported and researched, in particular, on wheat (Rees et al., 1984; Christopher et al., 2007; Khani et al., 2018) but the literature cites the presence of black point on other cereals as well, such as barley and rye (Machacek and Greaney, 1938; Jacobs and Rabie, 1987). The seed disease, the black point, causes the so-called “blackening of the embryo” or the black point, with implications on the quality of the seeds. A similar problem have been reported concerning the color of the grains, called purple seed stain (PSS) of soybean [*Glycine max* (L.) Merr.] (Li et al., 2019; Petcu et al., 2021). Research has

shown that some of the infected seeds suffer deterioration in the germination process, which reduces their quality and, therefore, their marketing (Williamson, 1997). Black point attack is caused by a complex of biotic and abiotic factors (Conner and Davidson, 1988; Lehmensiek et al., 2004; Christopher et al., 2007; Khani et al., 2018). Fungal discoloration of the embryo and whole seed of barley is a worldwide problem. Diseased kernels have turned dark brown to black on one or more sides or have a black appearance from the fungal mass covering them (Mathre, 1997). The attack of black point, also present in barley, was associated with fungal infections (De la Pena et al., 1999; Prokinova, 1999; Hudec, 2007). Neate and McMullen (2005) show that barley varieties are susceptible to black point attack, associating the pathogens *Alternaria* spp., *Bipolaris* and *Fusarium* sp. as being involved in the appearance of the attack and

recommends irrigation management and the cultivation of one of the few resistant varieties. In barley, the black point has implications in their downgrading for the malt industry, being a selection criterion. Seed black point causes severe losses in the barley industry and genetic improvement of barley black point resistance is complex, requiring an understanding of the genotype by environment interaction and the biochemical melanization mechanisms involved in black point development (Tah et al., 2010). Black point attack was reported on six-row barley and barley in two-rows variety, 2021 production, in Tulcea County, Romania by Cristea and Popescu (2022) (<https://www.facebook.com/RevistaSanatateaPlantelor/posts/pfbid02LEkHgNhoFxnWvyuTKAsMZ6V8SKcUfequZM6k8P8Lmba6v7f7TMs4eUreeM8LVxmUl>).

MATERIAL AND METHODS

The purpose of the surveys was to identify the species of fungi involved in the appearance of black point in six-row and two-row barley. The researches were carried out on barley and caryopsis, originating from Tulcea County, Romania. The biological material was represented by six-row barley of the Cardinal and Gerlach varieties and seeds of spring barley in two-rows, the Romanita variety. The samples were harvested in 2021. The constitutive variants were: V1 - six-row barley the Cardinal variety from the culture system during the conversion period - experimental field, V2 - six-row barley - the Cardinal variety from the culture system in the conventional system, experimental field, V3 - six-row barley the variety Gerlach, taken from silo (provided by SC Prest Geo-Dan SRL Tulcea, Agighiol collection) and V4 - barley in two-rows, Romanita variety, from the culture in conventional system, experimental field. Observations were made regarding the symptoms of the black point attack on infected seeds, determinations regarding the incidence of black point infected seeds in the analyzed samples, the identification of the mycoflora associated with black point seeds and the calculation of the average seed

infection rate with the fungal microorganisms associated with the attack. To determine the incidence of seeds affected by black point, samples of 150 seeds were made and the frequency was calculated according to the formula: $F = n \times 100/N$, where F - frequency, n - seeds with black point attack, N - total seeds analyzed. The infected seeds were distributed 5 seeds in Petri dishes with a diameter of 90 mm, in which 20 ml/dish of the PDA (potato-dextrose-agar) culture medium was distributed. The variants were constituted: v1 - untreated seeds (n, c - control); v2(as) - light disinfection with sterile water (as); v3 - disinfection with 70% ethanol solution (e), each variant being placed in three repetitions. The identification of the mycoflora was carried out after the specific fructifications of the fungi detected with the Zeiss Primo star microscope. The percentage of infected seeds was calculated (Hajihassani et al., 2012), as the average rate of seed infection with detected fungi, using the formula: Mean rate of seed infection = (Number of seed on which a fungal species identified / Number of seed tested) $\times 100$.

RESULTS AND DISCUSSION

Research on the presence of black point attack on six-row barley and barley in two-rows was reported by Cristea and Popescu (2021) (<https://www.facebook.com/RevistaSanatateaPlantelor/posts/pfbid02LEkHgNhoFxnWvyuTKAsMZ6V8SKcUfequZM6k8P8Lmba6v7f7TMs4eUreeM8LVxmUl>) (Figure 1). The differences noted between the seeds without black point and with black point in six-row barley and barley in two-rows confirm the presence of the attack (Figure 2). The observations regarding the presence of the black point attack showed that the affected six-row barley and barley in two-rows seeds presented a chocolate brown/reddish brown to black spot in the embryo region, which can advance towards the median area of the seeds, often shriveled (Figure 3) compared to the seeds healthy (Figure 2). The presence of brown or black spots in the embryo area or the chocolate brown coloration of the seeds has been described in

reference research (Burrows, 2005; Neate and McMullen, 2005). Also, Tah et al. (2010), show that black point (BP) is a brown or black coloring of the end of the embryo. Mathre (1997) states that diseased seeds are dark brown to black on one or more sides or have a black appearance. Describing the

pigmentation of cereal seeds, Li et al. (2003) also shows the black point of the seeds. The associated mycoflora and the etiology of black point disease in six-row barley and barley in two-rows have not yet been investigated in Romania.



Figure 1. Seeds with black point attack on six-barley (Gerlach-G and Cardinal-C varieties) and triticale (Romanita-R variety)



Figure 2. Seeds attacked by black point and healthy in the Cardinal (C), Gerlach (G) and Romanita (R) varieties of barley in two-rows

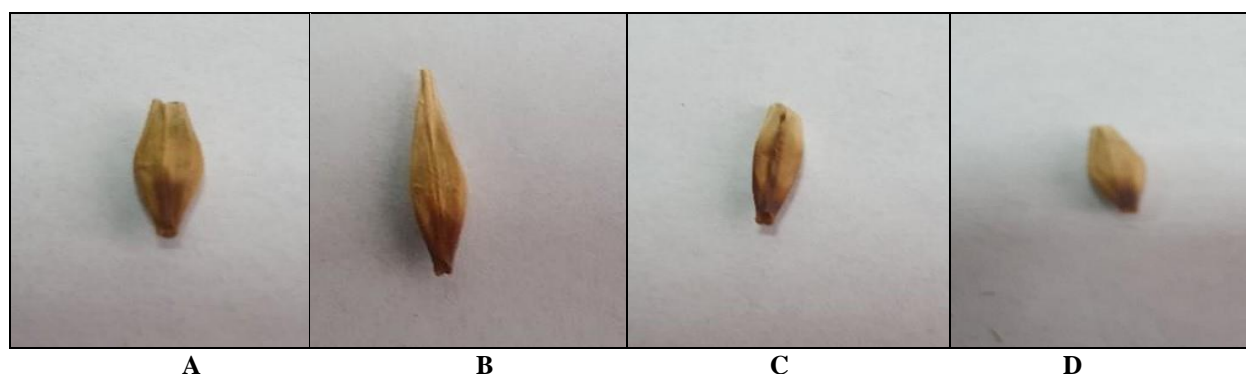


Figure 3. Symptoms of black point in six-barley and two-row barley: A seeds - the Cardinal variety (conventional); B - the Cardinal variety (conversion); C - the Gerlach variety; D - the Romanita variety

The frequency of caryopses with black point was determined from the analyzed samples and it was found that the highest incidence was recorded in the Gerlach variety with F=94%. In the Cardinal variety, the frequency was 14%, barley from the plot

during the conversion period. In the variant in which the Cardinal variety was cultivated conventionally, the incidence was 12%. In barley in two-rows seeds of the Romanita variety, the frequency of black point attack was 8% (Table 1).

Table 1. Incidence of black point attack in autumn barley and spring barley

Nr crt	Variety	Provenance	Black point (%)
1	Cardinal	Mahmudia, Tulcea/ experimental field, barley in the conversion period	14
		Mahmudia, Tulcea/ experimental field, barley in conventional culture	12
2	Gerlach	Seed collection silo	94
3	Romanita	Mahmudia, Tulcea/ experimental field, conventional culture	8

Black point attack on cereals has been associated with attack by common fungi http://www.herbiguide.com.au/Descriptions/hg_Black_Point_of_Barley.htm. The fungal microorganisms associated with the attack of black point in the analyzed barley varieties were *Alternaria* spp., *Fusarium* spp., *Drechslera*

spp., *Penicillium* spp., *Aspergillus* spp. In the case of the barley in two-rows Romanita variety, the fungal microorganisms associated with black point belonged to the genera *Alternaria*, *Fusarium*, *Drechslera*. Other microorganisms (bacterial colonies) also developed near the caryopses (Table 2).

Table 2. Fungal microorganisms identified

Species/ variety	Fungal microorganisms detected					
	<i>Alternaria</i> spp.	<i>Fusarium</i> spp.	<i>Drechslera</i> spp.	<i>Aspergillus</i> spp.	<i>Penicillium</i> spp.	<i>Rhizopus</i> spp.
Cardinal OB	+	+	+	+	-	-
Cardinal OF	+	+	+	+	-	-
Gerlach	+	+	+	+	+	+
Romanita	+	+	+	-	-	-

OB - barley in the conversion period; OF - barley in conventional system.

Fungi belonging to the genera *Fusarium*, *Alternaria*, *Bipolaris* are cited as being involved in the appearance of black point in six-row barley seeds (Neate and Mc Mullen, 2005). Research on the seed infection rate with the pathogen *Alternaria* spp showed that the highest value of the average infection rate, RI=53.33%, was determined in the Romanita barley in two-rows variety, in the control variant, followed by the Cardinal variety six-row barley from both culture systems, where the value of the average seed infection rate was 46.66%, also in the control variant. In the Gerlach variety, in the sterile

water variant, an average seed infection rate of 40% was recorded compared to the control variant of 33.33%. High values of the average rate of infection with *Alternaria* spp. were also recorded in the variants of disinfection with sterile water in the barley in two-rows with 46.66% and disinfection with ethanol solution 70%, where RI=40%. The lowest rate of infection with *Alternaria* spp. was calculated for the 70% ethanol version of the Gerlach variety. Regarding the fungi of the genus *Fusarium*, they were present on six-row barley and barley in two-rows seeds in all variants. The average infection rate of

the analyzed seeds was 86.66% for the Gerlach variety in the 70% ethanol disinfection variant and 66.66% for the Cardinal variety in the conversion period, with the same seed disinfection variant. It was noted the presence of colonies of other organisms (bacteria) that inhibited the mycelial growth of *Fusarium* pathogens during 3-6 days (Figure 4). In the Romanita spring barley in two-rows variety, the average seed infection rate with *Fusarium* spp. was 53.33% in the 70% ethanol version and 40% in the sterile water disinfection variant. In the control variant, it was found that the infection rate was 33.33% for the Cardinal six-row barley variety and 33.33% for the barley in two-rows variety respectively 40% for the Gerlach variety. Fungi belonging to the genera *Alternaria* and *Fusarium* were identified in the mycobiota of cereal seeds (Zaharia et al., 2022). The average seed infection rate with *Drechslera* spp. fungi was 20% in the Cardinal variety in conventional culture in the control and sterile water disinfection variants and 13.33% in the Romanita barley in two-rows variety and the

Cardinal variety in the culture during the conversion period. With the exception of the 70% ethanol variant in the Cardinal variety cultivated in a conventional system where the *Drechslera* spp. infection rate was 6.66%, in the other varieties detected with black point no colonies of *Drechslera* fungi developed in the ethanol 70% variant. *Alternaria* and *Helminthosporium* species are chiefly involved in the occurrence of black point disease or “kernel smudge” (Machacek and Greaney, 1938). *Aspergillus* spp. fungi had a weak colonization and the seed infection rate did not exceed 6.66%, being present in the case of barley varieties, in the control variant and in the Cardinal barley variety in the conversion period and in the disinfection variant with sterile water. In the Gerlach barley variety, a *Penicillium* spp. infection rate of 6.66% was also identified (Table 3, Figure 4). Seed-borne fungi, including species of *Alternaria*, *Fusarium*, *Aspergillus* and *Penicillium* have been considered as important pathogens of cereal grains (Raicu and Baci, 1978; Neegard, 1979; Cristea et al., 2008).

Table 3. Fungal microorganisms identified

Species/ variety		<i>Alternaria</i> spp. RI (%)	<i>Fusarium</i> spp. RI (%)	<i>Drechslera</i> spp. RI (%)	<i>Aspergillus</i> spp. RI (%)	<i>Penicillium</i> spp. RI (%)	Rhizopus spp. RI (%)
Cardinal OB	c	46.66	33.33	13.33	6.66	-	-
	as	33.33	40.00	13.33	6.66	-	-
	e	33.33	66.66	-	-	-	-
Cardinal OF	c	46.66	33.33	20.00	6.66	-	-
	as	33.33	46.66	20.00	-	-	-
	e	33.33	60.00	6.66	-	-	-
Gerlach G	c	33.33	40.00	13.33	6.66	6.66	-
	as	40.00	33.33	13.33	-	-	colonization plate
	e	13.33	86.66	-	-	-	-
Romanita R	c	46.66	33.33	20.00	-	-	-
	as	46.66	40.00	13.33	-	-	-
	e	40.00	53.33	-	-	-	-

OB = six-row barley in the conversion period; OF = six-row barley in conventional system; c - control variant; as - water sterile variant; e - 70% ethanol variant.

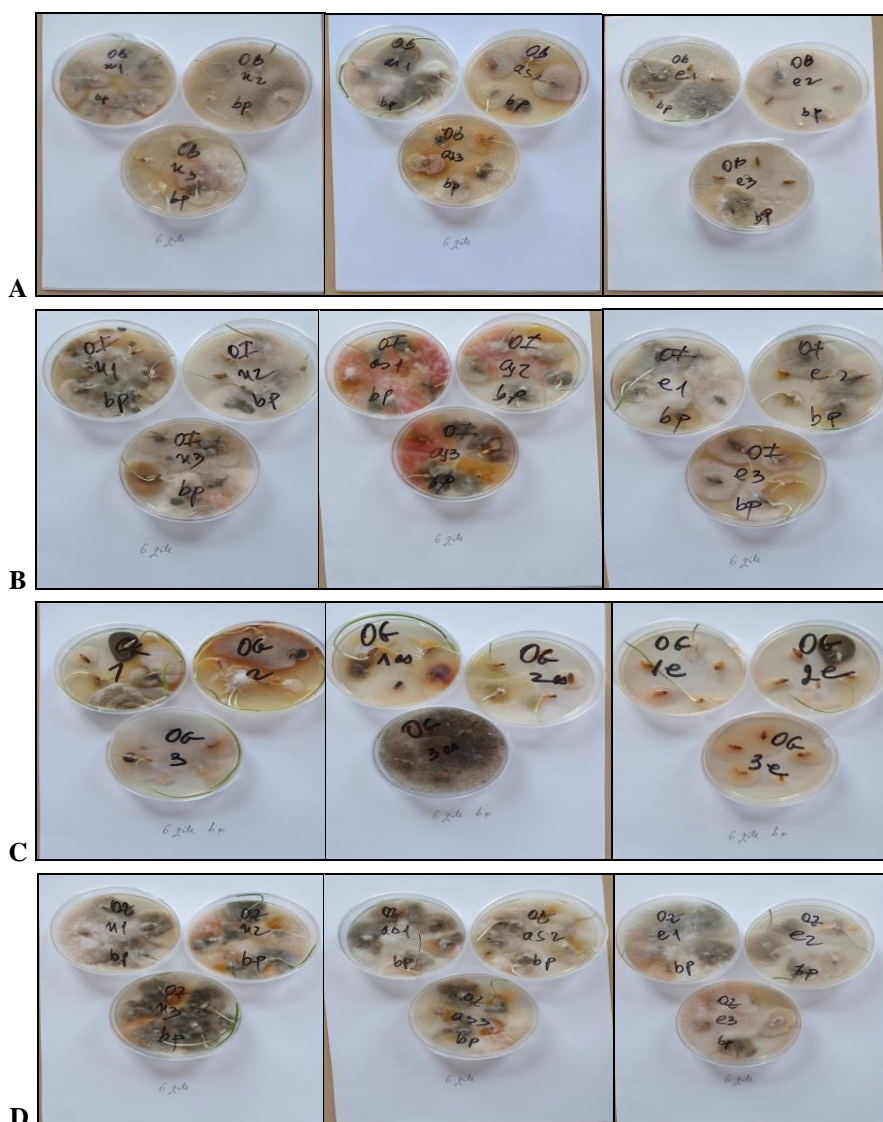


Figure 4. The development of fungal colonies associated with the attack of black point in the Cardinal six-row barley varieties - the variant in the conversion period (OB)-A, Cardinal- the variant in the conventional culture (OF)-B, Gerlach (OG)-C; barley in two-rows Romanita variety (OZ)-D

CONCLUSIONS

The attack of black point on two-row and six-row barley induces a chocolate-brown to black coloration in the embryonic area of the seeds. The mycoflora associated with the attack of black point on two-row and six-row barley was represented by fungi from the genera *Aternaria*, *Fusarium*, *Drechslera*. The presence of *Aspergillus* spp., *Penicilium* spp., *Rhizopus* spp. fungi was also found. The highest incidence of black point attack was reported in the Gerlach barley variety and the highest rate of seed infection was determined by *Alternaria* spp. and *Fusarium* spp. The disinfection with sterile water or 70% ethanol solution variant did not have a significant

effect on the fungi, *Alternaria* spp. and *Fusarium* spp. fungi survived, with higher values of the average infection rate also noted of the seeds.

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