MICROOGRANISMS ASSOCIATED WITH BLACK POINT DISEASE

Andrei-Mihai GAFENCU[1], Andreea-Mihaela FLOREA [1], Florin-Daniel LIPŞA[1], Eugen ULEA[1]

e-mail: agafencu@uaiasi.ro

Abstract

Black point disease is characterized by a brown to black discoloration of wheat kernels. The disease can be a problem in regions where are registered heavy rainfall during de early stage of kernels development. Black point disease is associated with many microorganisms, both fungi or bacteria, but those most usually responsible for these symptoms are species of the fungus *Alternaria alternate, Cochliobolus sativus, Fusarium graminearum, Aspergillus* spp., *Penicillium* spp. The aims of this study were to identify microorganisms associated with black point disease of wheat. The kernels of twenty-eight winter wheat varieties were analyzed for the purpose of determining the microorganisms associated with the black point.

Key words: wheat diseases, Black point, *Alternaria* spp., *Cladosporium* spp.

Numerous diseases affect the grain yield and quality of wheat. Such a disease is the black point of wheat. Black point occurs in all wheat-growing regions and is an economically serious disease in cereals that affects crop yield and value.

Black point is found in all regions where this plant is grown and is characterized by the change of color of wheat kernels, being observed especially in the area of the embryo (*figure 1*). The disease can be a problem when in the early phenophases of kernels formation are recorded high amounts of precipitations. (Gafencu A.M. *et al*, 2019; Özer N., 2005).

Black point hampers the seed germination, seedling growth, tillering, and grain yield. Therefore, many regions imposed regulations on the incidence of black point in wheat seeds. For example, a level of 2%-4% of black point-affected kernels is permitted in wheat samples for the food industry in the USA. In Australia, the upper limit was 5% for seeds storage in silos. In Romania, up to this moment, there is no official regulation regarding the incidence of the black point (Fernandez M.R. *et al*, 2011, 2000; Kaur J. *et al*, 2018; Khani M. *et al*, 2018; Gafencu A.M. *et al* 2019).

Incidences of black point are accelerated in the presence of environmental condition of heavy rain, high humidity, or extreme temperature during the early grain filling-stage (Conner R.L. *et al*, 1988; Gafencu A.M. *et al*, 2018).

Figure 1 **Black point symtomps in wheat kernels (Original)**

MATERIAL AND METHOD

Twenty-eight winter wheat varieties were used as research material for this study, which was conducted under field conditions from de Iasi Didactic Station - Ezareni Farm, during the vegetation seasons 2016-2017 and 2017-2018.

The fields were not treated with any fungicide controlling black point, or other diseases, whereas standard cultural practices were normally applied.

For the determination of the microorganism associated with the black point, 100 kernels from each wheat cultivar were analyzed (75 kernels with black point symptoms and 25 healthy kernels). The kernels were disinfected by immersion in sodium hypochloride (1%) for 3 minutes and then rinsed with sterilized distilled water. The kernels were placed in Petri dishes (9 cm in diameter) containing 20 ml PDA (Potato Dextrose Agar). Petri dishes were incubated at a temperature of 20^0C for 10 days. The arising colonies were counted and for identification, the fungi were morphologically and microscopically examined for features of mycelia, conidiophores and spores (El-Gremi S.M. *et al*, 2017; Toklu F. *et al*, 2008;, Li Q.Y. *et al*, 2019, Özer N., 2005*;*).

Referring to Ellis M.B. and Ellis J.P. (1985) the fungi were identified.

RESULTS AND DISCUSSIONS

Following the study of the diseased karyops, five genera of fungi were identified: *Alternaria* spp., *Cladosporium* spp., *Fusarium* spp., *Chaetomium* spp., and *Cochliobolus* spp. Of these microorganisms, *Alternaria* spp. was the predominant genus, being found in all cultivars analyzed. *Cladosporium* spp., *Fusarium* spp., *Chaetomium* spp., and *Cochliobolus* spp. were observed at a low frequency.

The data contained in *table 1* show the microorganism associated with the black point of wheat kernels harvested in 2017.

Alternaria spp. was observed in all cultivars, the number of isolated colonies varying according to the cultivar studied. Most colonies, 14.3 were isolated from wheat kernels from Bezostaya 1 variety. In the case of this wheat variety, 2 colonies of *Cladosporium* spp. were observed.

Table 1

**The average number of fungal colonies identified from wheat kernels,
harvested in 2017, with black point symptoms**

No.	Winter wheat variety	Black point incidence (%)	*Alternaria* spp.	*Cladosporium* spp.	*Fusarium* spp.	*Chaetomium* spp.	*Cochliobolus* spp.
1	Bezostaya 1	4.57±0.27	**14.3**	**2.0**	0.0	0.0	0.0
2	11424G1	8.73±0.35	**10.6**	0.0	0.0	0.0	0.0
3	Andrada	12.13±0.69	**8.6**	**0.6**	0.0	0.0	0.0
4	Codru	7.93±0.59	**7.6**	**2.3**	0.0	0.0	0.0
5	Dumbrava	10.13±0.61	**11.6**	**0.6**	0.0	0.0	0.0
6	Glosa	12.27±0.49	**12.6**	0.0	0.0	0.0	0.0
7	Izvor	29.27±0.98	**8.3**	0.0	0.0	0.0	0.0
8	Litera	21.37±1.37	**6.3**	**0.6**	0.6	0.0	0.0
9	Miranda FDL	20.20±1.27	**7.6**	**0.6**	0.0	0.0	0.0
10	Otilia	12.53±0.65	**7.3**	0.0	0.0	0.0	**0.3**
11	Pajura	25.83±1.53	**10.3**	**0.3**	0.3	1.0	0.0
12	Pitar	9.73±0.61	**5.3**	0.0	0.0	0.0	0.0
13	Semnal	26.50±0.97	**3.6**	**0.6**	0.6	0.0	0.0
14	T.109-12	7.53±0.34	**6.6**	**0.6**	0.0	0.0	0.0
15	T.118-11	9.03±1.56	**4.6**	**0.3**	0.0	0.0	0.0
16	T.123-11	3.30±0.32	**9.3**	0.0	0.0	0.0	0.0
17	T.124-11	7.67±0.20	**8.6**	**0.3**	0.0	0.0	**0.3**
18	T.143-11	13.27±0.35	**7.3**	0.0	0.3	0.0	0.0
19	T.19-10	5.40±0.25	**4.6**	**0.3**	0.0	0.0	0.0
20	T.95-12	5.93±0.03	**8.3**	**1.3**	1.3	0.3	0.0
21	Unitar	23.10±0.21	**5.6**	**0.3**	0.3	0.0	0.0
22	Ursita	11.17±0.24	**7.3**	**0.6**	0.0	0.0	0.0
23	Vestitor	5.97±0.30	**6.3**	**0.3**	0.0	0.0	0.0
24	Voevod	8.13±0.68	**12.6**	0.0	0.3	0.0	0.0
25	Voinic	24.03±0.85	**8.3**	**1.6**	0.0	0.0	0.0

Cladosporium spp. has also been identified from wheat kernels from the Andrada, Codru, Dumbrava, Glosa, Litera, Miranda FDL, Pajura, Semnal, T.109-12, T.118-11, T.124-11, T.19-10, T.95-12, Unitar, Ursita, Vestitor, Voinic varieties.

The third microorganism identified from wheat kernels with black point symptoms, harvested in 2017, was represented by *Fusarium* spp., whose presence was observed in seven cultivars: Litera, Pajura, Semnal, T.143-11, T.95-12, Unitar and Vestitor.

Chaetomium spp. was identified from the kernels with black point symptoms that came from the Pajura and T.95-12 varieties. Also, in the case of two varieties, Otilia and T.124-11, from the kernels with typically symptoms was isolated and *Cochliobolus* spp.

Of the cultivars analyzed, in neither case were not all the five fungi have identified, in the case of the Pajura variety and the wheat line T.95-12 four microorganisms were identified.

Following the analysis of wheat kernels from the wheat cultivars harvested in 2018, it was observed that the incidence of the black point attack was lower than the previous year, and of the five microorganisms identified after analyzing the kernels from 2017, in this year were observed only four of them: *Alternaria* spp., *Cladosporium* spp., *Fusarium* spp. and *Chaetomium* spp. *Aternaria* spp. was identified in all the kernels analyzed. The number of wheat cultivars were *Cladosporium* spp. was identified was reduced this year compared to the previous year. The cultivars for which the fungus was identified were represented by 11424G1, Codru, Dumbrava, Otilia, Pajura, Semnal, T.109-12, T.118-11, T.124-11, T.143-11, T.19-10, T.95-12, Unitar, Ursita, Voinic, Zamolxe. *Fusarium* spp. was identified in case of the following varieties Andrada, Miranda FDL, T.19-10, T.95-12, and Unitar.

Of the fungi identified after analyzing wheat kernels with black point symptoms harvested in 2018, *Chaetomium* spp. was identified just in two cases, represented by T.123-11 and T.143-11 wheat lines (*table 2*).

Table 2

The average number of fungal colonies identified from wheat kernels, harvested in 2018, with black point symptoms

No.	Winter wheat variety	Black point incidence (%)	*Alternaria* spp.	*Cladosporium* spp.	*Fusarium* spp.	*Chaetomium* spp.	*Cochliobolus* spp.
1	Bezostaya 1	3.20±0.30	4.6	0.0	0.0	0.0	0.0
2	11424G1	2.70±0.44	6.3	0.6	0.0	0.0	0.0
3	Andrada	2.70±0.46	12.6	0.0	0.6	0.0	0.0
4	Codru	2.83±0.41	5.0	0.6	0.0	0.0	0.0
5	Dumbrava	4.87±0.67	9.3	2.3	0.0	0.0	0.0
6	Glosa	3.30±0.25	14.0	0.0	0.0	0.0	0.0
7	Izvor	9.67±0.32	11.3	0.0	0.0	0.0	0.0
8	Miranda FDL	3.40±0.38	11.3	0.0	0.6	0.0	0.0
9	Otilia	5.60±0.55	5.6	1.0	0.0	0.0	0.0
10	Pajura	7.70±0.25	7.3	1.6	0.0	0.0	0.0
11	Pitar	3.70±0.29	2.6	0.0	0.3	0.0	0.0
12	Semnal	2.57±0.22	3.3	0.3	0.0	0.0	0.0
13	T.109-12	2.17±0.20	3.0	1.6	0.0	0.0	0.0
14	T.118-11	3.73±0.62	4.3	0.3	0.0	0.0	0.0
15	T.123-11	3.27±0.15	4.6	0.0	0.0	0.3	0.0
16	T.124-11	2.47±0.17	2.6	0.3	0.0	0.0	0.0
17	T.143-11	5.33±0.64	5.6	1.3	0.0	0.3	0.0
18	T.19-10	11.63±0.18	4.6	0.6	0.3	0.0	0.0
19	T.95-12	1.20±0.15	2.3	1.0	0.3	0.0	0.0
20	Unitar	2.90±0.10	3.3	1.0	0.6	0.0	0.0
21	Ursita	2.87±0.46	2.6	1.0	0.0	0.0	0.0
22	Voinic	4.77±0.38	5.0	1.6	0.0	0.0	0.0
23	Zamfira	2.07±0.41	3.0	0.0	0.0	0.0	0.0
24	Zamolxe	4.20±0.32	3.0	1.3	0.0	0.0	0.0
25	Zina	2.93±0.17	3.3	0.0	0.0	0.0	0.0

CONCLUSIONS

As a result of the study on microorganisms associated with the black point attack, five fungi were identified as responsible for this disease : *Alternaria* spp., *Cladosporium* spp., *Fusarium* spp., *Chaetomium* spp., and *Cochliobolus* spp.

Alternaria spp. was isolated for all wheat cultivars studied.

Cladosporium spp. was isolated in case of 17 wheat varieties harvested in 2017 and in the case of 16 wheat varieties harvested in 2018.

Fusarium spp. Was isolated in case of 6 varieties, in 2017 and also in 2018.

Chaetomium spp. was isolated from the wheat kerneles from 2 wheat varieties in both years.

Cochliobolus spp. it was isolated only in the case of wheat kernels harvested in 2017, being observed just in the case of two varieties.

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