

## IDENTIFICATION AND QUANTIFICATION OF FUNGI ASSOCIATED WITH SEEDS OF BARLEY, IN TERMS OF 2014

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### Abstract

*Seeds are considered very effective means of plant pathogens transport over long distances. The use of seed material, free from pathogens, is one of the basic conditions for obtaining healthy crops. If infested seed is used for sowing, then the seed will cause diseases in crop and crop yields will be reduced considerably. The study aimed to identify and quantify the fungi associated with barley (*Hordeum vulgare* L.) seeds. We have analysed samples of seeds of barley varieties grown in the area of South-Eastern Romania. The mycoflora spectrum determined from analyzed samples consists of a variety of parasitic and saprophytic fungi. Species of *Alternaria* and *Fusarium* genera were the most frequently isolated with an incidence of 93.3% and 80% respectively. We also identified *Curvularia lunata* and *Pyrenophora* species with an incidence of 20% and 13.3% respectively. The group of saprophytic fungi like *Oedocephalum* sp., *Penicillium* sp., *Rhizopus* sp., *Stemphylium* sp., *Trichoderma viride* and *Epicoccum purpurascens*, was also isolated.*

**Key words:** mycoflora, seeds, barley, varieties.

### INTRODUCTION

Barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) are two of the most important cereal crops in the entire world. Generally the most important factor in disease transmission from one year to another is the seed (Beratliel and Oprea, 1994). Infected seeds are the most frequent and richest source of transmission of pathogens, especially fungi (Alexandri et al., 1967; Hulea, 1973). The seeds quality as vegetal material of reproduction has a great influence on the crops' yield (Clear and Patrick, 1993). Barley seeds are rich in proteins, carbohydrates and mineral substances which represent a good medium for the development of microorganisms. Knowing the pathology of the seed makes it possible to prevent the introduction of new pathogen agents in the barley crop as well as to avoid the apparition of massive spread of infection in the field (Chong and Sheridan, 1982; Bateman and Kwasna, 1999; Kubiak and Korbas, 1999; Gheorghies and Cristea, 2001). Pathogens transmitted by seed, and those who like a vector seed, are a great problem which needs to

be dealt with taking into consideration various management strategies, including aspects concerning their biology (Bărbulescu and Popov, 1995). A clear identification and quantification of the pathogen transmitted through seeds is necessary in order to determine the means of control and the active substances which are to be applied (Cană et al., 2010). Association of several fungal, in some cases, can induce the black-point phenomenon characterized by blackening of the embryo (Cristea and Berca, 2003).

### MATERIALS AND METHODS

The study aimed to identify and quantify the associated fungi of barley (*Hordeum vulgare* L.) seeds. We have analyzed samples of barley seeds varieties grown in the area of South-Eastern Romania.

The samples which were examined were collected from various lots from local producers at the moment of harvest, in the climatic conditions of 2014. After harvesting, the samples were packed for transportation and storing them in order to maintain the physico -

chemical and microbiological analyzes up to the time of the laboratory in order to obtain more accurate results (Petcu, 2014). The biological material was represented by 15 barley samples such as: Alora baza, Amical, Andreea, Andrei, Cardinal, Compact, Friderichus, Hanzi baza, Maresal, Madalin, Orizont, Regent, Scarpia, Univers, Vanessa. The barley seeds were placed in Petri dishes with a diameter of 8 cm on PDA culture medium (potato-dextrose-agar), sterilized at 121°C/20 min). The culture medium was prepared after the classic recipe (Hulea, 1969; Constantinescu, 1974). For each variety was analyzed a total of 100 seeds arranged in three variants each with three repetitions. The seeds have been not disinfected before this stage because we want to estimate the presence of pathogens on seeds surface not into seeds tissues. It had been determined the frequency of affected seeds by the identified micromycetes. The dishes were incubated at a temperature of 22°C for 10 days. The identification of micromycetes was performed with a *Zeiss Primo Star* microscope based on scientific literature (Raicu and Baciu, 1978; Hulea and Iliescu, 1986).

## RESULTS AND DISCUSSIONS

The spectrum of identified pathogens includes fungi belonging to the *Ascomycotina* and *Deuteromycotina*, consisting of species of the genus - *Alternaria*, *Curvularia lunata*, *Epicoccum purpurascens*, *Fusarium*, *Oedocephalum*, *Penicillium*, *Pyrenophora*, *Rhizopus*, *Stemphylium* and *Trichoderma viride* (Table 1). The micromycetes *Alternaria* sp., *Penicillium* sp., *Rhizopus* sp., and *Fusarium* sp. were also detected on the seeds of other plant species in Romania (Cozea et al., 2011; Zala et al., 2011; Mardare et al., 2014; Pana et al., 2014).

It is noted that on the surface of the seeds, the *Alternaria* species were the most frequently 93.3% (Figure 1), the analyzed varieties developing colonies, except the Scarpia variety. Also, there was a high incidence of *Fusarium* species, which considered to be highly important pathogens for the seeds of barley; 80% of the species studied presented spores that developed

colonies of the genus (*F. graminearum*, *F. moniliforme*), except the Andreea, Maresal and Regent varieties.

*Epicoccum purpurascens*, was detected with a 60% incidence, *Rhizopus* sp. and *Stemphylium* sp. with 40% incidence, are also part of the saprophytic determined.

The *Pyrenophora* species were detected in the Andreea, Orizont and Univers varieties, with 20% incidence. *Penicillium* species were identified in 33.3% of the analyzed varieties (Compact, Maresal, Madalin, Scarpia, Univers) and the *Oedocephalum* sp. in 20% (Amical, Friderichus and Madalin). In Andreea and Cardinal varieties, *Curvularia lunata* and *Trichoderma viride* saprophytes were determined in 13.3% of the cases.

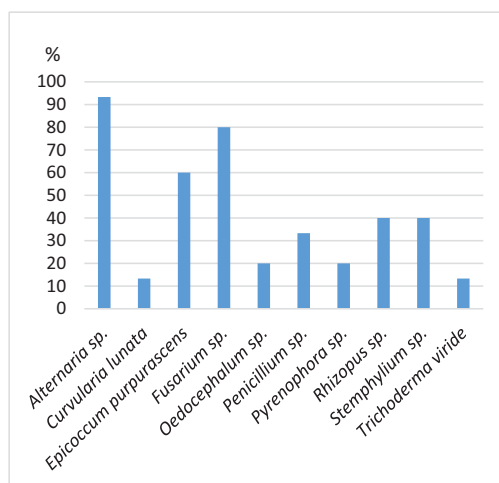


Figure 1. Incidence of pathogens spectrum on the seeds of barley analysed

Figure 2 presents aspects of the fungal colonization on barley seeds, in laboratory conditions on PDA culture medium.

Similar results were reported by other authors in the field of study of wheat seeds pathology (Cristea et al., 2008) as well as barley seeds pathology (Valceva, 2006; Karadjova, 1979 and Ivanov, 1980, cited by Georgieva-Andreeva et al, 2011, Cristea-Manole et al, 2015), who have found infections similar to those of the fungi in the lots of seeds from the north of Bulgaria and southern Romania.

Table 1. Identified mycoflora in barley seeds

Microscopic fungi	Variety														
	Alora baza	Amical	Andreea	Andrei	Cardinal	Compact	Friderichus	Hanzi baza	Maresal	Madalin	Orizont	Regent	Scarpia	Univers	Vanessa
<i>Alternaria</i> spp.	X	X	X	X	X	X	X	X	X	X	X	X	-	X	X
<i>Curvularia lunata</i>	-	-	X	-	X	-	-	-	-	-	-	-	-	-	-
<i>Epicoccum purpurascens</i>	X	X	-	-	X	-	X	X	X	X	-	X	X	-	X
<i>Fusarium</i> spp.	X	X	-	X	X	X	X	X	-	X	X	-	X	X	X
<i>Oedocephalum</i> sp.	-	X	-	-	-	-	X	-	-	X	-	-	-	-	-
<i>Penicillium</i> sp.	-	-	-	-	-	X	-	-	X	X	-	-	X	X	-
<i>Pyrenophora</i> sp.	-	-	X	-	-	-	-	-	-	-	X	-	-	X	-
<i>Rhizopus</i> sp.	-	-	X	X	X	-	-	-	-	-	X	X	X	-	-
<i>Stemphylium</i> sp.	-	-	-	-	X	X	-	X	-	X	-	-	X	X	-
<i>Trichoderma viride</i>	-	-	X	-	X	-	-	-	-	-	-	-	-	-	-

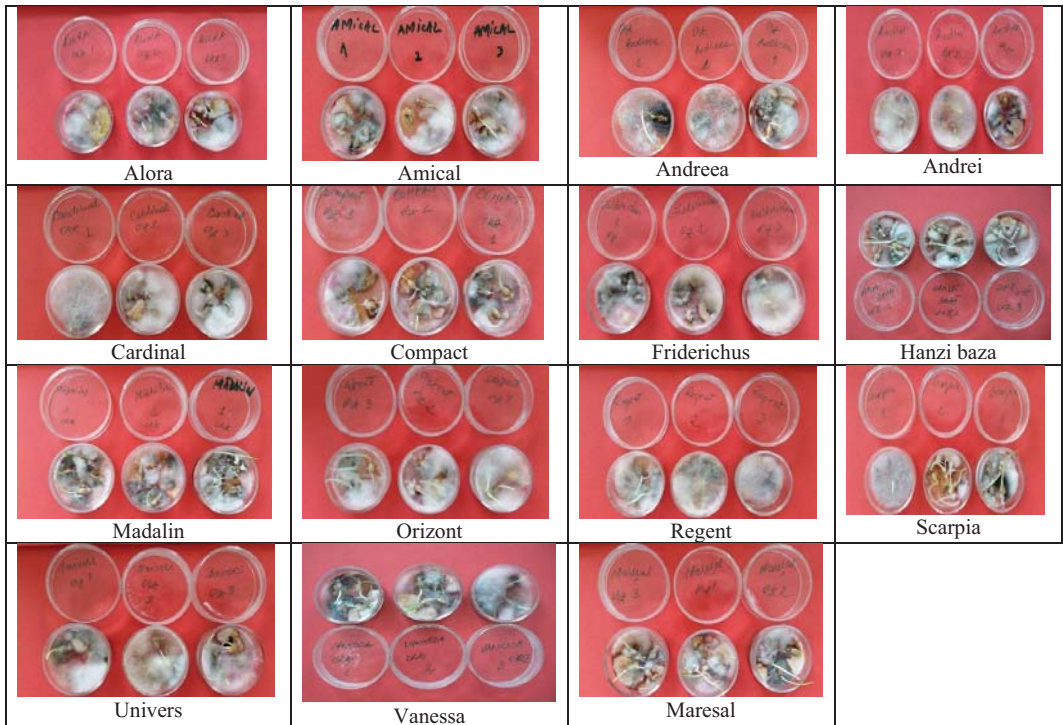


Figure 2. Fungal colonization of barley seeds

The varieties examined showed a variable number of fungi, which is between 3 and 7 pathogens. The lowest number of pathogens was identified Alora baza, Amical, Andrei, Maresal, Orizont and Vanessa varieties, and most pathogens were present on the seeds of the variety Cardinal (Figure 3).

## CONCLUSIONS

Our research confirmed the presence of a large number of fungi associated barley seeds.

The fungi isolated from the seeds barley were included in the genera: *Alternaria*, *Curvularia*, *Epicoccum*, *Fusarium*, *Oedocephalum*, *Penicillium*, *Pyrenophora*, *Rhizopus*, *Stemphylium* and *Trichoderma*.

The most frequent pathogenic species isolated belong to the genus *Alternaria* and *Fusarium* and they colonized the barley seeds in most of the varieties studied, with an incidence of 93.3% and 80% respectively.

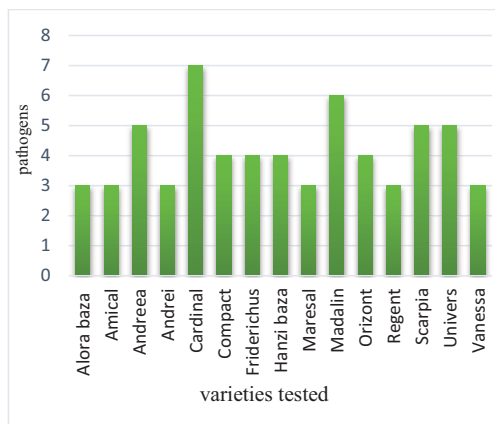


Figure 3. Fungi at seed level

## ACKNOWLEDGEMENTS

This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.

## REFERENCES

- Alexandri Al., Docea E., Olangiu M., Petrescu M., Pop, I., Radulescu E., Rafaila C., Severin V., 1967. *Tratat de fitopatologie agricola*, Vol.1. Ed. Academiei R.S.R. Bucuresti.
- Bateman GL, Kwasna H., 1999. Effects of number of winter wheat crops grown successively on fungal communities on wheat roots. *Applied Soil Ecology*, 13: 271-282.
- Bărbulescu A., Popov C., 1995. Recomandări privind tratamentul semințelor de grâu și orz împotriva bolilor și dăunătorilor. *Probl. prot. plant.*, V. 19-20, p. 75-80.
- Beratliet C., Oprea M. 1994. Ecosystem characteristics of agricultural products storages and health implication. *Plant Protection issues*, Bucharest, 13, 22, 44.
- Cană L., Nagy E., N. Goga, Stoica V., Popov C., 2010. Research on improvement of small-grain cereals protection strategy against seed and soil-borne pathogens, *Analele INCDA Fundulea*, VOL. LXXVIII, no 1, p. 131-138.
- Chong L.M., Sheridan J.E., 1982. Mycoflora of barley (*Hordeum vulgare* L.) seed in New Zealand. *New Zealand Journal of Botany*, 20: 187-189.
- Clear R.M., Patrick S.K., 1993. Prevalence of some seed borne fungi on soft white winter wheat seed from Ontario, Canada. *Plant Dis.Sur.*73: 143-149.
- Constantinescu O., 1974. *Metode și tehnici în micologie*. Ed. Ceres, București.
- Cozea A., Cristea S., 2011. Aspects regarding pathogen-enzymatic system interrelation at *Momordica charantia* naturalized in Romania. *Romanian Biotechnological Letter*, Vol. 16, No. 5, p. 6668-6672.
- Cristea-Manole M.S., Cristea S., Zală C., 2015. Research on mycoflora present in the caryopses of wheat (*Triticum aestivum*) in the S-E of Romania, in terms of 2014. *Romanian Biotechnological Letter*, Vol. 20, No. 1, p. 10183-10189.
- Cristea C., Georgescu M., Pătrașcu N., Groza O., Ion L., 2008. Research regarding the phatology and anathomy of the seed the extention of wheat kernel. *Scientific Papers, USAMV Bucharest, Seria A*, Vol.LI, p. 280-287.
- Cristea C.M., Berca M., 2013. Researches concerning the caryopses mycoflora of wheat to varieties grown in Modelu Location, Calarasi country, *Research Journal of Agricultural Science (RJAS)*, vol 43.
- Gheorghies C., Cristea S., 2001. *Fitopatologie*, Vol.1, Ed. Ceres, Bucuresti.
- Georgieva-Andreeva Mariela, Raykov Svilen, Tanova Krasimira, 2011. Study of the seed microflora in wheat and barley grown in environmentally friendly conditions. *Proceeding of the 4rd international symposium "New Research In Biotechnology"* Scientific Bulletin Series F XV, Biotechnology, Bucharest, p. 37-40.
- Hulea A., Negru Al., Severin V., 1973. *Main Seed Crop Diseases*. Ceres Publishing, Bucharest, p. 35-70.
- Hulea A., 1969. *Ghid pentru laboratoarele de micologie și bacteriologie*. Ed. Agrosilvica, Bucuresti.
- Hulea A., Ilescu P., 1986. *Determinator pentru identificarea mucegaiurilor potential toxigene*. Societatea de Medicina Veterinara din R.S.Romania, Bucuresti.
- Kubiak K., Korbas M., 1999. Occurrence of fungal diseases on selected winter wheat cultivars. *Postepy w Ochronie Roslin* 39 (2): 801-804.
- Mardare Elena Ștefania, Cristea Stelica, Zală R.C., 2014. Researches on the mycoflora of sunflower's achenes for the hybrids cultivated in Fetesti area, Ialomita country. *Scientific Papers, USAMV Iasi, Seria Agronomie*, vol, 57, nr 2, p. 213.
- Raicu C., Baciu D., 1978. *Patologia semintei*. Editura Ceres, Bucuresti.
- Până M., Cristea S., Cernat S., Negrilă E. 2014. The mycoflora of barley- the varieties extension certificated at ards- Teleorman. *Scientific Papers, USAMV Iasi, Seria Agronomie*, vol, 57, nr 2, p. 217.
- Petcu C.D., 2014. *Ambalaje utilizate in industria alimentara*, cap. *Materiale celulozice*. Ed Granada, Bucuresti, p. 56-60.
- Valcheva A., 2006. Study of mycological status of wheat for period 1994-2004. *Plant Science*, Sofia, 44: 35-39.
- Valcheva A., 2006. Study of fungi prevalent in barley. *Ecology and future*, Sofia, 2: 47-50.
- Zală C.R., Cristea Stelica, Groza O., Cristea Mihaela Carmen, 2010. Pathogen fungus of the sunflower seed (*Helianthus annuus* L.) and their impact upon germination. *Scientific Papers, USAMV Bucharest, Seria A*, Vol. LIII, p. 454-459.