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COMPARATIVE ASSESSMENT OF SEED MYCOSIS SUSCEPTIBILITY FOR 23 WHEAT CULTIVARS (*Triticum aestivum* L.)

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Abstract

Seed microflora is recognised for the influence it can have on crop health and subsequent quantitative and qualitative parameters of the yield. Aim of this study was to determine the mycosis susceptibility of wheat seeds belonging to 23 cultivars obtained from crops that received two fertilization regimes: basic fertilization in autumn and respectively supplementary fertilization in spring in addition to the basic fertilization from autumn. Results showed that seeds from the crop that benefited from supplementary fertilization had a higher average germination rate as well as a frequency decrease in pathogenic fungi. On average the germination rate for wheat was 92.61%, Alternaria sp. incidence was 24.67% and Fusarium sp. incidence was 21.74%. Cultivars showed heterogeneity regarding frequency of seed infection, suggesting a different susceptibility of the genotypes, but overall, the results obtained for Romanian cultivars were comparable with those for the cultivars from abroad. Results suggest that nutritional status of plants plays a role and supplementary fertilization can ensure healthier seed.

Key words*: caryopsis, grains, fungi, germination, incubation.*

INTRODUCTION

Mycoflora of wheat seeds can be rich and complex. Past research has revealed that numerous fungi belonging to various genera can be isolated from wheat seeds, such as: *Alternaria, Aspergillus, Bipolaris, Chaetomium, Curvularia, Drechslera, Epicoccum, Fusarium, Helminthosporium, Myrothecium, Nigrospora, Penicillium, Phoma, Rhizopus, Mucor, Sclerotium*, *Stemphylium* (Toklu et al., 2008; Pathak and Zaidi, 2013; Shad et al., 2019). In general, unsterilized seeds can present pathogenic, saprophytic (Suciu et al., 2020) or endophytic microflora (Hubbard et al., 2012). Phytosanitary condition of seeds, particularly microorganisms load can influence germination or can be responsible for the transmission to the crop of certain diseases (Pathak and Zaidi, 2013; Filatova et al., 2020). However, research has shown that some seed endophytic fungi can enhance resistance of wheat seeds to certain abiotic stress factors (Hubbard et al., 2012), while bacterial endophytes isolated from wheat seeds showed inhibitory effect against certain pathogenic fungi (Herrera et al., 2016). Latest findings open new prospects for the control of seed-borne pathogens. In wheat crop, seed-borne pathogens are estimated to be responsible for uneven plant development and a yield decrease ranging between 15-90% if infected seeds are sown in the field (Pathak and Zaidi, 2013). Most common pathogenic fungal species colonizing wheat seeds are from genera *Fusarium* and *Alternaria* (Ramires et al., 2018). Fungal seed colonizers from genera such as *Fusarium*, *Alternaria*, *Aspergillus* and *Penicillium* can be responsible for mycotoxin contamination of grains that pose a health threat (Spanic et al., 2020). Preventive strategies dealing with such pathogens have been crop rotation, optimised tillage, straw management, use of genotypes less susceptible to disease (Vogelgsang et al., 2008), fungicide use and other novel pre-sowing seed treatments (Filatova et al., 2020). Because the contamination of seeds with pathogens plays a major role for the success of the crop, the factors that influence the outcome of seed and

crop health are worth investigating due to their practical implications. Aim of this study was to determine the mycosis susceptibility of unsterilized wheat seeds from crops that received two fertilization regimes. Objectives of the study were:
- comparative assessment of germination,
- screening *Alternaria* sp. infection frequency,
- screening *Fusarium* sp. infection frequency.

MATERIALS AND METHODS

Biologic material for this research was represented by a selection of 11 autochthonous cultivars and 12 cultivars from abroad, that were chosen based on criteria of productivity and stability of production in conditions from Transylvanian Plain, Romania (Table 1). The seeds used in this study were from the yield of year 2019, from experimental field located at Agricultural Research and Development Station from Turda, Romania. The cultivation followed conventional technology for wheat. The fertilization regime had two variants: V_1 - basic fertilization in autumn with NPK 20:20:0 in doses of 250 kg/ha for ensuring a quantity of 50 kg/ha active substance of Nitrogen as well as Phosphorus; and V_2 – basic fertilization in autumn + supplementary fertilization in spring with 180 kg/ha nitro calcar that ensured 50 kg/ha Nitrogen. For the determination of germination and mycotic susceptibility of the wheat genotypes, seeds were placed for germination on moist filter paper in Petri dishes (Figure 1).

Table 1. Wheat cultivars (*Triticum aestivum* L.) used for screening seed mycosis susceptibility

Provenance		Cultivars
Autochthonous cultivars (Romania)	SCDA Turda:	Andrada, Apullum, Arieşan, Codru, Dumbrava, Turda 2000
	INCDA Fundulea:	Faur, Miranda, Pitar
	SCDA Suceava:	Magistral
	SCDA Lovrin:	Alex
Cultivars from abroad	Austria:	Capo, Fulvio, Gallio, Josef
	Hungary:	Mv Béres, Mv Kolo
	France:	Apache, Arlequin, Christine, Element, Renan
	Germany:	Exotic

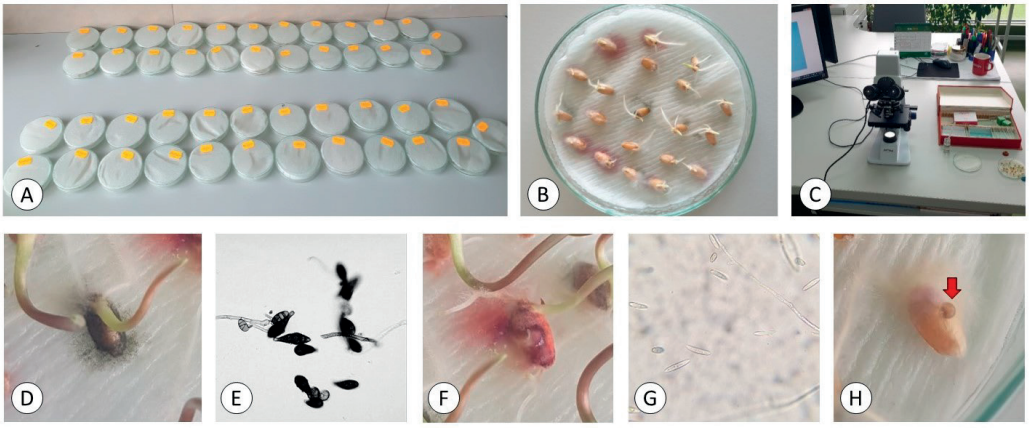

Figure 1. Working protocol and examination of wheat seedlings: a) Petri dishes with seeds during incubation, b) Petri dish after 4 days of incubation, c) microscopic analysis setup, d) macroscopic aspect of *Alternaria* sp. infection, e) microscopic observation of *Alternaria* sp., f) macroscopic aspect of *Fusarium* sp. infection, g) microscopic observation of *Fusarium* sp., h) arrow - bacterial exudate (original)

The Petri dishes were at first disinfected with alcohol and then water-soaked filter paper was placed in each. The Petri dishes with seeds were maintained closed at room temperature. After four days were conducted the macroscopic and microscopic analyses (Figure

1). Germination rate, and incidence of *Alternaria* sp. as well as *Fusarium* sp. was calculated as frequency (%) of germinated respectively infected seeds from the number of seeds placed at incubation – in the case of each experimental variant.

RESULTS AND DISCUSSIONS

Average germination rate for all cultivars regardless of fertilization regime was 92.61%, ranging between 80-100%. Also, average germination rate per cultivar was ≥ 90% in 18 out of 23 cultivars studied. Average germination rate associated with supplementary fertilization was 93.26%, while average germination rate associated with basic fertilization alone was lower (91.96%). The improvement brought by supplementary fertilization of the crop on germination rate of seeds was observed both for Romanian cultivars as well as for cultivars from abroad. Thus, average germination rate for the seeds of Romanian cultivars from the crop that benefited only of the basic fertilization (V_1) was 92.27%, while germination rate for seeds from the crop that enjoyed supplementary fertilization (V_2) increased to 93.64%. Similarly, the average germination rate for cultivars from abroad was 91.67% associated with basic fertilization alone (V_1), and 92.92% when supplementary fertilization was used for the crop (V_2). Two Romanian cultivars ('Arieșan', 'Dumbrava') as well as one cultivar from abroad ('Apache') had a germination rate of 100% regardless of fertilization regime of the crop (Figure 2). Supplementary fertilization increased the germination rate of the seeds to 100% for cultivars 'Alex' and 'Christine'. Lowest germination rate for seeds obtained from the plot that benefited only of basic fertilization (V_1) was identified for Romanian cultivar 'Pitar' (75%) and French cultivar 'Renan' (65%). But the same cultivars under supplementary fertilization had a germination ≥ 90%, indicating that fertilization can maximize the potential of these cultivars. Lowest germination rate for the seeds from the crop that benefited from supplementary fertilization besides basic fertilization (V_2) was identified in three cultivars: Romanian cultivar 'Codru' as well two cultivars from abroad 'Josef' and 'Mv Béres' - all three having a germination rate of 85% (Figure 2).

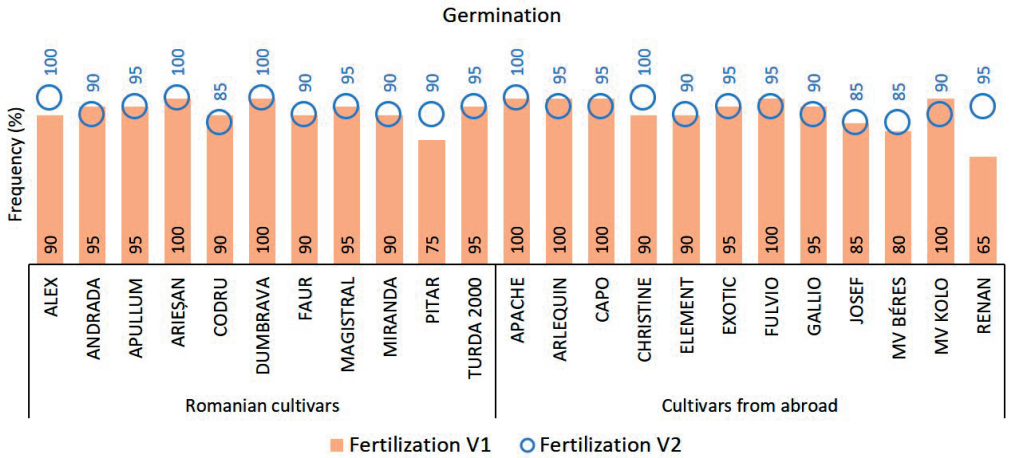

Figure 2. Germination frequency (%) for 23 wheat cultivars from crops with two fertilization regimes: V_1- basic fertilization in autumn, V_2- basic fertilization in autumn + supplementary fertilization in spring

Macroscopic and microscopic analysis of seeds after incubation allowed the identification of fungal pathogens (Figure 1d, 1e, 1f, 1g). Some seeds showed also signs of bacterial infection, observed based on bacterial exudate (Figure 1h) but overall incidence was low, less than 5%. *Alternaria* sp. infection (Figure 1d, 1e) presented an overall incidence of 24.67%, with a range between 5-40%. Seeds from the crop that had only basic fertilization had overall a

higher *Alternaria* sp. incidence of 25.22%, compared to seeds from the crop that benefited from supplementary fertilization (24.13%). This trend was observed both for average incidence in Romanian cultivars as well as for average incidence in cultivars from abroad. Thus, on average the seedlings of the Romanian cultivars had an *Alternaria* sp. incidence of 27.27% associated with basic fertilization regime (V_1), and this decreased to 25.45% for seeds from crop that benefited also of supplementary fertilization (V_2). Similarly, the average *Alternaria* sp. incidence for the cultivars from abroad was 23.33% corresponding to the crop with basic fertilization alone (V_1) and decreased to an average of 22.92% due to supplementary fertilization of the crop (V_2). Highest level of *Alternaria* sp. infected seedlings for seeds from the crop that benefited only of basic fertilization was 35%, and identified in cultivars 'Dumbrava', 'Faur', 'Turda 2000', 'Exotic' and 'Mv Béres' (Figure 3). Seeds from the crop that benefited from supplementary fertilization had a maximum incidence of 35% identified in Romanian cultivars 'Arieșan' and 'Codru', while French cultivar 'Apache' reached a level of 40% seeds affected by *Alternaria* sp. (Figure 3). Lowest frequency of *Alternaria* sp. (≤15%) was identified for cultivars 'Andrada', 'Christine', 'Fulvio', 'Gallio', when seeds were from the crop that had only basic fertilization (V_1). When crop enjoyed supplementary fertilization, the seeds of the cultivars 'Alex', 'Miranda', 'Capo', 'Christine', 'Gallio' had a low incidence of *Alternaria* sp. (Figure 3).

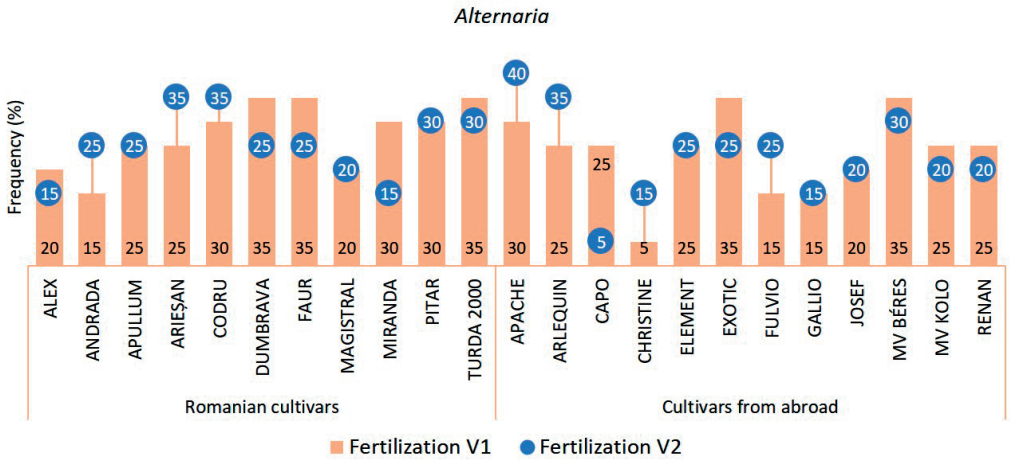

Figure 3. Frequency (%) of *Alternaria* sp. infection for 23 wheat cultivars from crops with two fertilization regimes: V_1 - basic fertilization in autumn, V_2 - basic fertilization in autumn + supplementary fertilization in spring

Fusarium sp. infection (Figure 1f, 1g) presented an overall incidence of 21.74%, with a range between 10-40%. Seeds from the crop that benefited only from basic fertilization had overall a higher *Fusarium* sp. incidence (22.39%), compared to seeds from the crop that benefited from supplementary fertilization (21.09%). This trend was observed for average incidence in Romanian cultivars as well, but not for the average incidence in cultivars from abroad. Thus, on average the seedlings of the Romanian cultivars had a *Fusarium* sp. incidence of 24.55% under basic fertilization alone (V_1), and this decreased to 20.45% for seeds from the crop that also had supplementary fertilization (V_2). By comparison, the average *Fusarium* sp. frequency of the cultivars from abroad was 20.42% for seeds from the crop with basic fertilization alone (V_1) and increased to an average of 21.67% for seeds from the crop that enjoyed also a supplementary fertilization (V_2). Highest frequency of *Fusarium* sp. infection (40%) of seedlings corresponding to seeds from crops that received only basic fertilization was observed for cultivars 'Dumbrava' and 'Mv

Béres'. Highest frequency of *Fusarium* sp. infection of seedlings corresponding to seeds from the crop that enjoyed supplementary fertilization was 35% and occurred in cultivar 'Miranda'. Lowest *Fusarium* sp. incidence (≤ 15%), was observed for cultivars 'Alex', 'Faur', 'Magistral', 'Turda 2000', 'Apache', 'Element', 'Exotic', 'Fulvio', 'Gallio', when seeds were from the crop that had only basic fertilization. When seeds were from the plot that received supplementary fertilization, the lower frequency occurred in cultivars 'Andrada', 'Arieșan', 'Faur', 'Magistral', 'Apache' 'Exotic', 'Gallio' (Figure 4).

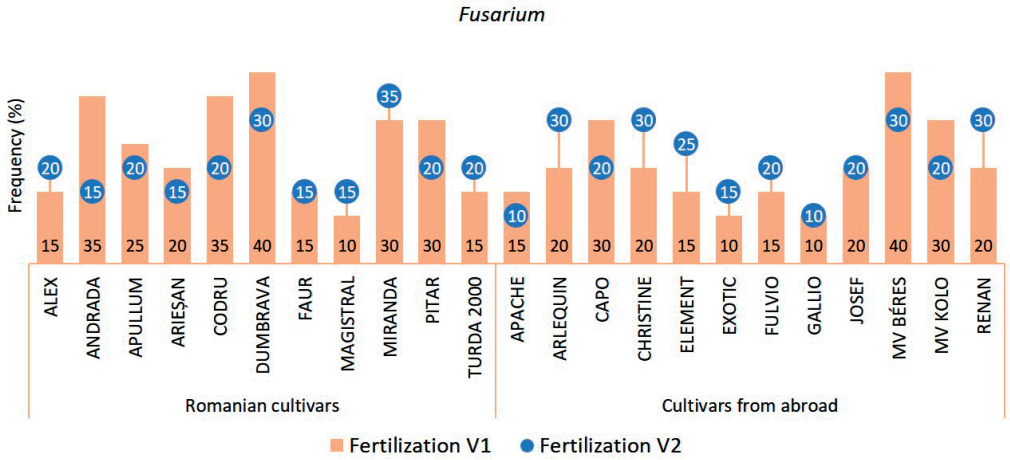

Figure 4. Frequency (%) of *Fusarium* sp. infection for 23 wheat cultivars from crops with two fertilization regimes: V_1- basic fertilization in autumn, V_2- basic fertilization in autumn + supplementary fertilization in spring

Results of the present research suggests that fertilization regime can have an influence both on the germination capacity as well as on mycotic load of the seeds. Thus, seeds that were from the plot that received besides the basic fertilization in autumn also a supplementary fertilization in spring, presented overall a higher germination rate as well as lower *Fusarium* sp. and *Alternaria* sp. infection incidence in seeds. This further infers that nutritional status of the plant can influence the seed quality as well as mycotic load of the seeds. Regardless of fertilization regime, several cultivars displayed a maximum germination rate ('Arieșan', 'Dumbrava', 'Apache') or a lower frequency for *Alternaria* sp. ('Christine', 'Gallio'), and *Fusarium* sp. ('Faur', 'Magistral', 'Apache', 'Exotic', 'Gallio').

Globally, the most serious and hazardous disease of wheat is Fusarium head blight disease (FHB) (Figueroa et al., 2018). In Europe, FHB is caused primarily by *Fusarium graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae* (Vogelgsang et al., 2008), and is associated with mycotoxin contamination of wheat grains. Although *Alternaria* spp. represents also a highly common occurring fungi on seeds worldwide, there are some conflicting views on the harmfulness of disease, and this is most likely due to diversity of this genus. *Alternaria* spp. found in seeds can be either saprotrophic, slightly phytopathogenic and is either producing or not producing mycotoxins (Gannibal, 2018). Frequent colonizers of wheat seeds are the species *Alternaria alternata*, *A. arborescens*, *A. infectoria*, *A. tenuissima*, and *A. triticina* (Ramires et al., 2018). There is evidence that infected seeds and seed transmission is the main source of infection with *Alternaria* sp. in wheat crops (Perelló and Larrán, 2013). Most often wheat kernels infected by *Alternaria* species are characterized by black-spot pigmentation in the underlying embryo region. This feature is associated with low quality of the flour and potential health risk due to some harmful fungal metabolites (Ramires et al., 2018).

Similar to the results of this study, previous research conducted in various countries, also

identified *Alternaria* sp. and *Fusarium* sp. among the most frequent seed-borne pathogenic fungi of wheat. Thus, a study in Turkey revealed that *Alternaria* had highest occurrence in wheat seeds regardless whether kernels placed for germination displayed black-point pigmentation, discoloration or had healthy aspect (Toklu et al., 2008). Another study on wheat seeds from Lithuania revealed that samples were colonized by *Alternaria, Fusarium* and *Penicillium* besides a few other fungi genera (Sinkevičienė and Šaluchaitė, 2020). In wheat samples from Argentina, *A. tenuissima* was the dominant fungi from genus *Alternaria* colonizing the seeds, followed by *A. infectoria*, *A. triticimaculans*, *A. triticina, A. alternata* and *A. chlamydospora* besides a few other related fungi (Perelló and Larrán, 2013). In Russian wheat, the frequency of *Alternaria tenuissimais* and *A. infectoria* was shown to reach levels up to over 70% (Gannibal, 2018). Organically grown wheat from Italy, had average seed infection rate less than 5% for either of the several *Fusarium* species identified, but *Fusarium poae* was one of the most abundant (Infantino et al., 2011). A long-term trial conducted in Switzerland also identified *F. poae* as the most prevalent *Fusarium* species in wheat seeds (Vogelgsang et al., 2008). As for the location of the pathogen, this was determined to be in seed coat of wheat for *Fusarium avenaceum* and *F. poae*, while *F. graminearum* was identified in seed coat and embryo (Hassani et al., 2019). By comparison, in cereal crops only the seed coat is affected by *Alternaria* infection, and the embryo can germinate, and under favourable conditions the seedling can survive and grow (Gannibal, 2018). One question is whether different fungi species from same genus that cause similar symptomatology could have a variable influence on seed germination. In this sense, Browne and Cooke (2005) succeeded to put in evidence the influence of infection with several *Fusarium* species on the average wheat seed germination relative to control with following results: *F. graminearum* (61.7%), *F. avenaceum* (65.5%), *Fusarium culmorum* (76.6%), *F. poae* (92.5%). Furthermore, some species of *Fusarium* appear to be more aggressive, since isolate mixture of *F. avenaceum* showed higher pathogenicity than *F. poae*, causing higher disease levels as well as higher toxin accumulation in wheat grains (Vogelgsang et al., 2008). Presence of the pathogens on seed is associated with both seed quality and safety (Ramires et al., 2018). An experiment proved that low levels of *Fusarium graminearum* seed infection was associated with higher wheat seed quality and significant positive correlation (0.24*) was found between seed infection and mycotoxin (deoxynivalenol) content (Argyris et al., 2003). Interesting perspectives have been identified regarding the potential treatments of wheat seeds against common fungal pathogens, as pre-sowing measures. Thus, out of 600 plant-associated bacterial isolates (e.g. *Pantoea* sp., *Pseudomonas* sp.), 16% isolates showed over 80% *Fusarium culmorum* disease suppression. Efficacy in this case was influenced by bacterial dose and suspension volume (Johansson et al., 2003). Same direction was explored by more recent authors that demonstrated that bacterial isolates (*Pantoea* sp., *Paenibacillus* sp.) from wheat seeds inhibited *F. graminearum* colonies on agar plates, with relatable results on seeds following same treatment (Herrera et al., 2016). Because crop rotation is often the recommended practice to reduce the inoculum source of fungal pathogens in wheat, some authors investigated whether there are potential alternative inoculum sources. Their results showed that *F. graminearum* isolated from wheat seeds, caused decreased germination energy in pea and lupine seeds with potential implications for crop rotation (Rasiukeviciute and Kelpsiene, 2018). It has been asserted that environmental conditions favour the growth and spread of fungi, that can ultimately compromise seed quality (Gannibal, 2018; Shad et al., 2019). But so far, influence of other factors such as fertilization regime on microorganism load were insufficiently explored. In this sense the present research provides a preliminary path for future investigations on the role of nutritional status of the plants from different genotypes on pathogenic fungi occurrence on seeds.

CONCLUSIONS

This research investigated the frequency of germination as well as *Fusarium* and

Alternaria presence on seeds for 23 wheat cultivars.

Results showed that average germination rate was 92.61%, overall *Alternaria* sp. incidence in seeds was 24.67% and overall *Fusarium* sp. incidence was 21.74% for seeds of 2019 crop obtained in conditions from Transylvanian Plain. Analysis showed that seeds from the crop that benefited from supplementary fertilization in spring besides basic fertilization in autumn, had an increase of average germination from 91.96% to 93.26%, as well as a decrease of pathogenic fungi incidence. Thus, average *Alternaria* sp. incidence decreased from 25.22% associated with basic fertilization alone, to 24.13% for seeds from the crop that benefited also of supplementary fertilization. Similarly, average *Fusarium* sp. incidence decreased from 22.39 % associated with basic fertilization to 21.09%. Cultivars displayed heterogeneity regarding incidence of seed infection associated with fertilization regimes, suggesting a different susceptibility of the genotypes as well as a potential influence of nutritional status of plants during vegetation on mycotic load of the seeds.

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