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ORIGINAL SCIENTIFIC PAPER

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SEEDBORNE FUNGI ON STORED ONION SEEDS

SUMMARY: Seed as a highly-valuable resource is preserved in collections for many years. Although the seed is kept under optimal conditions, monitoring of germination and the presence of fungi during seed preservation is of great importance. Therefore the aim of this paper is to examine the seed health status and germination of 43 onion accessions kept in the timespan for 15 years in the Institute of Field and Vegetable Crops collection. Germination of seed samples varied from 7–93%. The presence of fungi in the collection was determined on 33 tested samples. Fungi from the genera *Acremonium, Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Fusarium* and *Penicillium* were developed. The following *Fusarium* species identified on the seeds were *F. proliferatum*, *F. graminearum*, *F. sporotrichioides*, *F. solani*, *F. pseudograminearum* and *F. equiseti*. Based on factor analysis, *Fusarium* and *Penicillium* affected germination, while the occurrence of *Alternaria* species on onion seed is connected to the year of harvest.

KEYWORDS: onion, seed, fungi, germination, collection

INTRODUCTION

Onion (*Allium cepa* L.) is an important vegetable crop grown worldwide as well as in Serbia. In the agroecological conditions of Serbia, onion is grown at the relatively constant production areas, due to favourable natural conditions, tradition and daily use. The production area under this vegetable in Serbia in 2020 was 4,080 ha (Statistical office of Republic of Serbia).

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Onion could be produced from seeds and onion sets. Production from onion sets is typical for Southeast Europe, including Serbia (Gvozdanović-Varga, 2011), therefore the main focus was on fungi attacking onion sets and bulbs. However, special attention should be paid to storage of commercial seed because one of the major constraints in onion cultivation is the limited availability of vigorous seeds. On the other hand, breeders maintain onion genetic collections, also known as breeding or working collections. They contain seeds of accessions of various biological developmental stage, different geographic origin and genetic diversity. These collections should provide enough material for breeding and *ex situ* conservation. It is presumed that the conditions for seed preservation in breeders' collections are optimal.

The quality of onion seeds depends on many factors, such as environmental conditions during the growth of the mother plant and seed development, location of seeds on the plant, time and technology of harvesting and post harvest conditions (Dorna et al., 2013). The main reasons for the low quality of onion seeds besides the long flowering period and storage under suboptimal conditions are fungal infestations (Brocklehurst, 1985). However, it is known that breeders collect seeds from healthy vigorous plants. The quality of fully developed seed and its longevity are largely conditioned by abiotic factors in the storage (humidity, temperature, oxygen), but genetic and pre-storage factors should not be neglected, as well (Kalman et al., 2020; Solberg et al., 2020). Standardization of packaging and keeping conditions could contribute to the satisfying quality of onion planting (Rao et al., 2006).

Multiplication of the higher category of onion seed is expensive, labour demand and time-consuming due to a three or two-year period of production (Ozer and Koycu, 2004). Therefore, that process should be planned accurately, especially if we take into consideration that there is no need for sowing all the accessions from the seed collection every year or every second year. It is very important to monitor the current status of seed viability in the collected samples. The aim of this paper is to examine the seed health status and germination of accessions kept for 1–15 years in onion seed collection in the Institute of Field and Vegetable Crops.

MATERIAL AND METHOD

The presence of seed-borne fungi was analysed in 43 onion seed accessions within the collection of the Institute of Field and Vegetable Crops (Table 1). This survey included 13 varieties, two lines, one experimental hybrid and 11 landraces of *Allium cepa*. After the harvest, the seed samples were kept in the paper bags packed in the closed metal containers in the climate chamber at 5–6 °C, during the period 1–15 years.

Table 1. List of tested seed accessions

Accession / collection number	Year of production	Locality / Country of origin	Biological status of accession
Kupusinski jabučar (KJ)	2000; 2002; 2003; 2004; 2006; 2012; 2013	Serbia	variety
Holandski žuti (HY)	2004; 2005; 2006; 2013	Serbia	variety
Alek (A)	2004; 2005; 2006; 2007; 2010	Serbia	variety
Srebrenjak (S)	2001	Serbia	variety
Ljaskovski 58 (K 121)	2002	Bulgaria	variety
Junski srebrenjak (JS)	2005	Serbia	variety
Makoi bronz (MB)	2005	Hungary	variety
Favorit (K 107)	2006	Hungary	variety
Holland yellow (K 183)	2006, 2009	Crna bara, Serbia	variety
Makoi Feher (K 45)	2007	Hungary	variety
Ema (K 185)	2008	Hungary	variety
Istrian yellow (K 64 Ž)	2012	Croatia	variety
Istrian red (K 64 P)	2012	Croatia	variety
Ptujski red (K 211)	2014	Slovenia	variety
Kupusinski pogačar (KP)	2005, 2009	Serbia	breeding line
Nº 8301 (K 37)	2007	Bulgaria	breeding line
Ptujski x 185 (PT)	2010	Serbia	experimental hybrid
K 51	2007	Turopolje, Croatia	landrace
K 56	2007	Kistanje – Knin, Croatia	landrace
K 58	2007	Golubić – Knin, Croatia	landrace
K 65	2007	Istria, Croatia	landrace
K 76	2008	Breza – Sarajevo, Bosnia and Herzegovina	landrace
K 154	2008	Vrnjačka banja, Serbia	landrace
K 134	2009	Kardeljevo – Komin, Croatia	landrace
K 184	2009	Temerin, Serbia	landrace
K 191	2013	Trebinjski pogačar, Croatia	landrace
K 208	2014	Kupusina, Serbia	landrace
K 210	2014	Valpovo, Croatia	landrace

In order to analyse seed mycoflora, a standard phytopathological method was used. Onion seeds were sterilized in a 1% solution of sodium hypochlorite (NaOCl) for five minutes. Thereafter, seeds were rinsed twice with sterile water and incubated on a PDA medium at 25 °C in the dark. For each sample, ten seeds in three replication were analysed, 30 seeds in total. After seven days of incubation, fungi developed on the seed were identified morphologically by microscopic observations. Fungi from the genus *Fusarium* were identified to the species level according to the key (Leslie and Summerell, 2006). Germination

on PDA medium was recorded. Seed infection and germination rate were calculated according to the following formulas:

$$\text{Seed infection rate (\%)} = \frac{\text{Number of seeds infected by fungi}}{\text{Total number of seeds}} \times 100;$$

$$\text{Seed germination rate (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100.$$

Statistical analysis of data was done using software STATISTICA, ver. 13.2 (Dell, Inc., USA). Data obtained for germination were compared by Analysis of Variance (ANOVA) followed by the Bonferroni test ($p<0.01$). Factor Analysis was performed to reduce the number of variables and to detect relationships between variables. Rotated orthogonal components (varimax normalized method of rotation) with eigenvalues >1 were extracted (Kaiser, 1960) and the relative scores were determined. The occurrence of fungi was shown by graphs in Excel.

RESULTS AND DISCUSSION

Various fungal species have been determined in onion seeds produced in different climatic regions (Koycu and Ozer, 2007). The presence of fungi in the IFVCNS collection was determined on 34, out of 43 tested onion seed samples. Fungi from the genera *Acremonium, Alternaria, Aspergillus, Cladosporium, Epicoccum, Fusarium* and *Penicillium* were developed on the examined seeds. Species from the genus *Fusarium, Botrytis aclada* and *Aspergillus niger* are known as onion seed-borne pathogens (Chilvers and Du Toit, 2006; Southwood et al., 2015). The presence of numerous saprotrophic fungi from the genera: *Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Penicillium* and *Rhizopus* were also detected (Tylkowska and Dorna, 2001).

Fungi of the genus *Fusarium* were identified in eight samples with an infection rate of 3.3–23.3% (Figure 1). The highest number of *Fusarium* species, as well as the highest per cent of the seed infection, was found in sample A (2010). The following *Fusarium* species were identified on this seeds sample *F. proliferatum, F. graminearum*, *F. sporotrichoides, F. solani, F. pseudograminearum* and *F. equiseti.* The most frequent species was *Fusarium proliferatum* which appeared on seven seed samples (Table 2). Haapalainen et al. (2016) reported that *F. oxysporum* was frequently found in onions but only 15% of the isolates caused growth stunting in onion seedlings, while all *F. proliferatum* isolates tested were pathogenic to onion. Seed sample HY (2006) was infected with two *Fusarium* species (*F. proliferatum* and *F. oxysporum*), while on sample K37 (2007) *Fusarium compactum* was identified.

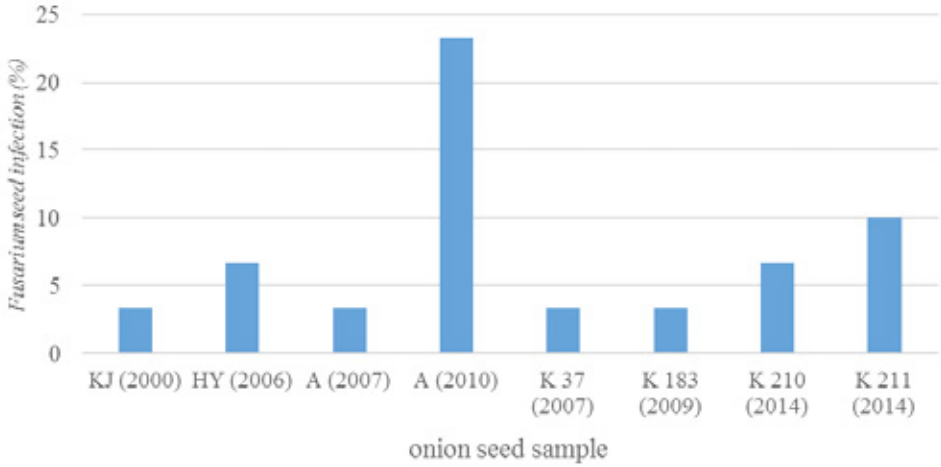

Figure 1. Fusarium onion seed infection

In Serbia, *Fusarium* species periodically cause significant diseases of onion, especially death and rot of seedlings (Klokočar-Šmit et al., 1988; Lević et al., 2009). Following species were reported on onion *F. oxysporum*, *F. solani*, *F. proliferatum*, *F. acuminatum*, *F. cepae*, *F. equiseti*, and *F. verticillioides* (Klokočar-Šmit et al., 1990; Lević, 2008). *Fusarium proliferatum* was reported as a predominant fungal species isolated from the roots and bulbs of onion plants in Serbia (Stanković et al., 2007). Recent findings of *Fusarium* sp. FIESC3, (such as members of FIESC a complex of morphologically similar species *F. equiseti / F. semitectum / F. incarnatum*) as a causal agent of pre-emergence dumping off, decay and rot of onion seed in Serbia was reported by Ignjatov et al. (2017). Apart from *F. proliferatum, F. equiseti*, *F. tricinctum F. sporotrichioides* and *F. poae* were first described in *Allium* sp. in Germany (Boehnke et al., 2015). *F. graminearum* was recorded on onion sets in Turkey (Koycu and Ozer, 1997). According to our knowledge, it is the first report of *F. sporotrichoides*, *F. pseudograminearum* and *F. compactum* on onion seeds in Serbia. The pathogenicity of these species should be tested.

Apart from the fungi of the genus *Fusarium* and other species, *Aspergillus niger* (the causal agent of black mould) can also be phytopathogenic, and its importance is reflected in the potential transmission of infection from seed to onion set (Koycu and Ozer, 1997). Species from the genus *Aspergillus* were isolated from ten onion samples, while *A. niger* was isolated from eight of them. The seed infection by *A. niger* was rated from 3–20% (Figure 2). The causal agent of black mould could be a predominant seed transmitted fungus on onion seeds and the problem is that visual symptoms are not observed because of latent infection (Saranya et al., 2017).

Table 2. Fusarium species isolated from onion seed

Variety Genotype /year	*Fusarium* species on onion seed samples (number of isolates)	seed infection (%)
KJ (2000)	*F. proliferatum* (1)	3.3
HY (2006)	*F. proliferatum* (2) *F. oxysporum* (1)	10.0
K 37 (2007)	*F. compactum* (1)	3.3
K 183 (2009)	*F. proliferatum* (1)	3.3
A (2007)	*F. proliferatum* (1)	3.3
A (2010)	*F. proliferatum* (1), *F. graminearum* (2) *F. sporotrichioides* (1), *F. solani* (1) *F. pseudograminearum* (1), *F. equiseti* (1)	23.3
K 210 (2014)	*F. proliferatum* (2)	6.7
K 211 (2014)	*F. proliferatum* (3)	10.0

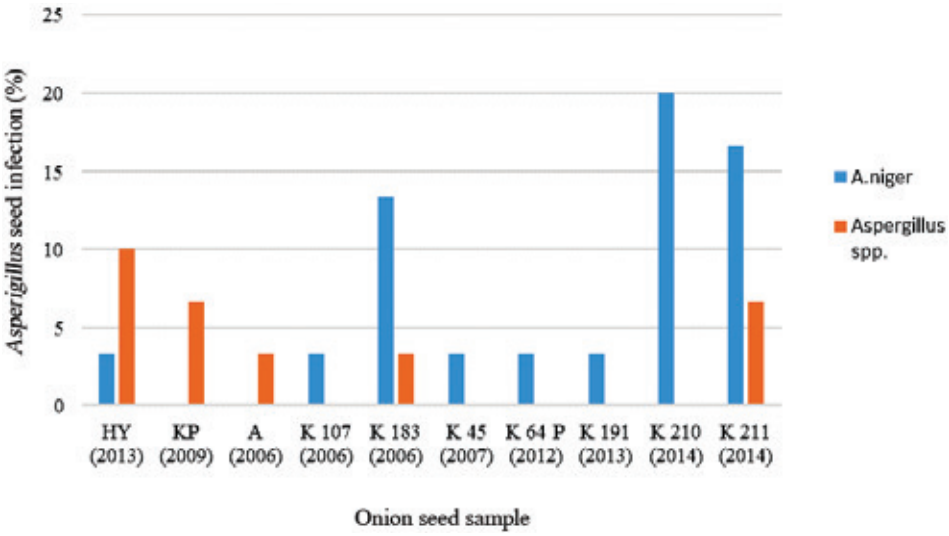

Figure 2. Aspergillus onion seed infection

Alternaria sp. were the most frequent and found on 15 onion samples. Three samples had infection 33%, three were between 20–27% and other samples were from 3–13% (Figure 3). Almost all samples which had *Alternaria* infection above 20% were grown in 2013 and 2014. The pathogenicity of *A. alternata* species on onion is described by Bihon et al. (2015), while *A. porri* is a well-known pathogen of onion and could be seed-borne (Kim et al., 2022). Fortunately, *A. porri* was not detected on the tested seed.

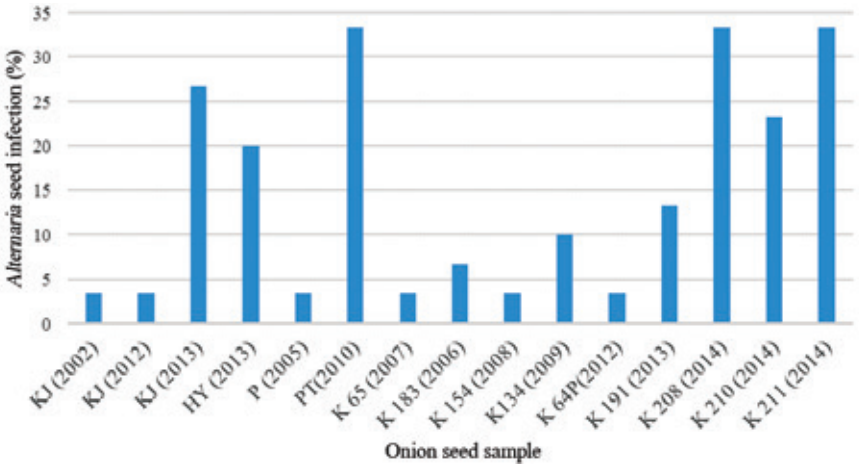

Figure 3. Alternaria onion seed infection

Penicillium infected 14 samples at a rate from 3 to 13.3% (Figure 4). A similar *Penicillium* seed-borne infection rate on onion seed in storage was reported by Adongo et al. (2015), although these authors did not mention the pathogenicity of these isolates.

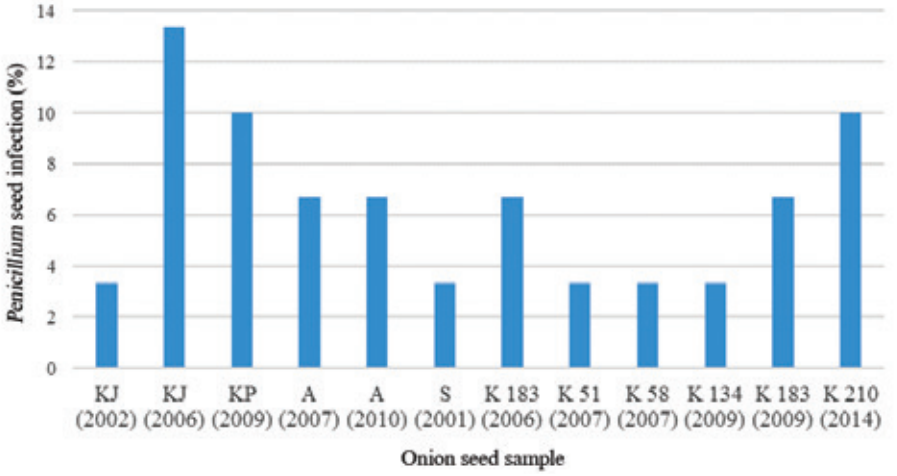

Figure 4. Penicillium onion seed infection

Seeds of K 191 (2013) and K208 (2014) had 16.6% infection with *Acremonium* sp. and other seven samples (K 64P (2012); K 64Ž (2012); K 154 (2008); K 37 (2007); KP (2009); HŽ (2004) and KJ (2006)) had an infection of 3%. *Epiccocum* sp. was found in three seed samples KJ (2006) 13% and A (2010) and K (208) 3%. *Cladosporium* sp. was isolated from three samples P (2005),

JS (2005) and K(208) with infection rate 3, 6.7 and 10% respectively. *Trichoderma* sp. was developed only on sample P (2005). These fungi were determined as saprophytic on onion seeds. Although these species are noted as saprophytic, their role in seed-borne disease of onion has not been revealed (Özer and Köycü, 2004).

Germination of tested genotypes varied from 7–93% (Table 3). Samples classification into homogenous groups was done according to the Bonferroni test. The highest germination had two genotypes stored for six and seven years (A (2007) and K 185 (2008)). Besides the high importance of environmental conditions, storage of the tested accessions (paper bags packed in the closed metal containers which were kept at constant temperature) strongly contributed to the seed viability preservation. It is in the accordance with Rao et al. (2006), who reported that the use of hermetically sealed containers, desiccants and low temperatures improves storability as several physiological and biochemical processes and products are being regulated during dry storage. Various authors indicated a different period in which onion seed retained high germinability in the storage. Doijode (1995) reported that onion seed could retain high germinability up to seven years when stored with silica gel in a moisture impervious container at 5–8 °C. Seeds with an initial germination percentage above 90%, kept at 10 °C, keep their germination potential after 12 months (Dorna et al., 2013).

The lowest germination had samples S (2001) and A (2010) (7 and 13% respectively). These genotypes are significantly different from those which achieved germination above 70% (Table 3). However, the low germination in these genotypes was caused by different factors. Genotype S (2001) had germination below 10% probably due to seed age, while sample A (2010) had 23% of *Fusarium* seed infection (Table 2).

Table 3. Germination of tested seed accessions

Germination (%)	Seed accessions
93	A (2007); K185 (2008)^{a*}
90	K 184 (2009)^{ab}
83	K76 (2008)^{abc}
73–80	K56 (2007); K134 (2009)^{abcd}
67–70	K107 (2006);K65 (2007); KJ (2012)^{abcde}
37–63	K210 (2014); K183 (2009); K64Ž (2012); KJ (2000); HY (2013); A (2006) KJ (2004) KJ (2013) K191 (2013) KJ (2003) MB (2005) K154 (2008) KP (2005) KJ (2006) K58 (2007); K37 (2007); K51 (2007); K45 (2007); KP (2009); K64P (2012)^{abcdef}
33	HY (2004); K 211 (2014); KJ (2002);JS (2005); HY (2005)^{bcdef}
27–30	K 121 (2002); K208 (2014); K183 (2006);A (2004)^{cdef}
23	A (2005), PT (2010); HY (2006)^{def}
13	A (2010)^{ef}
7	S (2001)^{f}

* values with different letters in the columns differ significantly at a significance level of $p<0.05$

Besides the other factors (such as environmental conditions during and after harvest) that were involved in germination, a genotype also influenced this trait. Ilić et al. (2006) reported that seed germination depends on the variety. On the other hand, the reduction in the germination of onion seed is attributed to its chemical composition and fragile seed coat which favours higher lipid peroxidation and fungal incidence (Amalfitano et al., 2019).

Table 4. Total seed infection of tested onion accessions

Total seed fungal infection (%)	Genotype/Variety
0	KJ (2003); KJ (2004); HY (2005); A (2004); A (2005); MB (2005); K65 (2007); K56 (2007); K76 (2008); K185 (2008);
1–5	KJ (2000); KJ (2012); HY (2004); A (2006); S (2001); K121 (2002); K107 (2006); K45 (2007); K51 (2007); K58 (2007); K64Ž (2012)
6–10	JS (2005); K37 (2007); KJ (2002); HY (2006); K154 (2008); K-184 (2009); A (2007); K183 (2009); K64P (2012)
11–30	KJ (2006); KJ (2013); KP (2005); KP (2009); K183 (2006); K134 (2009)
33–59	A (2010); K191 (2013); PT (2010); HY (2013);
60–67	K210 (2014); K211 (2014); K208 (2014);

Further explanation of the obtained results was done through the factor analysis. The Eigenvalues and variance explained by factors were indicated in the Table 5. Only the first five factors were presented because Eigenvalue was over one, and the total cumulative variance of those factors accounted 81.69%. The values in the Table 6, indicated the contribution of each variable to the factors. Only those factor loadings greater than 0.5 were considered important, these values are highlighted in bold. Factor 1 was strongly associated with year, *Alternaria, Aspergillus* and healthy seeds. *Acremonium* and *Cladosporium* were strongly associated with Factor 2.

Table 5. Eigenvalues and variance of the first five factors

Factor	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	3.357	30.516	3.357	30.516
2	1.796	16.329	5.152	46.845
3	1.521	13.827	6.673	60.672
4	1.269	11.539	7.943	72.211
5	1.043	9.482	8.986	81.693

Table 6. Factor analysis of evaluated parameters

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
year	**0.827**	0.209	0.040	-0.210	0.172
Alternaria sp.	**0.827**	0.326	-0.130	0.156	0.008
Acremonium sp.	0.227	**0.829**	0.075	-0.071	0.176
Penicillium sp.	0.145	-0.220	**0.872**	-0.032	0.017
Epicocum sp.	-0.078	0.315	**0.844**	0.078	0.032
Aspergillus sp.	**0.797**	-0.308	0.042	0.163	-0.020
Cladosporium sp.	0.086	**0.790**	-0.031	0.116	-0.319
Trichoderma sp.	-0.021	0.055	-0.035	-0.039	**-0.966**
Fusarium sp.	0.289	-0.266	0.320	**0.581**	0.030
healthy seeds	**-0.849**	-0.286	-0.286	-0.264	0.178
germination	-0.018	-0.161	0.095	**-0.923**	-0.016

Based on Factor analysis (Figure 5), the main species which affected germination were *Fusarium* and *Penicillium*. This was in accordance with the results of other authors (Palmero et al., 2012; Kintega et al., 2020) who reported that *Fusarium* species are seed-borne and pathogenic species. Among the saprophytic species, *Aspergillus* spp. and *Penicillium s*pp. could strongly influence the viability of the seeds during storage (Maude, 1996; Fontana et al., 2018).

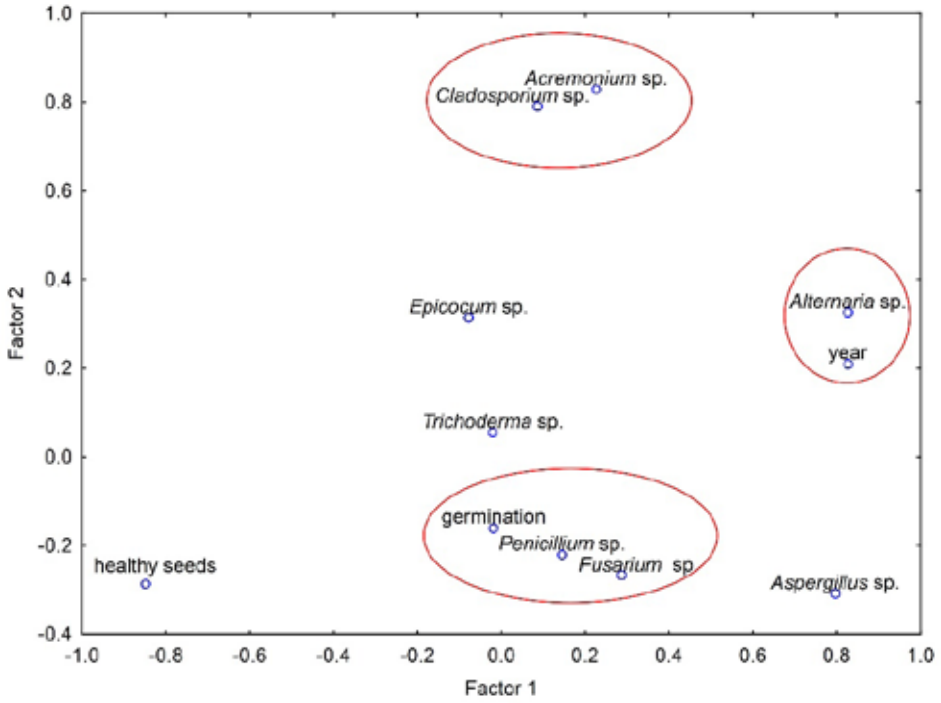

Figure 5. Biplot of evaluated traits in onion seed

Aspergillus niger was reported to reduce seed germination, seedling emergence and vigour (El-Nagerabi and Ahmed, 2001). However, samples K (210) and K (183) with *A. niger* infection 13.3 and 20% respectively, had high germination percent (67–70%) (Table 3). Additionally, the occurrence of *A. niger* was analyzed together with other *Aspergillus* species. Therefore *Aspergillus* species were not connected with germination (Figure 5).

The occurrence of *Alternaria* species on onion seed was connected to the year of harvest. The highest percentage of fungi from the genus *Alternaria* was observed in 2013 and 2014 (Figure 3). Additionally, the samples with the highest total seed fungal infection (33–67%) were harvested in 2010, 2013 and 2014 (Table 3). Meteorological conditions in 2013 and 2014 in Vojvodina, in the period of flower stems formation and beginning of flowering (May and June) were characterised by a doubled amount of precipitations compared to multiyear average and frequent showers. Similar weather conditions in July were followed by high temperatures during the period of seed filling. These conditions such as high humidity, and high temperatures favoured the development of *Alternaria* sp. However, in 2010 when there was the highest occurrence of *Fusarium* species on sample A (2010) the amount of precipitation in May and June was doubled and by 60% more than the multi-year monthly average respectively. In August and July of 2010, the weather was warm with regular rainfall (http://www.hidmet.gov.rs/ciril/meteorologija/agro.php). In Vojvodina, the early onion seed harvest is at the beginning of August (Gvozdanović Varga, 2011). These conditions during the vegetation period probably favoured *Fusarium* seed infection.

Acremonium and *Cladosporium* species grouped closely showing a clear distinction from other traits. Such grouping of these species indicated that they did not have any influence on the germination and health status of the seeds. Healthy seed is segregated from other traits.

CONCLUSION

Seed storage in the breeder's collections is a very important part of seed preservation for a long time. The main prerequisites for quality preservation are optimal storage conditions as well as high-quality seed. Additionally, the year of harvest is very important due to the presence of latent fungal infection on onion seed. Therefore the introduction of samples into the onion seed collection should be under the accurate analysis of their health status and germination.

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ПОЈАВА ГЉИВА НА УСКЛАДИШТЕНОМ СЕМЕНУ ЦРНОГ ЛУКА

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РЕЗИМЕ: Семе је веома важан ресурс који се чува у колекцији у вишегодишњем периоду. Иако се семе складишти у оптималним условима, веома је важно пратити клијавост и његово здравствено стање. Циљ овог рада је да се испита здравствено стање и клијавост четрдесет три узорка семена црног лука, који су чувани у колекцији Института за ратарство и повртарство 1–15 година. Клијавост црног лука варирала је у опсегу 7–93%. На тридесет три узорка је идентификовано присуство гљива из седам родова: *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Fusarium* и *Penicillium*. У оквиру рода *Fusarium* утврђено је присуство врста *F. proliferatum*, *F. graminearum*, *F. sporotrichioides*, *F. solani*, *F. pseudograminearum* и *F. equiseti*. На основу факторске анализе врсте које су утицале на клијавост су *Fusarium* и *Penicillium*, док је појава врста из рода *Alternaria* повезана са годином убирања семена.

КЉУЧНЕ РЕЧИ: црни лук, семе, гљиве, клијавост, колекција, *Fusarium*