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Detection of Seed Borne Fungi Associated with Some Cereals and Legume Crops of Seeds Grown in Main Season at Holetta Agricultural Research Center

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Abstract: Fungi are a major cause of postharvest deterioration of cereals and legumes. The current work was carried out using sixteen samples of eight crops wheat, barley, Teff, oat, lentil, fababean, mungbean and chickpea samples were collected to investigate the presence and incidence of seed borne fungi associated with them using PDA media. Results of the mycological analysis revealed that a total of 14 fungi belonging to 11 genera viz. *Penicillium* sp., *Aspergillus flavus*, *Aspergillus niger*, *Bipolaris* sp., *Botrytis* sp., *Eppicocum* sp., *Sclerotium* sp., *Alternaria* sp., *Fusarium graminaerum*, *Fusarium moniliformae*, *Fusarium oxysporium*, *Trichoderma* sp., *Tilletia* sp. and *Rhizopus* sp. were isolated from samples of the different crop seeds obtained from seed multiplication store, EIAR, HARC. Infection percentage varied from 5-100% in samples of seed multiplication store. In wheat variety wane and limu, in oat variety CI8237, CI8238, CI8239 and CI8240, in chickpea variety Natoli, Habru and Arerti, and in fababean variety Dosha showed 100% infection followed by Wolki of Fababean (97.5%), and one variety of Mungbean showed infection of 80%. One variety Iboni of Barley showed 75% infection and Dagem and Kora of Teff showed 37.5% and 12.5% infection. Bekoji variety of Lentil showed least infections of 5%. The obtained results revealed that seed-borne pathogens were present in most seed samples of important cereals and legume crops. Some of the identified fungi are potential producers of mycotoxins, thus their presence is important in terms of reduced food safety for humans and animals. Therefore, an early and accurate diagnosis and pathogen surveillance will provide time for the development and the application of disease management strategies.

Keywords: Crops, Incidence, Postharvest Deterioration, Seed Borne Fungi, Seed Health

1. Introduction

Seeds are considered as the root and foundation for crop production and breeding. They are also imperatively the strategic wealth for subsistence and development of human. The promotion of crop yield and quality greatly depends on the quality of seeds and often seed determines the yield. Seed health has been considered as an attribute of high quality and one of the most important premises for safe conservation [1]. Seed borne fungal, bacterial, and viral pathogens have some deleterious effects on seed, such as, reducing seed viability, vigor, germination capability, shortening longevity of conservation, and causing physiological changes. Furthermore, some seed borne pathogens are also seed-transmitted, which can cause severe diseases in the field after seed movement at a long distance [2].

The production is affected by diseases through reduction in yield caused by attack of fungal pathogens. Some of these fungal pathogens move into the field through the seeds, that is, they are seed-borne. Seeds are regarded as very effective means for transporting plant pathogens over long distances. Seed health plays an important role for successful cultivation and yield exploitation of a crop species. Among various factors that affect seed health, the most important are the seed associated fungi that not only lower seed germination, but also reduce seed vigor resulting in low yield. Seed-borne

diseases caused by fungi are relatively difficult to control as the fungal hyphae get established and become dormant. A seed associated pathogen present externally, internally or associated with the seed as contaminant, may cause abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection [3, 4].

Seed-borne infection of fungal pathogens are important not only for its association with seeds which cause reduction or failure in germination and causing disease to newly emerged seedlings or growing plants but also contaminate the soil by establishing its inocula permanently [5]. In this study, routine technique of seed health testing was applied to monitor and analyze the fungi that infect wheat, barley, teff, oat, lentil, fababean, mungbean and chickpea seeds stored in the seed multiplication store. Therefore, this study is designed to determine the identity and incidences of major fungi associated with crop seeds and establish and update the list of major seed-borne fungi associated with crop seeds in Holetta agricultural research center seed multiplication.

2. Materials and Methods

2.1. The Crop Seeds

Wheat, barley, Teff, oat, lentil, fababean, mungbean and chickpea seeds (totally 16 samples) were obtained from seed multiplication store at Holetta Agricultural Research Centre (HARC). These samples belonged to two, one, two, four, one, two, one and three varieties of wheat, barley, Teff, oat, lentil, fababean, mungbean and chickpea respectively grown in 2018 main season were used in this experiment after six months of storage.

2.2. Isolation of Fungi

Fifty seeds per sample were surface sterilized with 10% Chlorox solution to remove saprophytes for 3 min, followed by three times rinse in sterile distilled water for one minute each. Five surface sterilized seeds were then placed on each potato dextrose agar (PDA) media plates, and incubated for seven days at 25°C. Finally after eight days of incubation seeds on each petri-dish were examined under stereo microscope for the observation of presence of associated fungi, mycelia growth, fungal isolation and pure cultures of different out growing fungi were obtained by transferring fungal colonies to new PDA plates using sterile loop, and incubating the plates for seven days at 25°C. Pure cultures of each isolate was then stored at 4°C in vials containing 2.5 ml of sterile distilled water for further use. The number of infected seeds were counted and expressed in percentage and also proportion of samples that yielded its isolates was determined as follow:

$$\text{Frequency of occurrence (IN\%)} = \frac{\text{Number of seeds on which fungal species occurs}}{\text{Total number of seeds plated}} \text{ X } 100$$

$$\text{Isolation Frequency (IF\%)} = \frac{\text{Number of samples of occurrence of fungi species}}{\text{Total number of samples}} \text{ X } 100$$

2.3. Identification of Fungal Species

Pure cultures were obtained after repeated sub-culturing of fungi appearing on seeds on Potato Dextrose Agar (PDA) plates. The fungi were identified on the basis of spore morphology and colony characteristics using stereoscopic microscope. A list of morphological characters of taxonomic importance such as spore size, shape, septation, color and their arrangement on the conidiophores, character of the mycelium were compiled for each fungus. Identification of the fungus was performed using all the characteristics observed and identification reference manuals of Barnett & Hunter, and Singh [6, 7].

3. Result and Discussion

3.1. Fungi Associated with the Crops

In total, eleven fungi were isolated from wheat seeds. Fungi isolated from wheat seeds (Table 1) were *Alternaria* sp., *Aspergillus flavus*, *Aspergillus niger*, *Bipolaris* sp., *Eppicocum* sp., *Fusarium graminaerum*, *Fusarium moniliformae*, *Fusarium oxysporium*, *Penicillium* sp., *Rhizopus* sp. and *Trichoderma* sp. whereas six fungal species were found to be associated with barley seeds viz, *Alternaria* sp., *Aspergillus flavus*, *Bipolaris* sp., *Rhizopus* sp., *Sclerotium* sp., and *Tilletia* sp.. Fungi isolated from oat were four specie *Aspergillus flavus*, *Aspergillus niger*, *Alternaria* sp., and *Sclerotium* sp. whereas from tef were 3 fungi species *Alternaria* sp., *Aspergillus flavus*, and *Eppicocum* sp.. The only *Aspergillus flavus* was isolated from lentil. Four fungi species *Aspergillus flavus*, *Botrytis* sp., *Fusarium moniliformae*, and *Rhizopus* sp. were isolated from fababean whereas from mungbean 2 fungi species *Aspergillus flavus* and *Botrytis* sp.. Eight fungi species *Penicillium* sp., *Aspergillus flavus*, *Aspergillus niger*, *Alternaria* sp., *Botrytis* sp., *Fusarium moniliformae*, *Fusarium oxysporium*, and *Rhizopus* sp. were isolated from chickpea.

One of the dominant species isolated from the collected samples was *Aspergillus flavus*. The major distinction of this fungus was that its colonies are characterized by yellow to dark, yellowish-green pigments, consisting of a dense felt of conidiophores or mature vesicles bearing Phialides over their entire surface separating from the other species of Aspergillus [8-10]. This observation was also confirmed by [11], who reported *A. flavus* colonies as being initially yellow, turning to yellow-green or olive green with age and appearing dark green with smooth shape and having rapid growth.

Another dominant species of fungi isolated was *Alternaria* sp. which is characterized with dark grey colony

but may also be white, olive green, brown, or almost black and conidiophores are dark to olive brown and smooth, arise singly or un-branched. The distinctive character was light brown, tapered to a beak, conidia with transverse and longitudinal septa [10, 12]. The current study also confirmed the production of light brown, tapered to a beak, conidia with transverse and longitudinal septa and having quick growth.

Similar results were reported in a previous study, while testing the wheat germplasm conserved in gene bank. The predominant fungi isolated were Alternaria, Rhizopus, Penicillium, Aspergillus, Bipolaris, Cladosporium, Fusarium, Cladosporium, and Trichothecium [1].

Agreed with the presence of *Fusarium moniliformae*, *Alternaria* sp., *Bipolaris* sp., *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria* sp., *Penicillium* spp. and *Trichoderma* sp. associated with the seeds of rice varieties both in storage and in the field have been reported earlier [13, 14] and also confirmed with the study of Asela *et. al*; who reported that these fungi species *Fusarium oxysporium*, *Fusarium monoliformae*, *Fusarium graminaerum*, *Botrytis* sp., *Bipolaris victoriae*, *Bipolaris sorokiniana*, *Aspergillus flavus*, *Aspergillus niger*, *Phoma* sp., *Tilletia indica*, *Eppicocum nigrum*, *Cladosporium macrosporium*, *Alternaria triticina*, *Rhizopus* sp., *Penicillium chryosegenum* and *Nigrum* sp. were associated with the seeds of wheat [10].

The result revealed most of samples were infected by several species of fungi in the crop seed varieties tested. Some fungi, such as Alternaria, Aspergillus, Bipolaris, and Fusarium produced different fungal toxins, which could make changes in the chemical ingredients inside the seeds, reduce nutritive value and viability of seeds, and even cause seed death. In addition, some seed-borne fungi were also the causal agents of diseases of the roots, stems, and leaves of crops. The diseases transmitted by seeds resulted in an increase of difficulty in disease control and in the decline of yield and quality. The seed-borne pathogenic fungi keep long-term survival in seeds, when the seeds are conserved in the storage, under conditions of low temperature and relative humidity, which could have a harmful effect on germplasm viability and genetic integrity, and also can cause a potential threat to agricultural production when the seed is planted and propagated [1]. Also it was reported that fungal incidence was highly associated with two of the independent variables, namely, temperature and relative humidity of storage [10, 15]. Therefore, seed health testing is vital for management of seed-borne pathogen in the preserved seeds.

Table 1. *Isolation frequency of fungi from crops.*

Isolation Frequency (%)														
Crop	**variety**	***Afl***	***Anig***	***Alt***	***Bipo***	***Botr***	***Eppi***	***Fgra***	***Fmon***	***Foxy***	***Peni***	***Rhiz***	***Scl***	***Tind***	***Tric***
Barley	Iboni	100	0	100	100	0	0	0	0	0	0	100	100	100	0
Oat	CI8237	100	100	100	0	0	0	0	0	0	0	0	0	0	0
	CI8238	100	100	100	0	0	0	0	0	0	0	0	0	0	0
	CI8239	100	0	0	0	0	0	0	0	0	0	0	100	0	0
	CI8240	100	100	100	0	0	0	0	0	0	0	0	100	0	0
Wheat	Wane	100	100	100	100	0	100	100	100	100	0	100	0	0	0
	Limu	100	100	100	0	0	0	0	100	100	100	100	0	0	100
Teff	Dagem	100	0	0	0	0	0	0	0	0	0	0	0	0	0
	Kora	100	0	100	0	0	100	0	0	0	0	0	0	0	0
Lentil	Bokoji	100	0	0	0	0	0	0	0	0	0	0	0	0	0
Fababean	Dosha	100	0	0	0	100	0	0	100	0	0	100	0	0	0
	Wolki	100	0	0	0	100	0	0	100	0	0	100	0	0	0
Mungbean	Sanabor	100	0	0	0	100	0	0	0	0	0	0	0	0	0
Chickpea	Natoli	100	0	0	0	100	0	0	0	0	0	100	0	0	0
	Habru	100	100	100	0	100	0	0	100	0	100	100	0	0	0
	Arerti	100	0	100	0	100	0	0	0	100	100	100	0	0	0

Foxy = Fusarium oxysporium, Fmon = Fusarium monoliformae, Fgra = Fusarium graminaerum, Botr = Botrytis sp., Bipo = Bipolaris sp., Afl = Aspergillus flavus, Anig = Aspergillus niger, Tind = Tillitia indica, Eppi = Eppicocum sp., Alt = Alternaria sp., Rhiz = Rhizosporium sp., peni = Penicillium sp., Scl = Sclerotium sp., Tric = Trichoderma sp.

3.2. Fungal Contamination Frequency and Incidence

Mycological analysis revealed the contamination of crop seeds by different fungal species with different frequencies. The most predominant fungi isolated was *A. flavus* (100%) followed by *Alternaria alternata* (56.25%), *Rhizopus* sp (50%), *Aspergillus niger* and *Botrytis* sp. (37.5%), *Fusarium moniliformae* (25%), *Penicillium* sp., *Fusarium oxysporium* and *Sclerotium* sp. (18.5%), *Bipolaris* sp. and *Eppicocum* sp. (12.5%), *Fusarium graminaerum*, *Tilletia indica* and *Trichoderma* sp. were found (6.25%) (Table 1).

Variation regarding percentage infection of different fungal species was observed. Maximum fungal infection (100%) was observed in wheat variety wane and Limu, in oat variety CI8237, CI8238, CI8239 and CI8240, in chickpea variety Natoli, Habru and Arerti, and in fababean variety Dosha. One variety of fababean Wolki showed (97.5%) infection while one variety of Mungbean was 80% infected. Variety Iboni of Barley showed (75%) infection whereas variety Dagem and Kora of Teff 37.5% and 12.5% infection respectively. Also

variety Bekoji of Lentil showed least infection (5%) (Table 2). Infection percentage varied from 5-100% in seed storage of HARC, EIAR.

The result agrees with previous study, reported that most predominant fungi isolated were *A. flavus* (20%) followed by *Penicillium* spp. (18%), *Aspergillus niger* (13%), *Alternaria alternata* (11%) and *Rhizopus* sp. (7%) [13]. The result also coincides with the findings of Asela *et. al;* who reported that *A. flavus* was the dominate species encountered in the samples of wheat grains [10]. Tsedaley reported the highest frequency of *Aspergillus* spp. (40.4%) at farmer preserved seed [16]. Also contamination of wheat grains by fungal species at different locations and storage time with different frequencies was reported and highest fungal incidence (98.62%) was recorded after six months storage of wheat grain [10, 15].

Table 2. *Frequency of occurrence of seed associated fungi isolated from crops.*

Incidence (%)															
Crop	**Variety**	***Afl***	***Anig***	***Alt***	***Bipo***	***Botr***	***Eppi***	***Fgra***	***Fmon***	***Foxy***	***Peni***	***Rhiz***	***Scl***	***Tind***	***Tric***	**Total**
Barley	Iboni	30	0	10	5	0	0	0	0	0	0	10	15	5	0	75
Oat	CI8237	80	10	20	0	0	0	0	0	0	0	0	0	0	0	100
	CI8238	90	20	20	0	0	0	0	0	0	0	0	0	0	0	100
	CI8239	90	0	0	0	0	0	0	0	0	0	0	10	0	0	100
	CI8240	80	10	10	0	0	0	0	0	0	0	0	10	0	0	100
Wheat	Wane	60	7.5	15	5	0	7.5	7.5	5	10	0	22.5	0	0	0	100
	Limu	40	12.5	20	0	0	0	0	5	15	2.5	22.5	0	0	7.5	100
Tef	Dagem	37.5	0	0	0	0	0	0	0	0	0	0	0	0	0	37.5
	Kora	5	0	5	0	0	2.5	0	0	0	0	0	0	0	0	12.5
Lentil	Bokoji	5	0	0	0	0	0	0	0	0	0	0	0	0	0	5
Fababean	Dosha	22.5	0	0	0	95	0	0	2.5	0	0	7.5	0	0	0	100
	Wolki	15	0	0	0	75	0	0	0	0	0	7.5	0	0	0	97.5
Mungbean	Sanabor	45	0	0	0	35	0	0	0	0	0	0	0	0	0	80
Chickpea	Natoli	20	0	0	0	50	0	0	0	0	0	32.5	0	0	0	100
	Habru	35	2.5	15	0	37.5	0	0	5	0	5	20	0	0	0	100
	Arerti	35	0	12.5	0	60	0	0	0	10	7.5	12.5	0	0	0	100

Foxy = Fusarium oxysporium, Fmon = Fusarium monoliformae, Fgra = Fusarium graminaerum, Botr = Botrytis sp., Bipo = Bipolaris sp., Afl = Aspergillus flavus, Anig = Aspergillus niger, Tind = Tillitia indica, Eppi = Eppicocum sp., Alt = Alternaria sp., Rhiz = Rhizosporium sp., peni = Penicillium sp., Scl = Sclerotium sp., Tric = Trichoderma sp.

4. Conclusion and Recommendation

Fungi are a major cause of postharvest deterioration of cereals and legumes. Despite its importance, storage fungi has been one of the most poorly understood pathosystems in Ethiopia. As a result, the present study was conducted to determine the identity and incidences of major fungi associated with crop seeds and establish and update the list of major seed-borne fungi associated with crop seeds in Holetta agricultural research center seed multiplication store. The major objective of the study was to contribute towards improved crop production through effective and sustainable management of storage fungi in the country.

A total of 14 fungi belonging to 11 genera viz. *Penicillium* sp., *Aspergillus flavus*, *Aspergillus niger*, *Bipolaris* sp., *Botrytis* sp., *Eppicocum* sp., *Sclerotium* sp., *Alternaria* sp., *Fusarium graminaerum*, *Fusarium moniliformae*, *Fusarium oxysporium*, *Trichoderma* sp. *Tilletia* sp. and *Rhizopus* sp. were isolated from samples of the crops. A total of 11 fungi belonging to 8 genera viz. *Penicillium* sp., *Aspergillus flavus*, *Aspergillus niger*, *Bipolaris* sp., *Eppicocum* sp., *Alternaria* sp., *Fusarium graminaerum*, *Fusarium moniliformae*, *Fusarium oxysporium*, *Trichoderma* sp. and *Rhizopus* sp. were isolated from wheat samples whereas from barley samples 6 fungi belonging to 6 genera (*Alternaria* sp., *Aspergillus flavus*, *Bipolaris* sp., *Sclerotium* sp., *Tilletia* sp. and *Rhizopus* sp.) were isolated. A total of 4 fungi belonging to 3 genera viz. *Aspergillus flavus*, *Aspergillus niger*, *Alternaria* sp., *Sclerotium* sp. were isolated from oat samples whereas from tef samples 3 fungi belonging to 3 genera (*Alternaria* sp., *Aspergillus flavus*, and *Eppicocum* sp.) were isolated.

The only *Aspergillus flavus* was isolated from lentil sample. A total of 4 fungi belonging to 4 genera viz. *Aspergillus flavus*, Botrytis sp., *Fusarium moniliformae*, and *Rhizopus* sp. were isolated from fababean samples whereas from mungbean samples 2 fungi belonging to 2 genera *Aspergillus flavus* and *Botrytis* sp. were isolated. A total of 8 fungi belonging to 6 genera viz. *Penicillium* sp., *Aspergillus flavus*, *Aspergillus niger*, *Alternaria* sp., *Botrytis* sp., *Fusarium moniliformae*, *Fusarium oxysporium*, and *Rhizopus* sp. were isolated from chickpea samples. Infection percentage varied from 5-100% in seed multiplication store. In wheat variety wane and limu, in oat variety CI8237, CI8238, CI8239 and CI8240, in chickpea variety Natoli, Habru and Arerti, and in fababean variety Dosha showed highest (100%) infection whereas variety Bekoji of Lentil showed least infection (5%).

The obtained results revealed that seed-borne pathogens were present in most seed samples of important cereals and legume crops. Some of the identified fungi are potential producers of mycotoxins, thus their presence is important in

terms of reduced food safety for humans and animals. Therefore, an early and accurate diagnosis and pathogen surveillance will provide time for the development and the application of disease management strategies. Last but not least, looking at the alarming rate of crop production in the country to feed the ever increasing population, this study suggests that research on the biology, ecology and management of major fungi associated with crop should be given due attention in the country.

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