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Mycoflora associated with stored seeds of soybean

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Abstract

An investigation to detect the seed-borne fungi from seeds of eight soybean cultivars including E-358, DB-1601, 849-294D, Loopa, HS-16, 95-I, Faisal Soybean and Centennial was conducted. Soybean seed samples were collected from Oilseed Research Institute, Faisalabad. Blotter method was used for detection of the associated fungi of soybean seeds. A total of 9 fungal genera belonging to Zygomycetes, Euascomycetes and Deuteromycetes were found associated with the tested soybean seed samples. Each class had three fungal genera. The isolation frequencies of the fungi were variable among cultivars as well as between naturally infested and artificially disinfested seeds. Common fungal genera isolated during this study included *Absidia, Aspergillus, Mucor, Curvularia, Drechslera, Fusarium, Phoma, Rhizopus* and *Penicillium. Aspergillus, Penicillium* and *Fusarium* were identified more commonly from disinfested than that of non-infested seeds.

Key words: *Glycine max,* isolation frequency, seed-borne fungi.

Introduction

Soybean [*Glycine max* (L.) Merril] is cultivated as commercial crops in both Kharif and Rabbi Seasons in Pakistan on 89 ha. (FAO, 2009). Popularity of this crop is due to abundance high quality protein (43%) and cholesterol free rich source of oil (20%) and with high unsaturated fatty acids (Golbitz, 2003; Olguin *et al*., 2003; Belewu and Belewu, 2007). Soybean is classified as "poor storer" as it loses viability drastically under warm and humid conditions due to frequent invasion by storage fungi (Sharma, 1977; Mondal *et al*., 1981; Nandi *et al*., 1982). Fungi are the major cause of spoilage in stored grains and seeds. It is reported in the literature that during storage several microbes including bacteria, nematodes, fungi contaminate seeds, had adverse effect on seed quality (Mehrotra and Aggarwal, 2003). The species of *Aspergillus, Penicillium, Fusarium, Rhizopus* and *Alternaria* have been found commonly occurring as post-harvest molds in storage condition (Mehrotra and Aggarwal, 2003). Most of the species of *Aspergillus* are dominant and play vital role in the seed biodeterioration (Chavan, 2011).

In agriculture, seeds play very important role for the production of healthy crop. About 90% of the crops all over the world are produced by using seeds. Seeds in the field as well as in ill storage conditions interact with several microbes which deteriorate the seeds, both qualitatively and quantitatively, which is due to chemical breakdown of protein, oil, and fatty acids by the seed borne microbes (Welbaum, 2006). Fungi growing on stored grains reduce the germination rate, carbohydrate, protein, total oil content, increase moisture content and also enhancing other biochemical changes of grains (Bhattacharya, 2002). Such seeds are unfit for human consumption, are rejected at the industrial level, and also by growers as it results in huge losses in harvested yield (Anwar *et al*., 1995). The objectives of this study were to isolate and identify the storage mycoflora associated with seeds of commercially grown soybean cultivars and to assess their incidence and frequency.

Materials and Methods

Collection of seed samples

Eight different cultivars of soybean including E-358, DB-1601, 849-294D, Loopa, HS-16, 95-I, Faisal Soybean and Centennial were obtained from Oilseed Research Institute, Faisalabad. The seeds were harvested in 2010 and were stored in storage house of Oilseed Research Institute, Faisalabad.

Isolation and identification of fungi

The seeds were surface sterilized with 1% sodium hypochlorite solution for five minutes followed by three washings with distilled water. The seeds were air-dried under laminar flow for plating on blotter paper and potato dextrose agar (PDA). Four hundred seeds from each variety were randomly selected from each lot. Seeds were placed on Petri-plates having blotter paper and PDA according to ISTA rules. One hundred seeds of each variety were taken for each treatment with ten replicates. The Petri dishes were incubated at 30±1 ^{o}C with alternating 12 hours day and night for seven days. The fungi were isolated after incubation period from the seeds. Each fungus, visually differed from each other, was picked from seeds each variety and transferred to malt extract agar (MEA) media for culturing. The media plates were incubated at 25±1 ^{o}C for the formation of conidia of fungus for identification. The pure cultures were obtained by subsequent inoculations and multiplying the fungus on MEA media for identification.

The fungal cultures were identified by studying fungal colonies and its various morphological characteristic including their colors, sizes and shapes. The fungi were identified by using different keys (Booth, 1971; Ellis *et al.,* 1980; Sivaesan, 1990; Klich, 2002; Frisvad and Samson, 2004). Temporary slides were prepared from the pure cultures for photographs.

Results and Discussion

A total of 9 fungal genera belonging to three classes were obtained from seeds of eight cultivars of soybean. Each class had three fungal genera. The isolation frequencies of the fungi were variable among cultivars as well as between naturally infested and artificially disinfested seeds (Table 1). Three fungal genera including *Absidia*, *Mucor,* and *Rhizopus* species belonged to Zygomycetes. Isolation frequency of *A. glauca was*1% and only isolated from non-disinfested seeds of HS-16. It is a ubiquitous saphrophyte and has been found associated with wheat, sorghum, soybean, chickpea water melon, cauliflower and barley seeds (Dawar *et al*., 2007; Anjorin and Mohammed, 2009). Although there is no evidence that its presence on seed leads to deterioration of seed quality but *A*. *corymbifera* is known to cause diseases in human and animal involving contamination through skin-ground contact (Belfiori *et al*., 2007). Whereas both *Mucor* and *Rhizopus* were isolated from both disinfested and non-disinfested seeds. The isolation frequency of *Mucor* was 3% on disinfested seeds of Faisal Soybean and 9% and 4% on non-disinfested seeds of 95-I and 849-294D, respectively. The disinfested-seeds of three cultivars including Centennial, Faisal Soybean, and HS-16 had 2%, 4%, and 3% of *Rhizopus* isolation frequencies, respectively. Whereas, non-disinfested seeds of HS-16 had significantly (P≤0.05) higher isolation frequency compared to that of disinfested seeds. *Mucor* and *Rhizopus* (*R. stolonifer, R. solani,* and *R. arrhizus*) are generally non-pathogenic to seed but involved in fruit rot and post-harvest fungi of crops, vegetable, and fruits (Michailides and Spotts, 1990; Ismail *et al*., 2012). These two fungi have been reported to reduce germination (Howell, 2007; Anjorin and Mohammed, 2009) and *M. velutinosus* is known to cause skin disease in human beings (Sugui *et al*., 2011). *Rhizopus* spp. including *R. stolonifer* and *R. arrhizus,* have been well-known to cause soft rotting in fresh fruits, vegetables, flowers, bulbs, tubers and seedlings and head rotting in sunflower, which reduces sunflower seed quality and yield leading to 43 to 99% yield losses (Shtienberg, 1997; Clarke, 1999; Yildirim *et al*., 2010). Mehrotra and Aggarwal (2003) reported that such fungi could seriously retard seed germination through softening and necrosis of tissues, which adversely effects seed viability, wilting of plants, and stem flaccidity.

Isolation frequencies of *Aspergillus* spp. and *Penicillium* spp. were frequently appeared on soybean seeds. Isolation frequency of *Aspergillus* spp. was significantly greater ($P \leq 0.05$) on non-disinfested compared to that of disinfested soybean seeds of all cultivars evaluated. It has been reported on soybean seed and play vital role in the seed biodeterioration (Chavan, 2011). But these two genera produce gibberellins which is a growth regulating hormone in higher plants (Hasan, 2002; Hamayun *et al*., 2009).

Seventeen species from genus *Aspergillus* were identified from seed samples of all eight soybean cultivars included *A. euburneo-cremeus, A. ficuum, A. flavus, A. flavus* var. *columnaris, A. foetidus, A. fumigatus* var*. ellipticus, A. janus, A. nidulens* var. *aeristatus, A. niger, A. oryzae, A. phoenicis, A. sulphureus, A. tamarii, A. terricola, A. terreus, A.unguis* and *A. versicolor*. The association of *Aspergillus* spp. plays a vital role in the seed deterioration. Fungi growing on seed reduce germination rate, carbohydrate, protein, total oil contents and influence other biochemical changes in grains (Anwar *et al*., 1995; Bhattacharya, 2002; Afzal *et al*., 2010). Chavan (2011) found that in soybean fat contents were reduced by *A. flavus*, protein contents by *A. terreus*, *A. nigar*, *A. fumigatus*, and sugar contents by *A. versicolor*.

Isolation frequencies for *Penicillium* spp. were revealed from non-disinfested seeds of all seven cultivars including E-358, DB-1601, 849-294D, Loopa, HS-16, 95-I, Faisal Soybean and Centennial except Loopa. The non-disinfested seeds had significantly (P≤0.05) higher isolation frequency of *Penicillium* spp. over that of disinfected seeds. Four species of genus *Penicillium* including *P. chrysogenum P.*

digitatum, P. oxalicum and *P. rolfsii* were identified from seed samples. *Penicillium digitatum* and *P. chrysogenum* caused discoloration, rotting, shrinking, seed necrosis, loss in germination capacity, toxification and hampered the fat content of oilseeds (Chavan and Kakde, 2008).

Isolation frequencies of *Curvularia* spp. were observed on disinfested seeds of 95-1 and E-385 and on non-disinfested seeds of DB-1601 and E-358. Non-disinfested seeds of E-358 had significantly ($P \leq 0.05$) greater isolation frequency than that of disinfested seeds. Three species of genus *Curvularia* namely *C. clavata, C. lunata*, and *C. tuberculata* were identified, which reduce the seed germination (Prom, 2004). Kakde and Chavan (2011) reported that *C. lunata* infested seeds had increased level of fibre contents, which might be responsible for poor seed quality and quantity.

Three pathogenic fungal genera comprising of *Drechslera*, *Fusarium*, and *Phoma* isolated from soybean seeds belonged to Deuteromycetes. Isolation frequencies of *Derchslera* spp., causal agent of leaf spot diseases of several crop plants, were 2% on disinfested seeds of Centennial and 1% on non-disinfested seeds of E-358. Two species of *Drechslera (D. australiensis* and *D. hawaiensis)* were identified, their occurrence on soybean seeds has been documented (Pawar *et al*., 2012). *Fuarium* spp. important pathogens of wilt diseases of several crops were more frequently isolated from non-disinfested seeds than disinfested seeds of all eight cultivars except Faisal Soybean, which had zero isolation frequencies on non-disinfested as well as disinfested seeds. Four *Fuarium* spp. identified from soybean seeds included: *F oxysporum, F. acuminatum, F. equiseti,* and *F. semitectum. Fuarium* can commonly be isolated from seeds and has been reported pathogenic to soybeans (Nelson, 1999; Baird *et al*., 2001; Shovan *et al*., 2008). *Fusarium* spp. cause three types of diseases on soybean i.e. decay of pods, decay of stem base and roots and *Fusarium* wilt (McGee, 1992). *Fusarium* seed infection encourages the aflatoxin production, which had an impact on seed health and negative role in seed germination (Ozcelik *et al.,* 1990; Frisvad and Thrane, 2004). Most of the plant pathogenic *Fusarium* species are either soil-borne or seed-borne, which can damage seeds and seedlings and cause root rot. *Fusarium* spp. causing seed and root rot on corn, wheat, and soybeans include *F. oxysporum, F. solani, F. verticillioides, F. graminearum, F. culmorum, F. sublutinans, F. acuminatum, , F. merismoides, F. proliferaum, F. pseudograminearum,* and *F. semitectum* (Anjorin *et al*., 2008; Liu *et al*., 2012). Usually, *Fusarium* infects plants in combination with *Rhizoctonia, Phytophthora* and *Pythium* species, which kill seeds before germination and cause seedling death under suitable conditions for pathogens (Rizvi and Yang, 1996; Leslie *et al*., 2005; Broders, *et al*., 2007). *Phoma* spp. was isolated primary from non-disinfected seeds of soybean cv. 849-294D only. It has been reported to occur on soybean (Baird *et al*., 2001) however, no documentary evidence is available to show that this fungus affect seed quality or result in yield losses due to diseases (Kulik and Sinclair, 1999).

The objectives of the study were to determine the differences in isolation frequencies of common pathogens of seeds of soybean cultivars stored since 2010. Nine fungal genera comprised of field and storage fungi were isolated and identified from test seed samples. Isolation frequencies varied among cultivars. They can decrease plant populations that result in replanting and production losses. Several different pathogens can cause these diseases, and the most common tend to be *Aspergillus, Curvularia, Fusarium*, *Penicillium* and *Rhizoctonia*. They can kill and rot seeds before germination or cause seedling death, which might had devastating impact on physical quality and farm price of soybeans. Results of our study can be utilized for research on management of seed borne pathogens of soybean. Our findings may provide guidance for timely seed treatment with suitable fungicides to avoid losses inflicted by seed borne pathogens.

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Table 1: Frequency of fungi isolated from disinfested and non-disinfested seeds of soybean.

Isolated fungi	Frequency of occurrence of the isolated fungi (%)							
	95-I	**849-294D**	**Centennial**	**DB-1601**	**E-358**	**Faisal Soybean**	**HS-16**	**Loopa**
Zygomycetes								
Absidia sp.								
Disinfested	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	0.0a
Non-disinfested	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	1.0a	0.0a
Mucor spp.								
Disinfested	0.0b	0.0b	0.0a	0.0a	0.0a	3.0a	0.0a	0.0a
Non-disinfested	9.0a	4.0a	0.0a	0.0a	0.0a	0.0b	0.0a	0.0a
Rhizopus spp.								
Disinfested	0.0a	0.0a	2.0a	0.0a	0.0a	4.0a	3.0b	0.0a
Non-disinfested	0.0a	0.0a	0.0b	0.0a	0.0a	0.0b	6.0a	0.0a
Euascomycetes								
Aspergillus spp.								
Disinfested	9.0b	2.6b	10.5b	4.75b	3.6b	10.3a	10.0a	2.8b
Non-disinfested	10.6a	19.3a	17.2a	8.0a	12.8a	10.6a	6.3b	12.5a
Curvularia spp.								
Disinfested	1.0a	0.0a	0.0a	0.0b	1.0b	0.0a	0.0a	0.0a
Non-disinfested	0.0b	0.0a	0.0a	6.0a	7.0a	0.0a	0.0a	0.0a
Penicillium sp.								
Disinfested	0.0b	2.0a	0.0b	3.2b	0.0b	4.2b	0.0b	0.0a
Non-disinfested	11.2a	7.0a	4.0a	17.1a	14.0a	10.0a	4.0a	0.0a
Deuteromycetes								
Drechslera spp.								
Disinfested	0.0a	0.0a	2.0a	0.0a	0.0b	0.0a	0.0a	0.0a
Non-disinfested	0.0a	0.0a	0.0b	0.0a	1.0a	0.0a	0.0a	0.0a
Fusarium spp.								
Disinfested	12.b	10.0b	6.0b	7.3b	0.0a	1.0b	9.1b	2.1b
Non-disinfested	18.0a	20.2a	13.0a	17.1a	0.0a	6.0a	17.2a	7.1a
Phoma spp.								
Disinfested	0.0a	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Non-disinfested	0.0a	4.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a

Means followed by the same letter do not differ significantly at $P \leq 0.05$.

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