

DETECTION OF SEED BORNE MYCOFLORA IN MAIZE (*ZEA MAYS L.*)

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Abstract

Seed borne mycoflora of maize was tested by using blotter, agar plate and deep freezing methods as recommended by ISTA. Of the 100 samples collected from different places of Pakistan, a total number of 56 species belonging to 23 genera of fungi were isolated and identified. About 70% of the samples were infested with *Aspergillus flavus*, *A. niger*, *A. wentii* and *Penicillium* spp. Of the three methods used, agar plate method yielded the highest number of fungi as compared to blotter and deep freezing methods. Deep freezing method was the best for the detection of *Drechslera* spp., *Fusarium* spp., and *Penicillium* spp., while agar plate method was suitable for the detection of *Aspergillus* spp., *Cladosporium* spp., *Curvularia* spp., and *Rhizopus* spp. Out of 56 species, 22 species viz., *Arthrinium phaeospermum*, *Aspergillus foetidus*, *A. tubingensis*, *Curvularia clavata*, *C. intermedia*, *C. pallescens*, *Bipolaris maydis*, *Drechslera carbonum*, *Diplodia zea*, *Fusarium crockwellense*, *F. cladosporium*, *F. culmorum*, *F. graminearum*, *F. nivale*, *F. proliferatum*, *Penicillium citrinum*, *P. funiculosum*, *Phoma herbarum*, *Rhizopus oligosporum*, *Rhizoctonia solani*, *Syncephalastrum racemosum* and *Trichoderma harzianum* are new reports from Pakistan on maize seeds. However, the same fungal species have been reported on maize seed from various countries of the world such as USA, Thailand, India, Canada, Australia, France, Nepal, United Kingdom, Western Romania and Hungary.

Introduction

In Pakistan maize (*Zea mays L.*) is the most important cereal after wheat and rice. It is grown on 0.9355 million hectares annually with production of about 1.7371 m tones with an average yield of 1857 Kg/hectares (Anon., 2007). Maize is an important food and fodder crop. Its grains are important for the production of oil, starch and glucose (Krishnamurthi, 1969). It is an important component of both human and animal diet. During storage grains undergo quantitative and qualitative losses. The losses occur mainly because of improper storage. A large number of pathogenic fungi, bacteria, viruses and insects, infecting maize grain cause combined world wide annual losses of 9.4% (Shurtleff, 1980). Fungi affect the quality of grain through increase in fatty acid, reduction in germination, mustiness and finally spoilage of grain. The importance of fungi is also due to production of toxins that causes health hazard in human and animals. (Hiscocks, 1965). Fungal development in grains is influenced by temperature humidity and period of storage. Survey of literature shows that a number of fungi viz., *Alternaria alternata*, *Aspergillus* spp., *Bipolaris maydis*, *Fusarium moniliforme*, *Fusarium* spp., *Cephalosporium* spp., *Helminthosporium* spp., *Mucor* sp., and *Penicillium* spp., have been reported from maize seed (Hafiz, 1986; Amin *et al.*, 1985; Ahmad *et al.*, 1993; Anne *et al.*, 2000; Dasjardin *et al.*, 2006; Mohammed *et al.*, 2001; Tulin & Askun, 2006). In Pakistan fungal infection on maize seed was recorded upto 66-90% with an average of 71.1% from sound seeds and 83.7% from damage grains (Hafiz, 1986). The present report gives an account of the occurrence of seed borne mycoflora in maize seed samples collected from Pakistan.

Materials and Methods

Total number of 100 samples out of which 41 varieties (Islamabad white, Islamabad gold, Margala NARC, Soan-3, EV-1097, EV-7004, Rakaposhi, BS-2, BS-1, POP-2004, POP-2006, Golden, Sahiwal-2002, EV-5098, Agaiti-85, Agaiti-2002, EV-1098, Sadaf, EV-6089, EV-6098, Pak.Afgoyee, SAWAN-3, Mansehra BSO-1, EV-7004 NARC, EV-NARC Islamabad white, EV-3001-NARC, POP-2004 CCRI, Agaiti-2002, EV-1097 Islamabad gold, Agaiti-85, Margala-NARC, Azam, Sarhad wide, Kisan, Agaiti-2001, EV-1089, EV-7006, EV-98, POP-2004, Mansehra-1, EV-5089) and 59 samples of maize seeds purchased from different markets of Sindh (16), Punjab (15), NWFP (11), Balochistan (17) were used to detect the seed borne mycoflora. Using ISTA technique (Anon., 1993), 400 seeds from each sample were tested. For the standard blotter technique, untreated seeds and seeds after treatment with 1% Na (OCl)₂ were placed on three layers of moistened blotters, 10 seeds per Petri dish. For the agar plate method, the treated and untreated seeds were plated on potato dextrose agar (PDA), 10 seeds per Petri dish and the dishes were incubated at 24 ± °C in alternating cycle of 12 hr light and 12 hr darkness for 7 days. In the deep-freezing method, the treated and untreated seeds were incubated for 1 day each at 20 ± °C and at 0°C in a freezer followed by 5 days incubation at 24°C. Fungi growing on seeds were identified after reference to Barnett & Hunter (1972), Booth (1971), Ellis (1971), Nelson *et al.*, (1983) Raper & Fennel (1965) and Raper & Thom (1949).

Results and Discussion

A total number of 23 genera and 56 species of fungi were isolated from maize seeds (Table 1) viz., *Absidia hesseltinii* Van Tieghem, *Alternaria alternata* Nees., *Arthrinium phaeospermum* (Corda) M.B. Ellis, *Aspergillus candidus* Link., *A. flavus* Link ex Gray, *A. foetidus* (Nakazawa), *A. fumigatus* Fres., *A. niger* Van Tieghem., *A. ochraceus* Wilhelm, *A. parasiticus* Speare, *A. tubingensis* Schoberm Moss, *A. versicolor* (Vuill) Tiraboschi, *A. wentii* Wehmer, *Bipolaris maydis* Subram & Jain, *Botryodiplodia theobromae* Patch *Cephalosporium acremonium* Fres., *Chaetomium globosum* Kunze. ex Fr., *Chaetomium* sp., *Circinella* sp., *Cladosporium cladosporioides* (Fres) deVries, *Curvularia clavata* Jain., *C. intermedia* Boedijn, *C. lunata* (Wakker) Boedijn., *C. pallescens* Boedijn., *Drechslera carbonum* Nelson, *D. halodes* Subram & Jain., *D. tetramera* Nelson, *Fusarium crockwellense* Burgess Nelson & Toussoun, *F. cladosporium* Wollen. & Reinking, *F. culmorum* (W.G.Smith) Sacc., *F. equiseti* (Corda) Sacc., *F. graminearum*, Schwave., *F. moniliforme* Sheld., *F. nivale* (Frs) Cess., *F. oxysporum* Schlecht., emend.synd & Hans., *F. proliferatum* (Matsushimal Nirenverg), *F. semitectum* Berk & Rev., *F. solani* Mart., *F. subglutinans* Wr. & Reinle., *Macrophomina phaseolina*, (Tassi) Goid., *Monilia* sp., *Mucor* sp., *Nigrospora oryzae* (Berkand Br) Petch., *Nigrospora* sp., *Penicillium citrinum* Thom, *P. funiculosum* Thom, *P. oxalicum* Currien & Thom., *Penicillium* sp., *Phoma herbarum* Westend, *Rhizopus oligosporum* Saito., *R. oryzae* (Ehrnb. Exlink) Lind., *R. stolonifer* Fisher., *Rhizoctonia solani* Kuhn., *Syncephalastrum racemosum* Went & Prinsen Geerlings., *Trichoderma harzianum* Rifai, were isolated by blotter, agar plate and deep freezing methods (Table 1). Out of 56 species 22 species viz., *Arthrinium phaeospermum*, *Aspergillus foetidus*, *A. tubingensis*, *Curvularia clavata*, *C. intermedia*, *C. pallescens*, *Bipolaris maydis*, *Drechslera carbonum*, *Diplodia zeae*, *Fusarium crockwellense*, *F. cladosporium*, *F. culmorum*, *F. graminearum*, *F. nivale*, *F. proliferatum*, *Penicillium citrinum*, *P. funiculosum*, *Phoma herbarum*, *Rhizopus oligosporum*, *Rhizoctonia solani*, *Syncephalastrum racemosum* and

Trichoderma harzianum are new reports from Pakistan on maize seeds. Of the three methods used, the agar plate method yielded the highest number of fungi (23 genera and 56 species) as compared to blotter and deep freezing method. The deep freezing method was superior for the detection of deep seated as well as slow growing seed borne fungi like *Drechslera* spp., *Fusarium* spp., *Penicillium* spp., *Nigrospora oryzae*, *Monilia* sp., *Macrophomina phaseolina*, *Alternaria alternata*, *Syncephalastrum racemosum*. Mathur *et al.*, (1975) working on sorghum seeds also found that deep freezing method was most suitable for the detection of *Fusarium* species. Saprophytic fungi like *Aspergillus* species, *Cladosporium* species, *Curvularia* species, *Rhizopus* species, *Trichoderma* species were isolated in higher percentage by agar plate method. The agar plate method was found most suitable for the isolation of saprophytic fungi. Mathur & Neergaard (1970) and Khan *et al.*, (1988) preferred the use of agar plate method over the blotter method for the isolation of *Curvularia* spp., and *Drechslera* spp., from disinfested seeds of rice. However, in the present study *Drechslera* species were isolated in higher percentage by deep freezing method. *A. niger*, *A. flavus*, *A. wentii*, *Fusarium oxysporum*, *F. solani*, *Penicillium citrinum*, *Macrophomina phaseolina* and *Rhizopus oryzae* were most frequent and recorded from 86%, 78%, 74%, 67%, 65%, 60%, 55% and 50% from the seed samples showing an infection range between 100%, 0-96.6%, 8-77%, 0-96%, 0-93%, 0-85%, 1-64.3% and 4-82% respectively. *A. candidus*, *A. flavus* and *A. alternata* were detected in 100% samples showing infection range upto 20%, 24% and 28% respectively. *Penicillium* species were detected from 65% samples showing a high infection percentage of 4-85%. Variety POP 2004, BS-2 and BS-1 showed more fungal infection on which 21 fungal species were isolated. However, lowest fungal infestation was noted on variety Sahiwal 2002 and Pak Afgoyee.

Use of Sodium hypochloride helped in minimizing the incidence of superficial and fast growing as well as common seed borne fungi like *Aspergillus* spp., *Chaetomium* spp., *Cladosporium* spp., *Rhizopus* spp., *Cephalosporium* spp. Similar results were obtained by Dawar & Ghaffar (1991) on sunflower seeds. Surface disinfection of seed with 1% Na(OCl)₂ reduced the incidence of *Aspergillus* spp. However, other slow growing deep seated seed borne fungi like *Curvularia* spp., *Drechslera* spp., *Fusarium* spp., *Botryodiplodia theobromae* and *Macrophomina phaaseolina* were detected in greater frequency. These results are similar with the finding of Limonard (1968). Khan *et al.*, (1988) preferred the use of agar plate method over the blotter method for the isolation of *Curvularia* spp., and *Drechslera* spp., from disinfected seeds of rice. However, in the present studies *Drechslera* spp., were isolated in higher percentage in deep freezing method.

A number of fungi isolated in the present study are known to produce mycotoxins which are harmful for human health. Mycotoxins can cause severe damage to liver, kidney and nervous system of man even in low dosages (Rodricks, 1976). *Fusarium* and *Aspergillus* species are common fungal contaminants of maize and also produce mycotoxins (Bakan *et al.*, 2002; Verga *et al.*, 2005). *Aspergillus flavus* produces aflatoxin B₁, B₂, G₁ G₂ which are carcinogenic and produce liver cancer (Purchase, 1974; Diener & Davis, 1969; Pesta & Bondy 1990). *A. candidus* produce citrinin, harmful to kidney (Domsch *et al.*, 1980). *Fusarium solani* cause corneal ulcer while *F. oxysporum* produce Zeralenone α and β causing haemorrhage and necrosis in bone marrow. *F. proliferatum* and *F. verticillioides* cause epidemiologically human esophageal cancer (Desjardins *et al.*, 2006). Anne *et al.*, (2000), Curtui *et al.*, (1998) and Susan *et al.*, (2005) isolated several *Fusarium* species from maize seed viz., *Fusarium moniliforme*, *F. graminearum*,

F. proliferatum, *F. acuminatum*, *F. avenaceum*, *F. clamydosporium*, *F. equiseti*, *F. oxysporum*, *F. semitectum* and *F. torulosum* which produce mycotoxins viz., Toxins deoxynivalenol (DON), 3-acetyl DON, 15-acetyl DON, Fusarenon X(FX), T-2 Toxin (T2), Diacetoxyscir phenol (DAS), Zearalenon (ZEA), Fumonisin, Aflatoxin B₁, Ochratoxin A (OA) and Citrinin.(CT) respectively. Don and acetylene Don were the major mycotoxin in *Fusarium* species. *A. terreus* attacks human skin and nail and is parasitic on human ear (Domsch *et al.*, 1980). *A. wentii* produce kojic acid causing cardiovascular and brain disorder. There is need for proper storage of maize seed to minimize the fungal infestation and mycotoxin production during storage and provide disease free seeds for human consumption.

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