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Incidence of Seed-Borne Mycoflora in Wheat and Rice Germplasm

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***Abstract* – Seed-borne diseases cause significant losses in wheat and rice yield. Hundred accessions each of wheat and rice germplasm were screened to investigate the presence of seed borne fungi associated with them using blotters method. Accessions belonging to twenty five and thirty varieties for rice and wheat respectively were obtained from gene bank, PGRP, NARC. A total of 11 fungi belonging to 10 genera *viz*. *Penicillium* sp. *Aspergillus flavus*, *Aspergillus niger*, *Curvularia oryzae*, *Mucor* sp, *Pyricularia oryzae, Pythium* sp, *Alternaria alternata*, *Helminthosporium oryzae, Curvularia lunata* and *Rhizopus* sp were isolated from rice germplasm whereas from wheat germplasm twelve fungi belonging to 9 genera (*Alternaria alternata*, *Aspergillus flavus, Penicillium* sp., *Aspergillus niger, Aspergillus terreus, Mucor* sp., *Pythium* sp., *Fusarium* sp, *Aspergillus fumigatus*, *Curvularia lunata, Dreshslera halodes* and *Cladosporium cladospores*) were isolated. Infection percentage varied from 10-100 % in both seed lots. In wheat, accession no. 18903 and in rice, accessions no. 6595 and 6633 showed 100% infection.**

***Keywords* – Wheat, Rice, Germplasm, Seed Borne Fungi, Seed Health.**

I. INTRODUCTION

Plant germplasm resources 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. In order to conserve these genetic resources, gene bank has been established in Plant Genetic Resources Program, NARC, Islamabad and around 30,000 crop accessions have been conserved in the storage. 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].

Wheat and rice plants at all stages of growth are subject to numerous injuries and stresses. Each year about 20% of the wheat that otherwise would be available for food and feed is lost due to diseases [3]. There 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. Many diseases of wheat and rice are seed-borne like bakanae disease of rice (*Fusarium moniliforme* Sheld), loose smut [*Ustilago tritici* (Pers.) Rostrup], flag smut (*Urocystis tritici* Koern.), karnal bunt [*Neovossia indica* (Mitra) Mundkar] and ear cockle of wheat (*Anguillulina tritici* Gerv. & Bened.) [4]. Several fungal pathogens have been isolated from rice seeds, and have been reported to be responsible for a number of diseases right from the nursery to the field [5]. If untreated seeds are grown in the field then seed-borne pathogen of wheat are not only responsible to cause variation in plant morphology but it also reduce the crop yield up to 15-90% [6].

Fields of wheat and rice in Pakistan are widely surveyed during 1986-1989 for the presence of important seed-borne diseases. Major seed-borne diseases like loose smut (*Ustilago tritici*) and flag smut (*Urocystis tritici*) of wheat were widely prevalent in all the four provinces of Pakistan; partial bunt or karnal bunt *(Tilletia indica*) was found in the central, northern Punjab and NWFP whereas complete bunt or stinking smut (*Ti1letia foetida*) was confined in the uplands of Baluchistan and hilly areas of Punjab and NWFP. In the non-irrigated areas *Fusarium* sp., and in irrigated areas *Alternaria* spp., and *Drechs1era* spp., were associated with seed rot, foot rot and seedling blight of wheat. Blast disease of rice (*Pyricu1aria oryzae*) was a major disease among the seed-borne diseases of rice, in the central parts of Punjab and a minor disease in Sindh whereas brown leaf spot of rice (*Drechs1era oryzae*), kernel bunt of rice were prevalent in all the rice growing areas of Pakistan [7]. A seed associated pathogen present externally, internally or associated with the seed as

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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 [8, 9]. 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 [10]. In this study, routine technique of seed health testing was applied to monitor and analyze the fungi that infect wheat and rice accessions conserved in the gene bank.

II. Materials and Methods

A. Wheat and Rice Seeds

Wheat and rice seeds (100 accessions each) were obtained from gene bank (PGRP) at National Agricultural Research Centre (NARC), Islamabad. These accessions belonged to twenty five and thirty varieties of rice and wheat (data not shown) respectively.

B. Isolation of Fungi

The blotter test was used for the isolation of fungi associated with seeds [11]. Three 90 mm size discs of blotting paper was moistened with distilled water and placed at the bottom of 90 mm sterilized petri plate after draining excess water. Ten seeds of wheat and rice accessions were placed at equal distance in separate petri plates using a pair of forceps in triplicate. The plates were incubated at 20±2°C under alternate cycles of 12 hours NUV light and darkness. After eight days of incubation seeds were examined for presence of associated fungi. The number of infected seeds were counted and expressed in percentage as follow:

Frequency of occurrence (%)

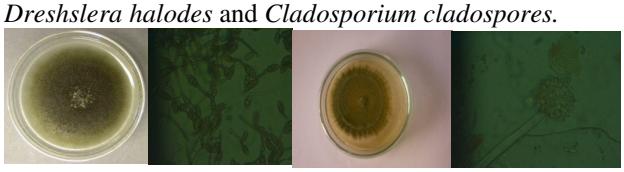
$$= \frac{\text{No. of seeds on which a fungal species occurs}}{\text{Total No. of seeds}} \times 100$$

C. Purification and Identification of Fungal Cultures

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-binocular microscope. A list of morphological characters of taxonomic importance such as spore size, shape, septation, colour and their arrangement on the conidiophores, appearance of the spore masses, character of the mycelium, density of the colony were compiled for each fungus. Identification of the fungus was performed using all the characteristics observed and identification reference manuals of Booth, Barnett & Hunter, and Watanabe.

III. Results and Discussion

In total, twelve fungi including both saprophytic as well as pathogenic were isolated from wheat seeds. Fungi isolated from wheat seeds (Fig.1) were *Alternaria alternata*, *Aspergillus flavus, Penicillum* sp., *Aspergillus niger, Aspergillus terreus, Mucor* sp., *Pythium* sp., *Fusarium spp, Aspergillus fumigatus*, *Curvularia lunata Dreshslera halodes* and *Cladosporium cladospores.*



a b c d

Fig. 1 Fungi isolated from wheat seeds. a: *A. alternata* colony; b: *A. alternata* conidia; c: *A. flavus* colony; d: *A. flavus*

Infection percentage varied from 10-100% in all the wheat accessions tested with accession no.18903 showing 100% infection followed by 018705 (90%), and five accessions numbers (018677, 018686 018703, 018704 and 011418) showed infection of 80%. Seven accessions (018687, 018976, 011411, 018905, 011419, 011398, and 12080) showed 20 % infection and 010733 showed least infection of 10%. Frequency of occurrence of each fungus in wheat seeds is presented in Figure 1. *Aspergillus flavus* (18%) was found to be the most predominant fungi followed by *Penicillium* spp (16%), *Alternaria alternata* (15%), *Aspergillus niger* (14%), *Mucor* sp (12%), *Pythium* sp (10%), *Curvularia lunata* and *Fusairum* sp. (4%), *Aspergillus terreus, Dreshslera halodes* and *Cladosporium cladospores* (2%) and *Aspergillus fumigatus* was found only 1%. Similar results were reported in a previous study [1] while testing the wheat germplasm conserved in gene bank of China. The pre-dominant fungi isolated were *Alternaria, Rhizopus, Penicillium, Aspergillus, Bipolaris, Cladosporium, Fusarium, Cladosporium*, and *Trichothecium.*

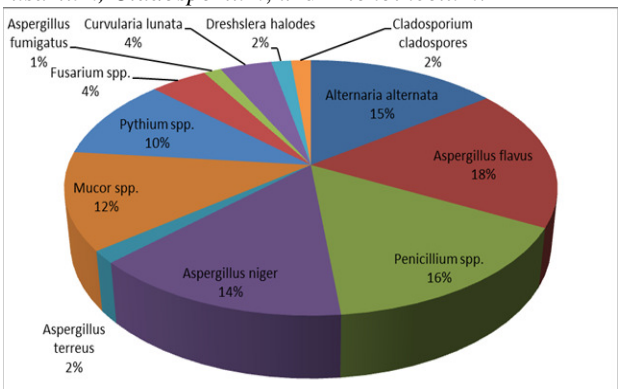


Fig.1. Frequency of occurrence of seed associated fungi isolated from wheat

Eleven fungal species were found to be associated with rice seeds *viz, Penicillium* sp. *Aspergillus flavus*, *Aspergillus niger*, *Curvularia oryzae*, *Mucor* sp, *Pyricularia oryzae, Pythium* sp, *Alternaria alternata*, *Helminthosporium oryzae, Curvularia lunata* and *Rhizopus* sp. Variation regarding percentage infection of different fungal species was observed. Maximum fungal infection (100%) was observed in accession no. 6595 and 6633. Two accessions (6520 and 6546) showed (90%) infection while eight accessions (6525, 6607, 6650, 6597, 6626, 6627, 6666 and 6638) were 80% infected. Accession

number 6547 showed least infection (10%). Frequency of occurrence of each fungus is presented in Figure 2. The most predominant fungi isolated was *A. flavus* (20%) followed by *Penicillium* spp (18%), *Aspergillus niger* (13%), *Alternaria alterrata* (11%), *Mucor* sp (10%), *Pythium* sp (9%), *Curvularia oryzae* ; *Rhizopus* sp (7%), *Helmintosporium oryzae* ; *Curvularia lunata* (2%) and *Pyricularia oryzae* was found only 1%. The presence of *Fusarium moniliforme, Alternaria* spp., *Helminthosporium* spp. and *Curvularia* spp., *Bipolaris oryzae*, *Fusarium oxysporum*, *Chaetomium globosum*, *Curvularia lunata*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Alternaria tenuis, Penicillium* spp, *Bipolaris oryzae, Curvularia lunata* and *Trichoderma harzianum* associated with the seeds of rice varieties both in storage and in the field have been reported earlier [5, 15, 16].

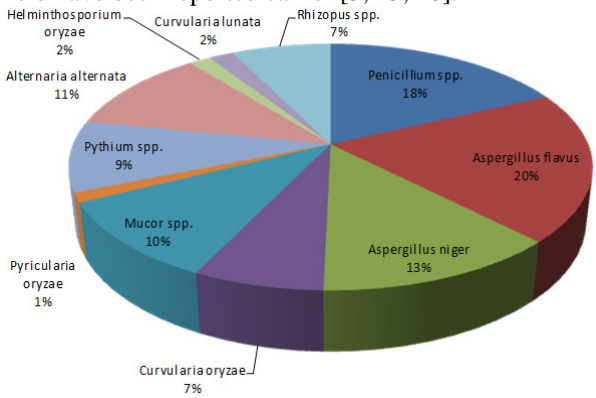


Fig.2. Frequency of occurrence of seed associated fungi isolated from rice

It is evident from the results (supplementary data) that none of the seeds were found to be free from mycoflora which advocates that irrespective of the environmental conditions and varieties the fungi develop the seed either superficial, semi deep or completely inside. Most samples were infected by several species of fungi in wheat and rice accessions tested. The seed-borne fungi could damage seeds at varying degree, causing seed shrinkage or color change. 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 invading the roots, stems, and leaves of wheat. 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 gene bank, 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 germplasm is planted and propagated after decades [1]. Therefore, seed health testing is vital for controlling seed-borne pathogens in the preserved germplasm.

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