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COMPARISON OF FUNGAL DIVERSITY OF LOCAL AND EXOTIC RICE (*ORYZA SATIVA* L.) GERMPLASM FOR THEIR SEED HEALTH

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ABSTRACT

Rice is one of the major cereal crop of the world. Its production potential is lower than in Pakistan because of rice seed mycoflora. Present study provides analysis of fungal mycoflora from rice seeds of 39 different germplasm lines (08 local and 30 exotic). Fungi were isolated from rice by agar and blotter paper methods. Surface sterilization of rice samples were done by using ethanol and sodium hypochlorite (NaOCl) (2% each). Total of 18 different fungal species belong to 8 different genus were isolated from tested exotic and local germplasm lines of rice. The diversity of fungal species were observed included *Alternaria juxtiseptata*, five species of *Aspergillus*, *Cladosporium cladosporoides*, three species of *Curvularia*, two species of *Drechslera biseptata*, *Fusarium globosum*, *Mucor* sp., *Penicillium oxalicum*, *Phoma* sp., *Rhizoctonia* sp. and *Rhizopus arrhizus. Curvularia lunata* had highest percent frequency in all the local germplasm lines although *Penicillium globosum*, *Rhizoctonia* sp., *Phoma* sp. were isolated in higher frequency from blotter paper method as compared to agar plate. In the preview of such high association of mycoflora with stored rice grains, a proper seed treatment is the need of the hour. Moreover, it must be evaluated for all the cultivated as well as research germplasm for proper control of these seed mycoflora for future utilization/availability, production of healthy and high yielding rice varieties to the farmers community.

Key words: Rice, mycoflora, agar plate method, blotter paper method, *Curvularia lunata*.

INTRODUCTION

Rice is a major world food commodity which feed about half of the world population (Nguefack, 2007). According to FAOSTATS, 722.76 million tonnes of rice was harvested over an area of 164.12 million ha worldwide (FAO, 2011). In Pakistan, rice is equally important and it ranks second among staple cereal food grains and it was sown over 2311 thousand ha with a production of 5541 thousand tonnes (Anonymous, 2013). Potential of rice production in Pakistan is lesser compared to the international community Seed borne fungi are one of the most prominent factor among many others which cause lower rice production in Pakistan (Butt *et al*., 2011).

Rice has many seed borne fungi associated which have been reported and some of these seed borne fungi may be pathogenic and lead to economically important diseases in rice such as bakanae disease of rice caused by *Fusarium moniliforme* Sheld (Guerrero *et al.*, 1972; Javaid and Anjum, 2006; Butt *et al*., 2011). Fungi have been reported to cause as much as 55 diseases and 43 of them are seed-borne or seed-transmittable (Neergaard 1979; Richardson 1979, Leeper, 1984; Ou 1985). Seed borne fungi are reported to be not only pathogenic but they also infect the soil and may establish as soil borne fungi; as well as most of the seed borne fungi belonging to *Aspergillus* and *Penicillium* genus develop during storage on the grains and cause reduction in seed vigor, germination potential and they many also cause grain discoloration and poor quality (Danquah and Mathur, 1976; Neergaard, 1986). Exotic germplasm and wild species of rice are very important with regard to breeding purposes and this germplasm is used for improving the local varieties for better agronomic traits and high production. But they may still have huge lot of fungi associated with them as reported in different researches carried out on the local rice germplasm. *Alternaria alternata*, *Alternaria longissima*, *Aspergillus* spp., *Cheatomium* sp., *Cladosporium* sp., *Cochlobolus lunatus*, *Colletotrichum* sp., *Curvularia lunata*, *Curvularia oryzae*, *Curvularia* sp. *Drechslera oryzae*, *Epicoccum* sp., *Fusarium moniliforme*, F. *oxysporum*, F. *semitectum*, *Helminthosporium oryzae*, *Myrothecium* sp., *Penicillium* sp., *Phoma* sp., *Rhizopus* sp., *Rhyncosporium oryzae*, *Sclerotium* sp., *Trichoderma* sp., *Trichoconus padwickii* and many other species of these and other genra have been isolated by the work of many research groups in Pakistan and around the world (Imolehin, 1983 & 1987; Khan *et al*., 1988; Kim and Lee, 1989; Odebunmi-Osikanlu, 1989; Wahid *et al*., 1993 & 2001; Khan *et al*., 2000; Javaid *et al*., 2002; Jhonson *et al*., 2004; Ibiam *et al*., 2006). Isolation of seed-borne fungi has been carried out by different groups on the both standard agar and blotter plate method as described by

ISTA and Rice Seed Health Testing Manual by IRRI. Both media have specificity for isolating some genra in greater percent frequency on one medium than the other as has been implicated through the work of Agarwal *et al*., (1972); Agarwal *et al*., 1989; Khan *et al*., (1988) and Khanam and Khanzada (1989) showing that *Drechslera* sp. and *Trichoconus padwickii* are generally isolated in higher frequencies from rice grains on blotter plates. Park *et al*., (2005) have used dichloran 18% glycerol agar and dichloran rose Bengal chloramphenicol agar (DRBC) for mycoflora analysis and isolated *Penicillium citrinum*, *Aspergillus candidus* and *Fusarium proliferatum* in high frequency.

The objectives of the current study were to compare the fungal diversity associated with local and exotic rice grain germplasm by different isolation methods (agar and blotter plate method). Furthermore seed health determined by some physical traits (seed length, seed width seed thickness, seed length width ratio and 1000 grain weight).

MATERIALS AND METHODS

Samples of 9 local varieties of rice *viz.*, Basmati 370, Basmati 198, Basmati 385, Basmati 2000, Basmati 515, Basmati Pak, Super Basmati, KSK-133 and IR6 were obtained from Rice Research Institute, Kala Shah Kaku and exotic rice germplasm of 30 different lines was obtained from United States Department of Agriculture (USDA), USA. Seeds from each rice samples were randomly selected and tested for different physical traits and isolations of pathogens. Physical characteristic of nine local varieties of rice were also measured. For this purpose, weight of 1000 seeds of each type was measured on a digital weighing balance, and three readings were taken for each sample. Seed length, seed width and seed thickness of these local rice varieties was measured with a digital Vernier Caliper.

Germination test for 9 local rice varieties was conducted in the glass petri plates of 9 cm diameter. Ten seeds of each sample were placed on double layer of blotter paper. The blotter paper was moistened with sterilized distilled water and the petri plates were kept in the germinator at 37^0C temperature with 80% relative humidity. Care was taken to keep the blotter papers moist. For the determination of germination index on blotter paper, data was recorded daily and calculation was made by the following formula;

$$\text{Germination index (G.I)} = n/d$$

Where, n = no. of seedlings emerging on day 's'

d = no. of days after sowing / plating

The isolation of fungi from the thirty exotic rice germplasm lines was done by standard agar plate method as well as by standard blotter paper method as described by International Seed Testing Association and IRRI Manual for Seed Health Testing of Rice. In the first set of experiment for local and exotic germplasm lines were inoculated with surface sterilization with 1% sodium hypochlorite (NaOCl) solution on standard PDA plates and in the control group seeds without surface sterilization were inoculated. In another set of experiment for comparison of sterilizing potential of sodium hypochlorite and ethanol, seeds of local germplasm lines treated with 2% solution of each were inoculated on PDA plates. The seed were soaked for 60 seconds in 2% solution followed by 3 washes with sterilized distilled water. In the final set of fungal isolation, surface sterilized seeds of nine local germplasm lines with 1% sodium hypochlorite were inoculated on PDA (agar plate method) and blotter papers (blotter method). Standard procedure was followed for inoculation of seed on these isolation media. Each of the experimental setup was incubated at 25±2°C for 7 days. After 7 days the cultures of different fungi were subcultured on water agar, PDA and MEA plates until pure culture were obtained. For all of these isolations, percentage frequency (F%) was calculated by the formula (Bajwa *et al.*, 2009).

$$\text{Frequency Percentage (F\%)} = \frac{\text{No. of seeds on which a particular fungi occur}}{\text{Total No. of seeds}} \times 100$$

RESULTS

Physical traits: Results of seed length, width and thickness of nine varieties of rice showed that Basmati Pak has healthiest grain. In addition, Basmati 515 and KSK-133 have also fine grain quality after Basmati Pak (Table 1). Among fine grain (basmati varieties) 'super basmati' had highest 1000 seed weight (25.567g) whereas Basmati 385 had the least weight of 18.2g. Super basmati has highest seed length to width ratio. Results of local varieties of rice are given in table 2 which shows that Basmati 385, KSK-133 and IR6 have 100% germination rate. The germination indices indicate the seed vigor on blotter paper. Among fine grain varieties, Basmati 385 had highest germination index whereas, Basmati 515 had lowest with 11.33. In case of coarse rice varieties, KSK 133 had higher germination index = 13.33 than IR 6.

Isolation of fungi from stored grains of local and exotic rice germplasm: In present study each fungal colony was purified and identified after 6-8 days on the basis of morphological characteristics (Domsch *et al.*, 1980; Ellis 1971; 1976). *Aspergillus ficuum*, *Aspergillus fumigatus*, *Aspergillus speluneus*, *Alternaria juxtiseptata*, *Cladosporium cladosporoides*, *Drechslera biseptata*, *Penicillium oxalicum* and *Rhizopus arrhizus* were exclusively isolated from exotic germplasm whereas, *Aspergillus phoenicis*, *Drechslera* sp. and *Mucor* sp. were exclusively isolated from local germplasm. Table 3 shows a comparison of percentage frequencies (F%) of

the fungi isolated and identified from local and exotic rice germplasm. *Curvularia lunata* shows highest F% in the local rice varieties with highest value 83.33% in KSK-133 and lowest in basmati 198 with 20%. The overall percentage frequencies (F%) of *Phoma* sp. and *Aspergillus phoenicis* was very high for Basmati 2000 with F% values of 56.7% and 63.3%.

Comparison of different sterilizing agents on seed-borne mycoflora of rice grains: Table-04 shows comparison of the sterilizing ability of the sodium hypochlorite (NaOCl) and ethanol at 2% concentration of the sterilization solutions. 2% sodium hypochlorite was more effective for the control of *Phoma* sp., *Aspergillus ficuum*, *Curvualaria clavata*, *Rhizoctonia* sp. *Rhizopus arrhizus* and *Mucor* sp.; whereas 2% ethanol showed control on growth and appearance of *Mucor* sp. and *Curvularia clavata*. The appearance of the most frequent seed-borne fungi i.e., *Curvularia lunata* was however, not controlled by any of the treatments given although the percent frequency (F%) was reduced to some extent in case of 2% sodium hypochlorite solution.

Comparison of different isolating media on frequency of isolated fungi: From table-05, the blotter paper method results showed high number of fungi as compared to agar plate method. The fungi like *Penicillium globosum*, *Rhizoctonia* sp., *Phoma* sp. were isolated in higher frequency from blotter paper method as compared to agar plate. But the percentage frequency of *Curvularia lunata* and *Drechslera* sp. was much lower on blotter paper method as compared to agar plate.

Table 1. Physical properties of local germplasm lines.

Variety	Length (mm)	Width (mm)	Thickness (mm)	Length/Width	1000 Seed weight (g)
Bas-370	9.6	1.99	1.82	4.82	19.433
Bas-385	9.41	1.91	1.78	4.96	18.2
Bas-198	9.27	1.89	1.75	4.89	18.467
Bas-2000	10.37	1.95	1.85	5.33	22.833
Bas-515	10.54	1.97	1.85	5.38	20.465
Bas-Pak	10.85	1.97	1.93	5.52	23.567
Sup Bas	10.59	1.88	1.78	5.76	25.567
KSK-133	9.78	2.28	1.93	4.28	22.567
IR6	9.57	2.2	1.88	4.36	20.767

Table 2. Germination percentages and germination indices of local germplasm lines on blotter paper

Variety	Germination Percentage (%)				Germination index
	24hr	48hr	72hr	96hr	
Basmati-370	0	66.67	90	90	12.33
Basmati-385	0	60	96.67	100	12.92
Basmati-198	0	53.33	93.33	93.33	12
Basmati-2000	0	56.67	90	96.67	12.33
Basmati-515	0	43.33	86.67	93.33	11.33
Basmati Pak	0	56.67	93.33	96.67	12.42
Super Basmati	0	46.67	90	93.33	11.68
KSK-133	0	66.67	100	100	13.33
IR-6	0	60	96.67	100	12.92

Table 4. Comparison of different sterilizing agents on isolation of mycoflora from rice germplasm

Name	2 % Sodium hypoclorite treatment (PDA)	2% ethanol treatment (PDA)	Without surface sterilization (PDA)
	Local Rice Germplasm		
Basmati 370	*Aspergillus flavus, Curvularia lunata, Drechslera sp., Phoma sp.*	*Aspergillus flavus, Aspergillus phoenicis, Aspergillus fumigatus, Curvularia lunata, Phoma sp.*	*Aspergillus flavus, Aspergillus phoenicis, Curvularia lunata, Curvularia clavata, Phoma sp.*
Basmati 198	*Aspergillus phoenicis, Aspergillus flavus, Curvularia lunata, Mucor sp.*	*Aspergillus flavus, Curvularia fumigatus, Mucor sp.*	*Aspergillus flavus, Aspergillus phoenicis, Curvularia lunata, Curvularia clavata, Drechslera sp., Phoma sp.*
Basmati 385	*Aspergillus phoenicis, Aspergillus flavus, Curvularia lunata, Phoma sp.*	*Aspergillus flavus, Curvularia fumigatus, Phoma sp., Rhizoctonia sp.*	*Aspergillus flavus, Aspergillus phoenicis, Curvularia clavata, Drechslera sp., Phoma sp.*
Basmati 2000	*Aspergillus phoenicis, Aspergillus flavus, Curvularia lunata, Mucor sp.*	*Aspergillus flavus, Aspergillus phoenicis, Aspergillus fumigatus, Curvularia lunata, Mucor sp. Rhizoctonia sp.*	*Aspergillus flavus, Aspergillus phoenicis, Curvularia lunata, Curvularia clavata, Drechslera sp., Phoma sp.*
Basmati 515	*Aspergillus phoenicis, Aspergillus flavus, Curvularia lunata, Drechslera sp., Phoma sp.*	*Aspergillus flavus, Aspergillus phoenicis, Aspergillus fumigatus, Curvularia lunata, Mucorsp., Rhizoctoniasp.*	*Aspergillus flavus, Aspergillus phoenicis, Curvularia lunata, Curvularia clavata, Drechslera sp., Phoma sp., Mucor sp.*
Super Basmati	*Aspergillus phoenicis, Aspergillus flavus, Curvularia lunata, Phoma sp.*	*Aspergillus flavus, Aspergillus phoenicis, Curvularia lunata, Mucor sp.*	*Aspergillus flavus, Aspergillus phoenicis, Curvularia lunata, Phoma sp*
Basmati Pak	*Aspergillus phoenicis, Aspergillus flavus, Curvularia lunata, Phoma sp.*	*Aspergillus flavus, Aspergillus phoenicis, Aspergillus fumigatus, Curvularia lunata, Rhizoctonia sp.*	*Aspergillus flavus, Aspergillus phoenicis, Curvularia lunata, Phoma sp.*
KSK-133	*Aspergillus phoenicis, Aspergillus flavus, Curvularia lunata, Mucor sp.*	*Aspergillus phoenicis, Aspergillus flavus, Curvularia lunata, Mucor sp., Rhizoctonia sp.*	*Aspergillus flavus, Aspergillus phoenicis, Curvularia lunata, Drechslera sp., Phoma sp.*
IR6	*Aspergillus flavus, Curvularia lunata,*	*Aspergillus flavus, Aspergillus phoenicis, Aspergillus fumigatus, Curvularia lunata, Mucor sp.*	*Aspergillus flavus, Aspergillus phoenicis, Curvularia lunata, Phoma sp., Monilia sp.*
	Exotic Rice Germplasm		
140-4-1-2-5	*Penicillium oxalicum*	*Aspergillus flavus, Penicillium oxalicum*	*Aspergillus flavus, Penicillium oxalicum*
45-5-18	-	-	-
79	*Aspergillus flavus, Cladosporium cladosporoides*	*Aspergillus flavus, Cladosporium cladosporoides*	*Aspergillus flavus, Cladosporium cladosporoides*
923	*Aspergillus speluneus, Curvularia lunata*	*Curvularia lunata*	*Aspergillus speluneus, Curvularia lunata*
Arabi	*Phoma sp.*	-	*Phoma sp.*
Basmati-802	*Drechslera biseptata*	*Drechslera biseptata*	*Drechslera biseptata*
Bellevue	*Phoma sp.*	-	*Phoma sp.*
Century Patna-231	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*
Ekarin		*Aspergillus flavus*	*Aspergillus flavus*
Embrapa-1200	*Aspergillus flavus*	*Aspergillus flavus, Rhizopus sp.*	*Aspergillus flavus, Rhizopus sp.*
Estrella	-	-	-
Gulfmont	-	-	-
IR-64	*Aspergillus flavus*	*Aspergillus flavus, Aspergillus fumigatus*	*Aspergillus flavus, Aspergillus fumigatus*

Jefferson	*Aspergillus flavus,*	*Aspergillus flavus, Aspergillus ficuum*	*Aspergillus flavus, Aspergillus ficuum*
KerangSerang	-	*Aspergillus ficuum*	*Aspergillus ficuum*
L-203	-	-	-
La Plata Gena F.A	-	*Phoma sp.*	*Phoma sp.*
Mayhia	-	-	-
Muse Tu Rum	-	*Rhizopus arrhizus*	*Rhizopus arrhizus*
N 11 0 61-71	*Curvularia lunata*	*Curvularia lunata*	*Curvularia lunata*
Presidio	-	-	-
Saber	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*
Sabine	-	-	-
Shima	*Penicillium oxalicum*	*Penicillium oxalicum*	*Penicillium oxalicum*
Sierra	-	*Rhizopus arrhizus*	*Rhizopus arrhizus*
Tehran	-	*Aspergillus ficuum*	*Aspergillus ficuum*
Texmont	-	-	-
Villaguay P.A	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*
Xiangzhaoxian No. 15	-	-	-
Yukare	*Aspergillus flavus, Alternaria juxtiseptata, Cladosporium cladosporoides*	*Aspergillus flavus, Aspergillus ficuum, Alternaria juxtiseptata, Cladosporium cladosporoides*	*Aspergillus flavus, Aspergillus ficuum, Alternaria juxtiseptata, Cladosporium cladosporoides*

Table 3 : Frequency percentage of fungi isolated from local and exotic rice germplasm

Name	**Fungi identified**													
Variety/line	*Aspergillus ficuum*	*Aspergillus flavus*	*Asergillus fumigatus*	*Aspergillus phoenicis*	*Aspergillus speluneus*	*Alternaria juxtiseptata*	*Cladosporium cladosporoides*	*Curvularia lunata*	*Drechslera biseptata*	*Drechslera* sp.	*Mucor* sp.	*Phoma* sp.	*Penicillium oxalicum*	*Rhizopus arrhizus*
Local Rice Germplasm														
Basmati 370	-	16.7	-	-	-	-	-	33.3	-	6.7	-	20	-	-
Basmati 198	-	6.7	-	3.33	-	-	-	20	-	-	3.3	-	-	-
Basmati 385	-	23.3	-	6.7	-	-	-	16.7	-	-	-	20	-	-
Basmati 2000	-	10	-	63.3	-	-	-	53.3	-	-	3.3	56.7	-	-
Basmati 515	-	10	-	16.7	-	-	-	36.7	-	10	-	13.3	-	-
Super Basmati	-	30	-	16.7	-	-	-	60	-	-	-	23.3	-	-
Basmati Pak	-	16.7	-	3.3	-	-	-	66.7	-	-	-	43.3	-	-
KSK-133	-	6.7	-	10	-	-	-	83.3	-	-	13.3	-	-	-
IR6	-	10	-	6.7	-	-	-	80	-	-	-	-	-	-
Exotic Rice Germplasm														
140-4-1-2-5	-	6.7	-	-	-	-	-	-	-	-	-	-	13.3	-
45-5-18	-	-	-	-	-	-	-	-	-	-	-	-	-	-
79	-	10	-	-	-	-	10	-	-	-	-	-	-	-
923	-	-	-	-	6.7	-	-	13.3	-	-	-	-	-	-
Arabi	-	-	-	-	-	-	-	-	-	-	-	6.7	-	-
Basmati-802	-	-	-	-	-	-	-	-	20	-	-	-	-	-
Bellevue	-	-	-	-	-	-	-	-	-	-	-	13.3	-	-
Century Patna-231	-	6.7	-	-	-	-	-	-	-	-	-	-	-	-
Ekarin	-	10	-	-	-	-	-	-	-	-	-	-	-	-
Embrapa-1200	-	26.7	-	-	-	-	-	-	-	-	-	-	-	6.7
Estrela	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gulfmont	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IR-64	-	13.3	10	-	-	-	-	-	-	-	-	-	-	-

Jefferson	30	6.7	-	-	-	-	-	-	-	-	-	-	-	-
KerangSerang	26.7	-	-	-	-	-	-	-	-	-	-	-	-	-
L-203	-	-	-	-	-	-	-	-	-	-	-	-	-	-
La Plata Gena												10		
F.A	-	-	-	-	-	-	-	-	-	-	-		-	-
Mayhia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Muse Tu Rum	-	-	-	-	-	-	-	-	-	-	-	-	-	10
N 11 0 61-71	-	-	-	-	-	-	-	10	-	-	-	-	-	-
Presidio	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saber	-	-	-	-	-	-	-	-	-	-	-	-	-	13.3
Sabine	-	20	-	-	-	-	-	-	-	-	-	-	-	-
Shima	-	-	-	-	-	-	-	-	-	-	-	6.7	-	-
Sierra	-	-	-	-	-	-	-	-	-	-	-	-	-	6.7
Tehran	10	-	-	-	-	-	-	-	-	-	-	-	-	-
Texmont	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Villaguay P.A	-	6.7	-	-	-	-	-	-	-	-	-	-	-	-
Xiangzhaoxian No. 15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Yukare	10	10	-	-	-	13.3	13.3	-	-	-	-	-	-	-

Table 5. Comparison of the two common isolation methods of fungi from seed

Name	**Fungi isolated from (with 1% sodium hypochlorite treatment)**	
	Blotter paper	**Agar plate (PDA)**
Basmati 370	*Curvularia clavata*, *Curvularia lunata*,	*Aspergillus flavus*, *Curvularia lunata*, *Drechslera sp.*, *Phoma sp.*
Basmati 198	*Aspergillus flavus*, *Aspergillus phoenicis*, *Curvularia clavata*, *Curvularia lunata*,	*Aspergillus flavus*, *Aspergillus phoenicis*, *Curvularia lunata*, *Mucor sp.*
Basmati 385	*Aspergillus phoenicis*, *Curvularia lunata*, *Rhizoctonia sp.*	*Aspergillus flavus*, *Aspergillus phoenicis*, *Curvularia lunata*, *Phoma sp.*
Basmati 2000	*Aspergillus flavus*, *Aspergillus phoenicis*, *Curvularia clavata*, *Curvularia lunata*, *Rhizoctonia sp.*, *Drechslera biseptata*,	*Aspergillus flavus*, *Aspergillus phoenicis*, *Curvularia lunata*, *Mucor sp.*, *Phoma sp.*
Basmati 515	*Aspergillus flavus*, *Aspergillus phoenici*, *Curvularia clavata*, *Curvularia lunata*, *Drechslera sp.*, *Fusarium globosum*,	*Aspergillus flavus*, *Aspergillus phoenicis*, *Curvularia lunata*, *Drechslera sp.*, *Phoma sp.*
Super Basmati	*Aspergillus flavus*, *Curvularia lunata*, *Drechslera sp.*, *Rhizoctonia sp.*	*Aspergillus flavus*, *Aspergillus phoenicis*, *Curvularia lunata*, *Phoma sp.*
Basmati Pak	*Aspergillus flavus*, *Curvularia lunata*, *Rhizoctonia sp.*	*Aspergillus flavus*, *Aspergillus phoenicis*, *Curvularia lunata*,

		Phoma sp.
KSK-133	*Aspergillus phoenicis, Curvularia clavata, Curvularia lunata,*	*Aspergillus flavus, Aspergillus phoenicis, Curvularia lunata, Mucor sp.*
IR6	*Aspergillus phoenicis, Curvularia clavata, Curvularia lunata, Rhizoctonia sp.*	*Aspergillus flavus, Aspergillus phoenicis, Curvularia lunata, Mucor sp.*

DISCUSSION

The present study was focused on the rice seed health testing with major aspect of isolation of the seed-borne fungi. 8 different genra of fungi and 18 species were isolated from 39 local and exotic rice germplasm lines *viz.*,, *Alternaria juxtiseptata*, *Aspergillus ficuum*, A. *flavus*, A. *fumigatus*, A. *phoenicis*, A. *speluneus*, *Cladosporium cladosporoides*, *Curvularia lunata*, C *clavata*, *Curvularia* sp., *Drechslera biseptata*, *Drechslera* sp., *Fusarium globosum*, *Mucor* sp., *Penicillium oxalicum*, *Phoma* sp., *Rhizoctonia* sp. and *Rhizopus arrhizus.*

Our findings are in coherence with the findings of other groups working with similar lines of local or exotic germplasm. *Alternaria alternata*, *Alternaria longissima*, *Aspergillus* spp., *Cheatomium* sp., *Cladosporium* sp., *Cochlobolus lunatus*, *Colletotrichum* sp., *Curvularia lunata*, *Curvularia oryzae*, *Curvularia* sp. *Drechslera oryzae*, *Epicoccum* sp., *Fusarium moniliforme*, F. *oxysporum*, F. *semitectum*, *Helminthosporium oryzae*, *Myrothecium* sp., *Penicillium* sp., *Phoma* sp., *Rhizopus* sp., *Rhyncosporium oryzae*, *Sclerotium* sp., *Trichoderma* sp., *Trichoconus padwickii* and many other species of these and other genra have been isolated by the work of many research groups in Pakistan and around the world (Imolehin, 1983 & 1987; Khan *et al*., 1988; Kim and Lee, 1989; Odebunmi-Osikanlu, 1989; Ista, 1993; Wahid *et al*., 1993 & 2001; Khan *et al*., 2000; Javaid *et al*., 2002; Ibiam *et al*., 2006). Some of the *Curvularia* species have been reported to be pathogenic to grasses especially for wheat, rice and maize and may lead to epidemics (Sivanesan 1987; Scheffer 1997; Berbee *et al*., 1999). Butt *et al*. (2011), Utobo *et al*. (2011) and Khanum and Khanzada (1989) have reported *Curvularia lunata* in different rice varieties and have labelled it as seed-borne. Although the percentage frequencies have been lower as compared to percentage frequencies found in the present study. The change in the dominating species on field to *Curvularia lunata* can be attributed to the change of climatic conditions (Masters and Norgrove, 2010).

Alternaria alternata, *Aspergillus* spp., *Cladosporium* sp., *Cochlobolus lunatus*, *Curvularia oryzae*, *Drechslera oryzae*, *Fusarium moniliforme*, *Helminthosporium oryzae*, *Penicillium* sp., *Phoma* sp., *Rhizopus* sp., *Trichoconus padwickii* (syn: Alternaria padwickii) have been consistently isolated from the local varieties from Pakistan in the past 3 decades and they have definitive role as pathogenic to rice. (Butt *et al*., 2011; Khan *et al*., 2000; Bhutta and Hussain, 1998; Khan *et al*., 1988). Similarly Utobo *et al*. (2011) isolated 9 genera of fungi associated with the stored rice grains *viz.*, *Trichocomis padwickii* (syn: *Alternaria padwickii*), *Aspergillus niger*, *Curvularia lunata*, *Helminthosporium oryzae*, *Fusarium moniliforme*, *Alternaria oryzae*, *Rhizopus oryzae*, *Penicillium* sp., and *Pyricularia oryzae*. *Trichoconis padwickii*, *Helminthosporium oryzae* and *Fusarium moniliforme* had highest incidence in the local check varieties as well as hybrid varietites. We also evaluated common isolation methods *viz.*, blotter and agar plate method and found that *Penicillium globosum*, *Rhizoctonia* sp., *Phoma* sp. were isolated in higher frequency from blotter paper method *Curvularia lunata* and *Drechslera* sp. from agar plates. Khan *et al*. (1988) has also reported the blotter method was the most suitable method for detecting *Alternaria alternata, A. tenuissima*, *Aspergillus niger, A. flavus, A. terreus*, *Chaetomium globosum* and *Curvularia lunata* on rice seeds; whereas agar plate showed higher percent frequencies of *Drechslera* spp. and *Curvularia* spp. were also isolated. However, Agarwal *et al*. (1972) found out that blotter paper method is a better method for the isolation of *Drechslera oryzae* and *Trichocomis padwickii* than the agar plate method. The surface sterilization of the seeds before inoculating on the agar plates with 2% ethanol and 2% sodium hypochlorite (NaOCl) revealed that sodium hypochlorite be stronger sterilizing agent and the percent frequencies were reduced significantly compared to the ethanol treatments of the seeds. This observation is supported by studies of Khomvilai *et al*. (2005) which reports sodium hypochlorite as a strong antifungal agent for control of pure strains of *Saprolegnia parasitica* Coker (NJM8604), a typical fungus that causes Saprolagniasis, isolated from the cultured coho salmon *Oncorhynchus kisutch*. Sitara and Akhtar (2007) has also used 10% sodium hypochlorite as fungicide along with Antracol (70% WP), Aliette (80% w/ w), Ridomyl Gold (MZ 68% WP), Neem seed powder @ 0.1%, 0.2% & 0.3%. Although found sodium hypochlorite to be the least effective in control of the 11 species of fungi *viz.*,

Aspergillus niger, *A. flavus, A. wentii, Chaetomium* sp., *Drechslera* sp., *Fusarium chlamydosporum, F. oxysporum, F. moniliforme, F. semitectum, F. nivale, Nigrospora sp., Phoma*sp. and *Rhizopus* sp. isolated from maize seeds. Badoni and Chauhan (2010) compared two most common sterilizing agents, sodium hypochlorite and mercuric chloride for surface sterilization of explants of potato cultivar Kufri Himalini after sprouting. The sprouts were treated with sterilizing agents when they were 0.5 to 1 cm in three treatments 2, 5 and 8 minutes for each. The explants were inoculated on MS medium without hormones and data recording was done till 30 days. The results showed that sodium hypochlorite treatment for 8 minutes was most effective and had no effect on explants. Chun *et al*. (1997) has also reported that sodium hypochlorite was most effective in disinfection and seedling germination of rice seeds. They found that bacteria from rice seeds were completely removed by immersing in household bleach solutions (50% bleach and 2.6% NaOCl) adjusted to pH 7.0 in 0.5 M potassium phosphate, while fungi were eliminated at pH 5.0 and below. Leaper (1986) has reported sporicidal effects of ethanol and a few other primary alcohols against *Bacillus subtilis*.

Conclusion: The diversity and characterization of different fungal species isolated from diverse rice germplasm lines were classified for future studies to the scientific community. The study may be utilized for the screening of various rice germplasm lines on the basis of various isolated fungal species and classification of the genotypes with respect to their various kinds of pathogen. This study may also equally helpful for the scientist and farmers community for the classification on the basis of resistant, tolerant and susceptibility of rice genotypes against various pathogens and also provides the information for further investigation in to new insight in scientific field.

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