SEED ASSOCIATED MYCOFLORA OF RICE FROM KYMORE REGION, CENTRAL INDIA

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ABSTRACT: Traditional and improved rice cultivars collected from different parts of Kymore region Madhya Pradesh in India were raised for studying grain discoloration and screening of seed borne mycoflora. Eleven species of fungi were isolated from discolored grain of the samples using standard blotter paper method. The most predominant species observed was *Bipolaris oryzae,* followed by *Curvularia lunata* and *Alternaria padwickii*. The seed mycoflora shows significant diversities in all the cultivars. High assemblages of mycoflora were observed in traditional than improved cultivars. The association of the mycoflora with various cultivars indicated seed contaminations and thus reducing seed quality.

Keywords: *Rice, germination, grain discoloration, quality seeds, seed borne mycoflora*

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Rice (*Oryza sativa L*) is the principal food grain crop cultivated in 237 million acres around the globe. Rice is the central for the food security of over half the world population (Cheema et al., 1991) providing staple food for almost 2 billion people (Sakthivel, 2001).India occupies maximum area of rice (28%) in the world and rank second to China in production (22.1%). The production of rice in India is 105.31 million tons and productivity is 2372 kg/ha, and in Madhya Pradesh the production is 3.78 million tons and productivity 1106 kg/ha which is far below than the average national productivity (Rao, 2011). There are various biotic and abiotic stresses which are responsible for low yield but major constraints to production are the infectious diseases. Plant diseases are a great challenge in commercial agriculture and pose real economics threats to both conventional and organic farming. Natural contamination with mycotoxins has been reported for most of the major agricultural commodities in the world including rice (Ciegler, 1978). Rice has served as a host for a number of diseases and insect-pests, 54 in the temperate zone, and about 500 in tropical countries (Papademetriou, 2000). Rice crop is attacked by several bacterial, fungal, viral pathogens and pests. Among them blast, bacterial blight, tungro virus are considered as major diseases (Sakthivel, 2001).

Seeds play a major role in agriculture production because over 90% of crop productions are propagated via seeds (Agrios, 1988). Seed of high genetic purity and planting quality is vital to increase productivity level of rice varieties (Panduranga Rao et al., 2004). Seeds are also the major carriers of plant pathogens such as bacteria, viruses and especially the fungi that affect storage life. The fungi are one of the major causes of seed quality deterioration as well as decreased crop yields (Thobunluepop, 2008). The fungi are disastrous as they reduce seed vigor and weaken the plant at its initial growth stages (Butt et al., 2011), and also decreases seed germiability causing seed discoloration and, producing toxins that may be injurious to man and domestic animals and may reduce seed weight (Uma and Wesely, 2013). In case of severe infection by the fungi the seed completely deteriorates and the grain may become unsuitable even for animal consumption due to production of mycotoxic substances (Islam and Borthakur, 2012). The study of seed health testing is important (Thobunluepop, 2008; Ibiam*et al*., 2008; Jayaraman and Kalyanasundaram, 1994; *Gopalakrishnan et al., 2010*) as it assure the safe movement of seed of different crops for research or trade because many harmful organisms are carried with the seed, and have the potential to cause severe damage to crop production and crop seed for international trade (Archana and Prakash, 2013).

Rice grains may be infected by various organisms before or after harvest causing seed damage in the form of discoloration (Neergard, 1977). The fungi associated to rice seeds can be categorized as "field fungi" which are more or less parasitic and infect the grains before harvest (Neninger et al., 2003) and "storage fungi" which usually are saprophytes and develop after harvest (Christensen and Kaufmann, 1965). Fungal infestation generally results in the reduction of grain quality, change in color, taste, smell, reduction in nutritional value, free fatty acids (FFA) germination ability (Dharmaputra, 1997), and loss in viability of rice seeds (Oren and Bass, 1982). The laboratory studies have shown that the fungi are capable of producing hundreds of toxic chemicals, most of which are not included in routine analyses and their toxic effects are often insidious and may go undetected, the true dimensions of the mycotoxin problem are unknown (Ciegler, 1978). The isolation and identification of fungi from discolored rice grains from various parts of the world has been reported before (Thobunluepop, 2008; Neergard, 1977; Danquah et al., 1976; Inoba, 1980; Imolehin, 1983; Sharma *et al*., 1987; Notteghem et al., 1995; Tonon et al., 1997; Broggiand Moltó, 2001;Habib et al., 2012; Hajano et al., 2011; Ora et al., 2011; Sadia, 2012; Ahmed et al., 2013; Pinciroli et al., 2013).

Several fungal pathogens have been isolated from rice seeds, and have been reported to be responsible for a number of diseases from the nursery to the field (Ibiam *et al.*, 2006). Rice seeds are very often infected by field fungi such as *Bipolarisoryzae, Trichoconispaddwickii, Fusariummoniliforme, Fusarium oxysporum, Fusarium semitectum, Pyricularia grisea, Curvularia lunata, Alternaria altemata* (Mia et al., 2002). The aim of this research work is to identify various seed mycoflora and their role in seed germination and seedling vigor for the assessment of the seed health standard and to design a defense mechanism in combating the pathogens and producing the quality grains and sustainable yield of the crop.

MATERIALS AND METHODS

Study of grain discoloration

Twelve rice cultivars including six improved (Vandana, Basmati, Govinda, Jaya, Kalinga and IR-64) and six traditional (Indrajal, Gurmatia, Dehula, Ajaan, Lochai and Newari), collected from various parts of Kymore regions of Madhya Pradesh were evaluated. The experiments were conducted in RBD with three replications in Agriculture land at Rewa during *kharif* season 2011. Recommended agronomic practices were adopted for raising crops. The seeds harvested from experiments were dried, kept in cloth bags and brought to Departmental laboratory for further analysis. Hundred seeds of each cultivar were examined for grain discolorations.

Detection and Identification of seed borne mycoflora

Detection of mycoflora associated with seeds of different cultivars was studied using standard blotter paper method (ISTA, 2011). One hundred seeds of each cultivar were counted at random. Seed samples were pre-treated with 1% sodium hypochlorite solution for five minutes to remove surface contaminants on the surface. The samples were placed on moist blotting paper (Whatman No.1) in glass Petri dishes at the rate of 25 seeds per Petri dishes. The Petri dishes were incubated at 25±1°C under 12/12 hrs alternating cycles of light and darkness for a week. Each seed was observed under stereomicroscope for fungal growth. The fungi were identified on the basis of sporulation, conidial characters and fruiting bodies which developed on the seed (Ahmed and Reddy, 1993).

Influence of seed borne mycoflora on Seed germination and mortality

The influence of seed borne mycoflora on seed germination was studied using standard blotter method (ISTA 2011). Twenty five seeds of each cultivar were tested for germination at room temperature. The seeds were placed on moist filter paper and covered by another filter paper. The experiment was performed in triplicate with five replications. Percentage germination of the seeds on the blotter paper was recorded 14 days after incubation.

The root and shoot lengths were measured after seven days of germination and seedling vigor index was calculated as:

Seedling Vigour Index = Percent seed germination x Seedling length (mm) (Abdulbaki and Anderson, 1973).

Statistical Analysis

The data obtained were analyzed using technique of ANOVA as given by Ronald E. Walpole

RESULTS AND DISCUSSION

Grain discolorations

All the grains of the tested cultivars were found infested by seed borne fungi but differed in the level of susceptibility. The traditional cultivars showed the highest degree of discoloration and the susceptibility ranges from 1.96 to 22.56% (Table 1). The cultivar Gurmatia shows maximum grain discoloration (22.56%), followed by Dehula (20.07%) and Newari (17.32%). However, the rate of infection in improved samples was comparatively less than traditional, but shows varied differences. Among the improved cultivars, Basmati shows maximum discoloration (8.01%) and IR-64 (1.96%) exhibits the least discolored grains. The results are in accordance with Du *et al*.(2011) who reported that the discolored grains in rice are observed in both dry and wet season, but more severe in wet season and are major problems for rice production.

Table 1: Percentage discolored grains of rice varieties

Varieties	% grain discoloration
Indrajal	16.02
Gurmatia	22.56
Lochai	9.94
Dehula	20.07
Ajaan	13.24
Newari	17.32
Basmati	8.01
Govinda	5.07
IR-64	1.96
Jaya	2.10
Vandana	3.21
Kalinga	4.12

Seed mycoflora

In the present study eleven fungi were found associated with the cultivars that include: *Alternaria padwickii, Alternaria alternata, Bipolaris oryzae, Curvularia lunata, Penicillium* sp., *Fusarium oxysporum, Fusarium moniliforme, Nigrospore oryzae, Spermospora* sp., *Cercospora geniculata* and *Trichothecium roseum* (Table 2). The average pooled data reveals that fungus *Bipolaris oryzae* shows maximum association (18.84%), followed by *Curvularia lunata, Alternaria padwickii* whereas fungus *Spermospora sp.* shows least association (0.44%) with the cultivars. Among these fungi, *A. padwickii and A. alternata*, were found in all cultivars before harvest and in storage. Among the mycofloras, *Bipolaris oryzae* shows highest levels of infection in the entire tested sample (18.84%) with maximum in traditional variety Gurmatia. The fungus *Curvularia lunata* and *Penicillium sp.* were more abundant in Gurmatia and all other traditional cultivars in comparison to the improved cultivars (Table 2). The genus *Fusarium* and *Nigrospora* shows least rate of infection in all the tested samples. The occurrence of fungi in discolored grain of rice has been reported by various researchers throughout the rice growing regions of the world (Thobunluepop, 2008; Imolehin, 1983; Sharma et al., 1987; Notteghem et al., 1995; Saifulla et al., 1998; Islam et al., 2012; Utobo et al., 2011). The study is similar to the findings of Imolehin(1983) who observed that *Helminthosporium oryzae* was the most frequently isolated seed borne fungus of the rice cultivars. The isolation of *Fusarium moniliforme* from seed samples indicates poor seed quality causing grain discoloration (Roy and Baruah, 1972). The findings are in accordance with Misra et al. (1995) who isolated a total of 36 fungal forms in different rice varieties under storage conditions using standard blotter and agar plate methods from Philippines. Pacin et al. (2003) recorded *F. oxysporum* as the most prevalent toxigenic fungal species, followed by *F. verticillioides* and *A. flavus from f*reshly harvested rice in Ecuador.

In the present study the frequency of the occurrences of the fungi was high in traditional than improved cultivars. Among the traditional cultivars the maximum number of fungi were isolated from Gurmatia (15.39%), followed by Dehula (13.17%) and Newari (11.21%). The improved cultivars also exhibited significant variation in the occurrences of seed-borne fungi. The rate of infection was highest in Basmati (6.79%), followed by Govinda (5.94%) and Kalinga (4.88%), and the least infection was noted in IR-64 (1.53%). In the present investigation it was observed that majority of the improved cultivars and a few traditional cultivars exhibits less seed infestation which may be attributed to improved genetic make-up of the hybrid cultivars (Utobo et al., 2011). Further the occurrence of mycoflora in the cultivars in low to high range were also noted, which is mainly due to time duration in storage period as it is a decisive factor in moisture content, storage mycoflora and aflatoxin B_1 (AFB_1) and causes maximum deterioration, increase in frequency of incidence as well as concentration of AFB_1 (Jayaraman and Kalyanasundaram, 1994).

Table 2: Percent occurrence of fungi isolated from discolored grains of rice varieties during harvest

Varieties	Fungi isolated from discolored grains of rice varieties											Mean
	Alternaria padwickii	*Alternaria alternata*	*Bipolaris oryzaeta*	*Curvularia lunata*	*Penicillium sp.*	*Fusarium oxysporum*	*Fusarium monoliforme*	*Nigrospora oryzae*	*Spermospora* sp.	*Cercospora geniculata*	*Trichothecium roseum*	
Indrajal	16.3	7.5	24.6	18.6	8.3	10.5	12.1	7.1	1.0	6.4	1.8	10.38
Gurmatia	23.1	10.2	33.7	26.1	14.1	17.4	21.1	10.0	2.1	8.2	3.2	15.39
Lochai	11.4	5.1	21.2	13.9	5.9	6.5	10.7	5.0	0.0	4.5	0.5	7.7
Dehula	20.2	9.5	27.0	23.5	11.9	14.2	18.3	9.1	1.6	7.4	2.1	13.17
Ajaan	13.7	6.4	22.8	16.3	7.5	7.9	11.2	6.2	0.0	5.8	1.5	9.03
Newari	14.9	8.1	25.7	21.3	10.8	12.0	13.1	8.6	0.5	7.0	1.3	11.21
Basmati	10.1	4.5	18.5	12.4	5.6	6.1	9.3	4.2	0.0	3.4	0.5	6.79
Govinda	8.7	3.9	17.6	11.3	4.6	5.5	7.1	3.7	0.0	2.9	0.0	5.94
IR-64	2.1	1.1	5.0	3.0	1.2	1.5	1.9	1.0	0.0	0.0	0.0	1.53
Jaya	6.1	2.4	7.4	6.8	3.2	4.3	5.2	2.2	0.0	2.0	0.0	3.6
Vandana	7.4	2.7	10.6	7.9	3.6	4.7	6.3	2.9	0.0	2.2	0.0	4.39
Kalinga	8.0	3.6	11.9	8.3	4.0	5.1	6.9	3.1	0.0	2.8	0.0	4.88
Mean	11.84	5.42	18.84	14.12	6.73	7.98	10.27	5.26	0.44	4.39	0.90	

Seed germination and seedling vigor

The traditional and improved cultivars show remarkable variation in seed germination efficiency and seedling vigor index (Table 3). Almost all seed samples were infected by seed borne fungi with a high or low degree of infection. In the present investigation the traditional cultivar Gurmatia shows least seed germination (60.6%), followed by Dehula (76%) and Indrajal (82%), whereas the improved cultivars Basmati and Vandana exhibits least and IR-64 shows maximum seed germination efficiency (97.3%). The seed mortality varied with the rate of infections. The traditional exhibited the highest mortality compared to improved cultivars. The cultivar Gurmatia shows highest mortality (39.4%), followed by Dehula (24%) and Indrajal (18%), and among improved cultivars, Basmati and Vandana exhibited the highest mortality whereas least mortality was exhibited by IR-64 (2.7%).

In the present research the reduction in seed germination in various tested cultivars were observed which may be due to fungi *Helminthosporium oryzae a*s reported earlier (Danquah et al., 1976; Rath, 1974; Misra and Singh, 1969). The reduction in germination efficiencies in cultivar Gurmatia along with other traditional cultivars is mainly attributed to predominance of brown spot fungus *B. oryzae* that produces non-specific phytotoxins ophiobolin A and ophiobolin B during spore germination, inhibiting root elongation (Xiao *et al*., 1991), producing abnormalities in the seedlings (Vidhyasekaran et al., 1986) and resulting in rapid cell death (Condon et al., 2013). Uma and Wesely (2013) suggested that the fungi are responsible for reduction in the quality of rice due to high moisture and temperature conditions before its harvest and decreases seed germiability, causes seed discoloration and may reduce seed weight. Islam *et al*. (2012) reported that the rice seeds infected by the fungi germinate poorly and could be a major source of inoculums for new crops raised from them.

Table 3: Seed germination, mortality and seedling vigor index of local and hybrid varieties of rice

Varieties	Germination %	Mortality %	Seedling length (mm)		No. of secondary roots	Seedling vigor index (SVI)
			Root length	Shoot length		
Indrajal	82	18	50	40	14	738
Gurmatia	60.6	39.4	60	40	15	606
Lochai	84	16	65	40	21	882
Dehula	76	24	44	40	18	638.4
Ajaan	84	16	50	47	21	814.8
Newari	84	16	49	29	16	655.2
Basmati	91.3	8.7	50	50	22	913
Govinda	92	8	70	50	37	1104
IR-64	97.3	2.7	82.4	60	30	1385.55
Jaya	93	7	80.2	54	37	1224.8
Vandana	91.3	8.7	70	60	26	1186.9
Kalinga	92	8	70	50	25	1104
SEm±	0.25	0.32	0.04	0.15	0.38	
CD (5%)	0.81	1.02	0.13	0.48	1.23	
CV(5%)	0.11	0.68	0.22	0.19	3.11	

In the present research significant variation were noted in root length, shoot length and number of secondary roots in the tested cultivars. The root length and shoot length of the samples ranged from 44 to 82.4 mm and 29 to 60 mm, respectively. The traditional cultivars exhibits minimum, whereas the improved cultivars maximum root and shoot length. Similarly, the emergence of secondary root also follows the same trend and the numbers of secondary roots in traditional and improved cultivars were in the range of 14- 21 and 22-37, respectively. The study is similar to the findings of Utobo et al. (2011) who suggested that improved rice cultivars showed higher vigor in terms of germination, root length, root weight, shoot length, shoot weight and vigor index when compared to traditional cultivars.

In the present study the Seedling Vigor Index (SVI) also shows a remarkable variation in all the tested samples (Table 3). The SVI were found maximum in improved than traditional cultivars. Among the traditional cultivar Gurmatia shows least seedling vigor (606%), followed by Dehula and Newari, 638.4% and 655.2%, respectively. The improved cultivars exhibited SVI in the range of 913 to 1324.32%. Basmati shows minimum SVI (913%) and the highest SVI were represented by IR-64 (1324.32%). The variations in seedling vigor are mainly due to seed mycoflora which utilizes the stored food of the seed for their growth and development. The grain discoloration has resulted in reduction in seedling vigor as reported earlier in various crops (Manoharachary and Kunwar, 2006; Agarwal and Sinclair, 1997; Sarkar and Saxena, 2007). Uma and Wesely (2013) suggested that the fungal pathogens are disastrous as they reduce seed vigor and weaken the plant at its initial growth stages. Ora et al. (2011) observed that lowest pathogenic incidence recording cultivars showed lowest mortality and highest seed germination and seedling vigor index. Moreover, there are reporting of huge economic losses and rice yield due to incidence of *Bipolaris oryzae*, *Alternaria padwickii, Pyricularia oryzae, Curvularia* and *Fusarium spp.* (Thobunluepop, 2008; *Gopalakrishnan* et al., 2010).

CONCLUSION

This study has isolated eleven species of mycoflora from infected grains of rice during harvest with *Bipolaris oryzae,* showing highest frequency of occurrences in the cultivars. This research emphasizes on qualitative evaluation and for designing quality management practices during harvest of rice grains to avoid contamination and deterioration of the seeds.

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