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Prevalence of microflora associated with different rice varieties and its impact on sowing seed quality

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Abstract
A total of 28 rice varieties were procured from Plant Breeder (Rice), JNKVV, Jabalpur. The collected seeds were placed in a butter paper envelop and stored at low temperature (4^0C) to avoid any further deterioration. The rice seeds were tested for the association of mycoflora and its impact on sowing seed quality like seed germination, seed emergence and seedling vigour. The mycoflora that were associated with diseases, were identified based upon the symptoms and structure and fruiting bodies produced either under lab or field conditions. Effect of mycoflora was determined by standard ragdoll method, standard blotter method and seedling vigour index was calculated based on the seedling length by adopting the formula as recommended by Abdul- Baki and Anderson (1973). The association of *Curvularia lunata* ranged from 2.0-17.0%, *Alternaria alternata* 2.0-14.0%, *Helminthosporium oryzae* 2.0-11.0 % and *Fusarium moniliforme* 2.0-13.0%. Association of *Pyricularia oryzae* and *Trichoconiella padwickii* were not recorded. Significant reduction in seed germination and seedling vigour was observed in seed infected with *Curvularia lunata* (19%) *and Altenaria alternata* (24%) in mahamaya and varalu varieties respectively.

Keywords: Rice, seed borne fungi, mycoflora, *Curvularia lunata*, standard blotter, seed germination

1. Introduction
Rice plays an important role in supporting over three billion people around the world with more than 6.7 billion bowls of rice consumed every day. (IRRI Annual Report 2010, Sushil Pandey 2011). Therefore it is a challenge for agriculture to increase food production to meet the food demands and human sustainability. The production of rice in India, however, increased gradually because of significant development in agriculture, research, education, extension etc. Besides increasing crop production, one of the greatest challenges is to control the disease of rice. Rice pests are any organism or microbes with crop potential to reduce the yield or value of this crop (Jahn *et al*. 2007). In Madhya Pradesh, majority of rice grown areas is under rainfed conditions, and predominance of small holders with low adaptive capacity causing more vulnerable to climate change. At JNKVV, Jabalpur investigations on rice diseases have been taken up.

The growth and productivity of rice is dependent mainly on the influence of both biotic and abiotic factors. A number of plant pathogens infect the crop (Singh 2004; Agrios 2009). Important fungal pathogens are blast (*Magnaporthe grisea*), sheath blight (*Rhizoctonia solani*), Bakanae disease (*Fusarium moniliformae*), false smut (*Ustilaginoidea virens*) and brown spot (*Helminthosporium oryzae*) (Ou 1985) [21]. The increasing pressure of biotic factors has adversely affected the production and productivity of quality grain and seed. Number of rice diseases have been observed that are creating a problem for profitable sustainable production.

Several pathogens are seed borne in rice (Neergaard 1997). Under changing climatic conditions and with the advent of development of hundreds of varieties, free flow of the genetic stock and due to intense cultivation, several pathogens have come up with varied degree of potential. Several microorganisms have been found associated with rice seeds. The microorganisms are responsible for causing various diseases as seed rot, seedling decay, seedling abnormalities, seed discoloration, chaffiness, pre and post emergence mortality. With this view the investigation has been undertaken to understand the status of mycoflora associated with rice seeds, its detection and impact on sowing seed quality.

2. Materials and Method
2.1 Location of the site
The field investigations were conducted at Rice Research Experimental Area, Adhartal Tank farm, Department of Plant Breeding & Genetics, JNKVV, Jabalpur.

The studies on rice varieties were conducted in supervision of Rice Breeder (Dr. Koutu at Seed Breeding Farm). The investigations were conducted in rice crop grown at Jabalpur that lies between $22^0 21'$ and $80^0 58'$ East longitude at an altitude 411.78 meter above the mean sea level.

2.2 Identification of seed associated mycoflora

Association of mycoflora with seeds of 28 rice varieties was determined. The mycoflora that were associated with diseases, were identified based upon the symptoms and structure and fruiting bodies produced either under lab or field conditions. The identity of the associated microorganisms was confirmed through various keys developed by other scientists.

2.3 Collection of seeds

The seeds of 28 rice varieties were procured from Plant Breeder (Rice), JNKVV, Jabalpur. The collected seeds were placed in a butter paper envelop and stored at low temperature (4^0C) to avoid any further deterioration.

2.4. Detection of fungi associated with seeds

1. Standard blotter method

The rice seeds were tested for the association of mycoflora. Three circular blotter paper of the size of Petri dishes (90 mm diameter) were cut with the help of scissor. The blotters were dipped in sterile water for few seconds. The excess of water was drained-off. The wet blotters were placed in each pre-sterilized Petri dish with the help of sterilized forceps. In each Petri dish 25 seeds were placed in a such manner that sixteen were at outer circle, eight in a inner circle and one in the centre. The seeded Petri dish were incubated in the growth chamber. Untreated seeds of each sample were analyzed. Petri dish were examined on fifth day of incubation. Mycoflora were directly detected under stereo binocular microscope.

2.5 Effect of mycoflora on germination

Influence on fungal infection, on seed germination was determined.

1. Standard Ragdoll method

Standard Ragdoll method (ISTA 1993) [14] was used for the testing the effect of disease on germination of rice seeds. Seed samples were selected from the harvest of diseased plants. In this method, 400 seeds of varieties were used. The towel (blotter) papers were moistened with sterile water. Excess of water was removed. The paper were stretched and placed over a clean surface of table on a paper, 50 seeds were arranged on the half portion of the towel paper. Seeds were covered with the other half portion of the paper and rolled over. A wax paper was wrapped on rolled paper towel and both ends were tightened with rubber band, it prevented the runoff water as well as helped in maintaining of humidity require for germination. The rolled towel papers were kept in a slanting position. The seeded towels were placed in a seed germination/walk-in-chamber (Fig III), at 25^0C with relative humidity about 85%. Seedlings were examined on 14^{th} day of incubation and germination percentage was calculated (Fig I).

2. Standard blotter method

In this method, three pieces of circular blotter paper were placed in a pre-sterilize Petri dish. The blotter papers were moistened with distilled and sterile water. Excess of water was removed from the Petri dish. In a plate 25 seeds were arranged with the help of forceps on the top of the blotter. Seeded plates were incubated in the growth chamber, germination of the observed after 7 days of incubation (Fig II).

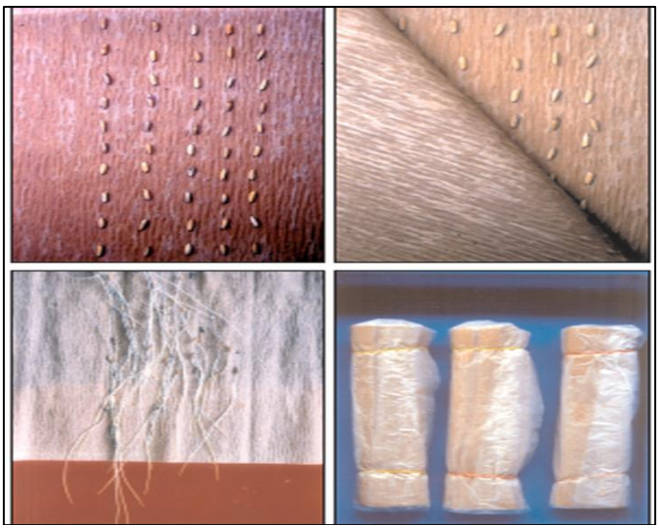

Fig 1: Standard paper towel method (ISTA 1993)

Fig 2: Standard blotter method (ISTA

Fig 3: Incubation chamber

Effect on seedling vigour index

Seedling vigour index was calculated based on the seedling length by adopting the formula as recommended by Abdul- Baki and Anderson (1973).

Seedling vigour index = (Mean root length+ mean shoot length) × percent seed germination)

For the purpose ten seedlings of each category grown between towel papers were randomly selected during the germination test. Shoot length were measured from the collar region to the point of attachment whereas root length was measured from collar region to the tip of the root.

3. Results

Seeds of 28 varieties were collected from Rice Breeder, Department of Plant Breeding and Genetics and the mycoflora were detected by standard blotter method. Data presented in Table 1 indicate that maximum (19.0%) association of *Curvularia lunata* was recorded with seeds of Mahamaya, *Alternaria alternata* (24.0%) with seeds of Varalu. The association of *Helminthosporium oryzae* ranged from 2.0-14.0% with maximum in WGL 32183. None of the seed sample exhibited the association of *Trichoconiella padwickii* and *Pyricularia oryzae*. Association of *Fusarium moniliforme* ranged from 2.0-21.0% maximum with seeds of MTU 1081. The seed germination ranged from 40.0-86.0 %.

Table 1: Mycoflora associated with seeds of different rice varieties obtained from the research fields and detected by standard blotter method (ISTA, 1996)

Varieties	Per cent disease incidence						% seed germination
	Curvularia lunata	*Alternaria alternata*	*Helminthosporium oryzae*	*Trichoconiella padwickii*	*Fusarium moniliforme*	*Pyricularia oryzae*	
Chandrahasini	11	7	7	0	2	0	83
Dhanteshwari	12	5	0	0	2	0	82
Eramallelu	10	11	0	0	11	0	80
Falguni	5	0	0	0	3	0	85
IR 36	12	17	0	0	15	0	0
IR 64	14	12	0	0	14	0	80
JGL 3828	5	0	5	0	15	0	80
JGL 3844	3	0	0	0	10	0	86
JR 201	2	0	2	0	7	0	82
Kavya	8	8	0	0	2	0	85
Kranti	11	13	11	0	13	0	80
Mahamaya	17	5	0	0	14	0	75
MR 219	10	0	0	0	11	0	79
MR 220	14	0	5	0	0	0	79
MTU 1010	5	10	5	0	0	0	75
MTU 1081	5	0	3	0	11	0	85
PA 6129	7	0	2	0	11	0	80
Pratiksha	2	0	4	0	0	0	80
Pusa 1121	2	0	5	0	0	0	85
Pusa 1401	0	0	0	0	15	0	85
Pusa 1460	0	0	0	0	5	0	86
Pusa Basmati	0	0	0	0	3	0	86
Surekha	0	0	2	0	5	0	84
Varalu	0	5	0	0	7	0	85
WGL 14	2	2	11	0	7	0	82
WGL 21	0	11	5	0	8	0	85
WGL 32100	12	10	9	0	9	0	79
WGL 32183	11	5	14	0	10	0	75
Range	2-17	2-17	2-14	0	2-15	0	75-86

3.1 Impact of mycoflora on sowing seed quality

Effect on seed germination

The influence of healthy and diseased seeds on seed germination was determined by placing the seeds between the blotter (Ragdoll) and top of the blotter (Standard blotter method) and the results are presented (Table2).

Table 2: Influence of seed mycoflora on germination of rice seeds as tested by standard blotter method and standard paper towel method

Sample	Per cent seed germination		Per cent reduction	Per cent seed germination		Per cent reduction
	Paper towel method			Top of paper method		
	Healthy	Diseased		Healthy	Diseased	
Mahamaya	85	76	10.58	83	75	9.63
Initial seed infection *Curvularia lunata* 19%						
Varalu	84	73	13.09	84	73	13.09
Initial seed infection *Alternaria alternata* 24%						
MTU 1081	87	75	16.47	85	72	15.29
Initial seed infection *Fusarium moniliforme* 21%						

Data presented in Table 2 indicate that maximum reduction (18.60%) in seed germination was observed in MTU 1081(16.47%) when the seeds were placed between blotter and it was followed by 13.09% (Varalu). When the seeds were placed on the top of the blotter maximum reduction was observed in MTU 1081(15.29%).

3.2 Effect on seed emergence

Influence of healthy and diseased seeds on seed emergence was tested by sand and soil method. The seeds were sown in sand, soil and emergence was counted on 5th day.

Table 3: Influence of seed mycoflora on seed emergence of rice seeds as tested by seed sowing in sterile sand and seed sowing method

Sample	Per cent seed emergence		Per cent reduction	Per cent seed emergence		Per cent reduction
	Sand method			Soil method		
	Healthy	Diseased		Healthy	Diseased	
Mahamaya	89	81	8.98	87	80	8.04
Initial seed infection *Curvularia lunata* 19%						
Varalu	85	75	11.76	82	74	9.75
Initial seed infection *Alternaria alternata* 24%						
MTU 1081	88	79	10.22	86	77	10.46
Initial seed infection *Fusarium moniliforme* 21%						

Data presented in Table 3 indicate that maximum reduction (11.76%) was observed in Varalu and 10.22% MTU 1081, respectively when the seeds were sown in sterile sand. When the seeds were sown in sown in soil maximum reduction was observed in MTU 1081 (10.46%). Minimum reduction in seed emergence was observed in Mahamaya (8.04%) (Table 3).

3.3 Effect on vigour

Table 4: Influence of mycoflora on root and shoot length of rice seeds as tested by standard paper towel method (ISTA, 1996)

Sample	Healthy				Diseased			
	Shoot length	Root length	Seed germination	Vigour	Shoot length	Root length	Seed germination	vigour
Mahamaya	12	8	85	1700	10	7	76	1292
Varalu	13	8.5	84	1806	9	8	73	1241
MTU 1081	13	13	87	2262	12	10	75	1650

The effect on infection on seedling vigour of rice seeds was determined by multiplying shoot length and root length with seed germination of both healthy and diseased seeds. It was observed that seeds of MTU 1081 showed high vigour (2262) in healthy seeds, whereas in diseased seeds vigour of MTU 1081 was higher (1650) (Table 4).

4. Discussion

Association of mycoflora with seeds was detected for all the samples procured from farmers and Rice Breeder, Department of Plant Breeding and Genetics by employing Standard blotter method (ISTA 1996). Two hundred seeds from each location were placed on top of the blotters placed in Petri dishes and observations were made after incubation of six days. The association of mycoflora was confirmed under stereoscopic binocular microscope on the basis of habit characters and finally confirming by making slides as observed under compound microscope. Mycoflora were identified with the help of available keys.

It was concluded that four mycoflora were dominant and variable association was noticed in the seeds samples. Association of *Pyricularia oryzae* and *Trichoconiella padwickii* were not recorded. The association of *Curvularia lunata* ranged from 2.0-17.0%, *Alternaria alternata* 2.0-14.0%, *Helminthosporium oryzae* 2.0-11.0 % and *Fusarium moniliforme* 2.0-13.0%. Seed associated mycoflora have been investigated by Agarwal *et al*. (1972) [5] and Reddy and Khare (1979) [24]. Association of *Curvularia lunata, Fusarium moniliforme, Alternaria alternata, Helminthosporium oryzae, Trichoconiella padwickii* with seeds is reported. Grain discoloration, false smut and bunt have also been reported.

Association of *Curvularia lunata* (19%) exhibited 10.58 per cent reduction in seed germination and 8.98% reduction in seed emergence in variety Mahamaya. In variety Varalu association of *Alternaria alternata* (24%) showed 13.09% reduction in seed germination and 11.76% reduction in seed emergence. Whereas, similar association of mycoflora with 21% of initial seed infection in MTU 1081 showed 16.47% and 10.22% reduction in seed germination and seed emergence.

The effect of infection on seedling vigour was determined by multiplying mean of shoot length and root length with seed germination of both healthy and diseased seeds. It was observed that seeds of MTU 1081 showed high vigour (2262) in healthy seeds, whereas in diseased seeds vigour of MTU 1081 was higher (1650).

5. Conclusion

Investigations were made to determine the association of mycoflora with rice seeds. Association was detected by Standard blotter method (ISTA, 1996). Two hundred seeds from each location were placed on top of blotters in Petri dishes and observations were recorded after incubation of six days. The association of mycoflora was confirmed under stereoscopic binocular microscope on the basis of habit characters and finally confirming by making slides as observed under compound microscope. In all, mycoflora namely *Curvularia lunata, Alternaria alternata, Helminthosporium oryzae, Fusarium moniliforme* were observed. Seed associated mycoflora show significant impact on yield and quality, hence assessment of seed associated mycoflora is very much important.

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