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Detection of Seed-Borne Mycoflora associated with Paddy Seeds and its Influence on Seedling Health

R. Karan*, P. Renganathan, P. Balabaskar and T. Suthin Raj

Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, India

**Corresponding author*

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A B S T R A C T

Rice is an important food crop across the world. Good quality seed is the major criteria for better yield and production. Seed samples collected from the Cuddalore, Mayiladuthurai, Tanjore, Trichy and Karur district were tested for the presence of seed borne fungal mycoflora and their percentage of incidence using Blotter paper and Agar plate techniques. This experiment shows the higher incidence of *Bipolaris oryzae* in the collected samples followed by *Curvularialunata, Aspergillus* and *Alternaria alternata,* whereas *Pyricularia grisea, Sarocladiumoryzae, Ustilaginoidea virens* and *Fusarium sp.,* were found in a relatively low level. The JGL 1798 variety had a high mean incidence of seedborne fungi whereas the BPT 5204 had a least mean incidence of the pathogen. The JGL 1798 variety recorded a minimum germination (68%), maximum seed mortality (32%), maximum percentage of abnormal seedling (34%), minimum root length (6 cm), shoot length (5.5 cm) and Vigour index (782).

Introduction

Rice (*Oryza sativa* L.) is one of the stable food crops in the world. Among the rice growing countries, China and India ranks top in its Production. Globally, 167 million hactare area is planted with rice, yielding 782 million tonnes (FAO 2019). India is the second largest producer of rice after China whose production is 112.91 million tonnes (GOI 2019). The seed quality and seed health are important criteria for higher grain yield and enhanced crop growth (Haque *et al.,* 2012). Seed infection of pathogens reduced the germination percentage, viability of seedlings and vigour of seedlings (Hamin *et al.,* 2014). Most of major rice diseases are seedborne in nature. Thus, rice is associated with many microorganisms including Fungi, bacteria and viruses (Mew TW and Gonzales P 2002). The

present investigation was carried out to detect the seed borne mycoflora associated with farm saved seeds of rice and its influence on seed health.

Materials and Methods

Collection of Samples

Seeds are collected from the farmers' fields at Sivapuri, Keelamungiladi, C Mutlur, Vallampadugai, Kollidam, Annamalai Nagar, Mayiladuthurai, Jeeyapuram, Thiruverumbur, Valayapatti and Kumaramangalam. Different variety of both stored and freshly harvested seeds are collected during the samba season of rice cultivation in 2019

Detection of seed borne mycoflora

Blotter Paper method

According to the International Rules for Seed Testing (ISTA 2001), the standard blotter paper technique was used for the detection of seed borne fungal mycoflora. 25 seeds were placed on the glass petri plates containing three layers of moist filter paper and incubated at 28°C for 7 days. Each seed was observed under a compound microscope for the presence of fungal spores (Agarwal and Sinclair 1997)

Agar Plate technique

In the agar plate method, two hundred seeds were tested for each maintaining four replications. Seeds were surface sterilized using 1% sodium hypochlorite followed by 70% ethanol. They were placed in the plates containing PDA medium and incubated at room temperature for 7 days under 12 hrs of alternate cycles of light and dark period. Fungi associated with seeds are identified based on colony characters and morphology of sporulation structure using compound microscope (Agarwal and Sinclair 1997)

The percentage of incidence was calculated using the following formula:

$$\text{Percentage of Incidence} = \frac{\text{No of seeds affected by a pathogen}}{\text{Total no of seeds}} \times 100$$

Influence of seed borne mycoflora in seedling health

The influence of seed borne mycoflora in seedling health was tested using the Standard Roll Paper Towel method (ISTA 1976). The germination paper was soaked in water for 2 to 4 hrs to moist it evenly and to remove water soluble toxic substances present in it. Randomly selected 100 seeds were evenly placed equidistantly between the two sheets of Paper towel, rolled carefully, ensuring no pressure on seeds, wrapped with a polythene sheet to reduce surface evaporation and kept in germination chambers in an upright position. Each treatment was replicated thrice. They were incubated at room temperature (28±2^{o}C) for 14 days. The normal seedlings were selected at random from each replication and the shoot and root length from the collar at the tip of the primary root was measured and the respective mean values were recorded. The Vigour index (VI) was calculated using the formula suggested by Abdul Bakri and Anderson (1973)

VI= (Root length + Shoot length) × (Germination Percentage).

Results and Discussion

Detection of seed borne mycoflora

A total of 36 samples from 5 major varieties were collected from 12 places belonging to 5 districts of the Cauvery Delta Region of Tamil Nadu. The results are depicted in Table 2. The

experiment revealed that seed samples contain the infection of *Aspergillus, Alternaria alternata, Bipolaris oryzae, Curvularia lunata, Pyricularia grisea, Fusarium* sp., *Sarocladium oryzae, Ustilaginoidea virens* in both Blotter Paper and Agar Plate techniques.

The Blotter Paper Technique showed a highest percentage of incidence of *Bipolaris oryzae*(21%) followed by *Aspergillus* sp., (15.40%), *Curvularia lunata* (12.80%) and *Alternaria alternata* (10.70%). The following species viz., *Pyricularia grisea* (1.80%), *Fusarium* (5%), *Sarocladium oryzae* (5.40%) and *Ustilaginoidea virens* (6.8%) were present in relatively low levels. Among the verities tested, JGL 1798 and CR 1009 had a highest mean incidence of seed borne fungi (10.75%) followed by ADT 43 (10.13%), CO 51 (9.5%) while a lowest incidence was found in BPT 5204 (8.19%).

The Agar plate technique also showed the highest incidence of *Bipolaris oryzae* (22.80%) followed by *Curvularia lunata* (16.20). Others viz., *Aspergillus* (13%), *Fusarium* (10.2%) and *Alternaria alternata* (9.20%) had a significant level of incidence. Among the verities tested, JGL 1798 had a highest mean incidence of seed borne fungi (11.88%) followed by CO 51 (11.50%), ADT 43 (11.13%), CR 1009 (10.50%) and a lowest incidence was found in BPT 5204 (9.88%).

The results are agreement with Gopalakrishnan *et al.*, (2010) who conducted a survey at Tamil Nadu to identify the seed borne fungal genera associated with paddy seeds. In this survey, they were identified as the 8 fungal genera viz., *Aspergillus, Alternaria, Bipolaris, Chaetomium, Sarocladium, Curvularia, Fusarium,* and *Trichoderma*. Among them, *Bipolaris oryzae* was a predominant one which was associated with the 58.89% of the seed samples. Similarly, Naveenkumar *et al.*, (2016) also isolated a total of 9 genera namely, *Curvularialunata, Alternaria padwiki, Fusarium moniliforme, Pyricularia oryzae, Helminthosporium oryzae, Rhizopus oryzae, Sarocladium oryzae, Aspergillus niger* and *Trichoderma* species. Out of those, *Helminthosporium oryzae* was having the highest percentage of incidence in 62.36% of the seed samples. Ora *et al.,* reported 12 species associated with rice seeds. Among those species, *Bipolaris oryzae* was a predominant one. Ahemed *et al.,* (2013), Habib *et al.,* (2012), Reena and Solanki (2017), Kumari *et al.,* (2017) and Pawar *et al.,* (2016) also found similar results from their respective experiments.

Influence of seed borne mycoflora in seedling health

The present investigation revealed that fungal species associated with paddy seeds have greater impact on seedling health. An increase in the prevalence of seed borne pathogenic fungi reduces the germination percentage, whereas, increased the seed mortality, percentage of abnormal seedlings and vigour index. Among the various varieties tested, JGL 1798 recorded a minimum germination percentage (68%) whereas BPT 5204 recorded maximum a germination percentage (81%). The percentage of abnormal seedling was high in JGL 1798 (34%) and low in BPT 5204 (21%). The seed mortality was high in JGL 1798 (32%) and low in BPT 5204 (19%).

A maximum root length (14 cm) and shoot length (11 cm) was observed in BPT 5204 and a minimum root (6 cm) and shoot (5.5 cm) length was observed in JGL 1798. A maximum vigour index was observed in BPT 5204 (2025) whereas a minimum vigour index was observed in JGL 1798 (782). JGL 1798 had a highest percentage of seed borne fungi incidence. Hence it has low a germination and seedling health.

Table.1 Details of seed samples collected for experiment

District	Place	Variety	Source
Cuddalore	Annamalai nagar	CO 51	AU Agronomy Farm
	Sivapuri	CO 51	Farmers holding
	Keelamungiladi	CR 1009	Farmers holding
	C Mutlur	CR 1009	Farmers holding
Mayiladuthurai	Vallampadugai	CO 51	Farmers holding
	Kollidam	ADT 43	Farmers holding
	Mayiladuthurai	ADT 43	Farmers holding
Tanjore	Aduthurai	ADT 43	TRRI
Thiruchirapalli	Thiruverumbur	BPT 5204	Farmers holding
	Jeeyapuram	BPT 5204	Farmers holding
Karur	Valayapatti	BPT 5204, JGL 1798	Farmers holding
	Kumaramangalam	BPT 5204, JGL 1798	Farmers holding

Table.2 Percentage of incidence of seed borne mycoflora

	Blotter paper technique						**Agar plate technique**					
Name of the fungi	BPT 5204	CO 51	ADT 43	CR 1009	JGL 1798	Mean	BPT 5204	CO 51	ADT 43	CR 1009	JGL 1798	Mean
Aspergillus sp.	15.0^{a}	10.0^{c}	15.0^{b}	17.0^{b}	20.0^{b}	15.4	10.0^{c}	15.0^{c}	12.0^{c}	13.0^{c}	15.0^{b}	13.0
Alternaria alternata	11.5^{b}	10.0^{c}	11.0^{d}	10.0^{c}	11.0^{d}	10.7	8.0^{d}	7.0^{e}	10.0^{d}	11.0^{d}	10.0^{c}	9.2
Bipolarisoryzae	15.0^{a}	23.0^{a}	20.0^{a}	25.0^{a}	22.0^{a}	21.0	20.0^{a}	25.0^{a}	23.0^{a}	21.0^{a}	25.0^{a}	22.8
Curvularialunata	11.0^{b}	16.0^{b}	12.0^{c}	10.0^{c}	15.0^{c}	12.8	15.0^{b}	17.0^{b}	18.0^{b}	15.0^{b}	16.0^{b}	16.2
Pyricularia grisea	0.0^{e}	1.0^{f}	3.0^{h}	3.0^{e}	2.0^{g}	1.8	1.0^{f}	2.0^{f}	5.0^{f}	3.0^{f}	2.0^{e}	2.6
Fusarium sp.	5.0^{c}	4.0^{e}	5.0^{g}	6.0^{d}	5.0^{f}	5	10.0^{c}	12.0^{d}	10.0^{d}	11.0^{d}	8.0^{d}	10.2
Sarocladiumoryzae	3.0^{d}	5.0^{e}	7.0^{f}	5.0^{d}	7.0^{e}	5.4	5.0^{e}	7.0^{e}	3.0^{g}	5.0e	8.0^{d}	5.6
Ustilaginoidea virens	5.0^{c}	7.0^{d}	8.0^{e}	10.0^{c}	4.0^{f}	6.8	10.0^{c}	7.0^{e}	8.0^{e}	5.0^{e}	11.0^{c}	8.2
Mean	8.19	9.5	10.13	10.75	10.75	9.86	9.88	11.50	11.13	10.50	11.88	10.98

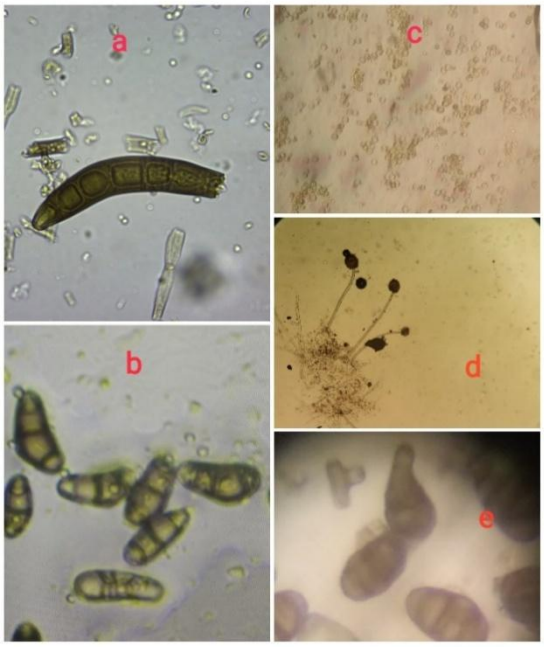
*Values in each column followed by the same letter are not significantly different according to the DMRT method (p=0.05)

Table.3 Influence of seed borne mycoflora in seedling health

Variety	Seed germination (%)	Seed mortality (%)	Abnormal seedlings (%)	Root length (cm)	Shoot length (cm)	Vigour Index
BPT 5204	81[a]	19[e]	21[e]	14[a]	11[a]	2025[a]
CO 51	77.5[ab]	22.5[d]	24[d]	11[b]	9[b]	1550[b]
ADT 43	73[bc]	27[c]	28.0[c]	10.5[b]	8.0[c]	1350.5[c]
CR 1009	70[c]	30[b]	31.5[b]	8.5[c]	6.5[d]	1050[d]
JGL 1798	68[c]	32[a]	34[a]	6[d]	5.5[e]	782[e]

*Values in each column followed by the same letter are not significantly different according to the DMRT method (p=0.05)

Fig.1 Microscopic observations



a. *Bipolaris oryzae* b. *Curvularia lunata* c. *Ustilaginoidea virens* d. *Aspergillus* sp., e. *Alternaria alternata*

The results are agreement with Teja *et al.,* 2018 who reported the seed lot infected by *C. lunata, A. alternate* having a low germination and seedling vigour of rice. Similar such results were observed by Pandey (2015) who reported the reduction of seed quality when they were contaminated with *B. oryzae*, *C. Lunata* and *A. padwickii.* He also reported that the Gurmatia variety which had a highest percentage of seed borne pathogen (22.56%) showed low germination (60.6 %), high mortality (39.4%) and low seedling vigour index (606). The results were also agreement with Deb and Khair (2018).

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