https://doi.org/10.56093/ijas.v90i7.105572

Indian Journal of Agricultural Sciences **90** (7): 1250–3, July 2020/Article

Storage of fungi with rice (*Oryza sativa*)-PRH 10 and their influence on seed quality

DEEPAK KUMAR, K N SINGH, MD SHAMIM, MAHESH KUMAR, MD WASIM SIDDIQUI, DEEPTI SRIVASTAVA, SANJEEV KUMAR, RAKESH KUMAR and PRAVIN KUMAR UPADHYAY*

Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh 224 229, India

Received: 13 November 2018; Accepted: 25 October 2019

ABSTRACT

Presence of fungi causing infestations deteriorates quality of husked and dehisced rice (*Oryza sativa* L.) seed. So, identifying storage conditions is crucial in food safety programs. Present study deals with isolation of fungi and their purification from rice to know the impact on seed storage quality. Four important fungi causing infestations *viz. Aspergillus flavus, A. niger, Penicillium sp*. and *Fusarium sp.* were isolated and characterized. Culture filtrate of fungi were studied on rice seed deterioration on the basis of seed germination percentage, root/shoot length, total carbohydrate, total protein content, α-amylase activity, lipoxygenase activity and storage proteins profiling by SDS-PAGE. Maximum deterioration in rice seed was observed with *A. flavus* spores filtrate inoculated seeds followed by *A. niger, Penicillium* sp. and *Fusarium* sp., respectively. Enzyme activity of α-amylase and lipoxygenase were significantly increased with seed storage and their ageing.

Key words: Aromatic rice hybrid, Seed deterioration, Seed quality, Storage fungi

Rice (*Oryza sativa* L.) is the most important staple food crop for more than half of the world's population (Kumar *et al.* 2019). With increasing world population, hybrid rice varieties may be a breakthrough, which could help to the achieve goal of self-sufficiency of food. Hybrid rice, PRH-10 is very fine grain, excellent cooking quality and most popular among Indian farmers (Singh *et al.* 2013). In India, huge amount of hybrid seeds of rice are being used without taking care of seed health/quality. Diverse climate conditions in India like moisture, temperature, relative humidity and rainfall at harvest stage leads to more prone to invasion by filamentous fungi and bacteria (Gautam *et al.* 2012). Contamination of food grain as rice is important issue for grain quality from consumer's health point of view. Biochemical changes leading to seed deterioration generally take place when seed moisture is favourable for growth of storage fungi. Colonization of storage fungi led to a number of physiochemical changes usually termed ageing, which include loss of seeds viability, pasting properties, colour, flavour and composition. Islam and Ahmed (2017) reported the consistent mixture of spore suspension contains fungal spores of *Fusarium* spp, *Trichoderma*, *Aspergillus flavus*, *Aspergilus niger*, *Helmithosporium*, *Cercospora*, *Alternaria*, *Bipolaris* and *Curvularia* of rice causes lethal symptoms. More recent and compared publications should be added to enrich scientific content. Present investigation was designed to find the important seed storage fungi and their effect on rice hybrid seed quality caused by invasion of different storage fungi.

*Corresponding author e-mail: pravin.ndu@gmail.com

MATERIALS AND METHODS

Sample collection: Fresh hybrid rice var. PRH 10 was collected from Genetics Division, IARI-New Delhi, India. Further seed, were sown in experimental field of Plant Molecular Biology and Genetic Engineering, ANDUA & T, Kumarganj, Ayodhya (2016 and 2017). Fresh mature seed of the rice were collected and stored for comparative analysis of different storage characters.

Seed sampling and surface sterilisation: After harvesting of fresh rice seed were analysed with three storage time period (7, 14 and 21 days, respectively), a representative sub-sample of 2.0 kg rice seeds, was reserved for analysis from top, middle and bottom part of each of two bags by using nobble trier and collected into a clean conical flask and carefully mixed. Two additional working sub-samples of rice (500 g each) from these sub-samples has been used for determination of fungal contamination were obtained and first surface-sterilised using 70% ethanol pre-rinse prior to a 0.8% chlorine treatment for 2 min. (Andrews 1996). Excess disinfectant was drained off from grains, followed by rinsing grains three times with sterilised tap water.

Isolation and identification of fungi: About 10-12 grains were inoculated randomly in Potato dextrose agar (PDA)

medium containing kanamycin (50 ppm) in three replicates. Petri plates were incubated at 28±2°C for 7 days. Observation of fungal growth and its colonies were routinely studied in binocular microscope (Olympus CH 20i, New York, USA) as described by Gilman (2001).

Preparation of fungi culture filtrates: Grown fungal colonies were further multiplied on sterilized yeast extract in sucrose medium (2% yeast extract, 4% sucrose, pH 6.5). Three replicates of each fungus with control (medium without inoculums) were incubated at 28±2°C for 15 days. Culture filtrate obtained after 15 days of incubation was further sterilized by passing them through a bacterial syringe filter (pore size 0.2µm, Cole Parmer, India Pvt Ltd, Mumbai, India) and used as culture.

Effect of fungal culture filtrate on seed quality: Surface sterilized healthy seeds were immersed in culture filtrates of isolated fungi for 4, 8, 12 and 24 hr. Inoculated seeds were air dried and subjected to germination test on moist filter paper. Treated samples were incubated upright in plastic trays at 25±2°C for 8 days, then examined for germination. Root and shoot length were analysed by paper towel method after 14 days (ISTA 1985). Each treatment carried out with three replications.

Effect of fungal culture filtrate on physiochemical parameters: Samples preparation for physiochemical analysis parameters treated with fungi spore filtrates were carried out according to Purushotham *et al.* (1996). Experiment were carried out in three replicates with controls (without inoculum) were incubated at 28±2°C for 21 days incubation period. These fungal culture filtrate treated seeds were used for analysis of carbohydrate, amylose content, protein content, α-amylase and lipoxygenase enzyme activity at different incubation time intervals.

Changes in total carbohydrate content: Seed samples (1.0 g) from each flask was removed at intervals of 7 days and used for total carbohydrate analysis. Samples were homogenized in 5% trichloroacetic acid in a mortar and pestle and centrifuged at 3000 g for 15 min. Supernatant was used for estimation of total carbohydrate content by phenol-sulphuric acid method (Dobois *et al.* 1956) at 495 nm in double beam UV visible spectrophotometer (UV 570 4SS, ECIL).

Changes in total amylose content: Total amylose content was measured by method described by Sadasivam and Manickam (2009) with slight modification. Finally OD was measured at 590 nm with help of double beam UV-spectrophotometer (UV 570 4SS, ECIL, Hyderabad, India). Same procedure was also adopted to create the standard amylose graph.

Changes in total protein content: Total protein were extracted from 1.0 g seed sample as described by Galani *et al.* (2011) and analysed by method of Lowry *et al.* (1951) at 660 nm.

Changes in α- amylase activity: Alpha-amylase activity was quantified by dinitro-salicylic acid method of Bernfield (1955). Developed colour was read at 540 nm in specific UV visible spectrophotometer. The reducing sugar released was calculated as maltose units.

Changes in lipoxygenase activity: Extraction of lipoxygenase from treated seeds was carried out according the protocol of Basak and Johari (2002) with some modifications.

SDS-PAGE profiling of seed storage proteins: Extracted seed proteins from different treatments characterized by SDS-PAGE with some modifications using vertical gel electrophoresis unit. Banding pattern was analysed as described by Das *et al.* (2010). Similarity coefficient = number of same bands (Number of common bands+ number of different bands) was used for calculating similarity between control and treated seeds. Both control and treated were considered similar, if similarity coefficient value was 1.0 and different if its value was 0.

All the data were analysed for analysis of variance with the help of software RAUSTAT 2003 and MINITAB 17.

RESULTS AND DISCUSSION

Effect of fungal culture filtrate on seed quality

Germination: Germination of seeds was affected by culture filtrates of all test's fungi (Table 1). Significant mean

Table 1 Effect of culture filtrate of different stored fungi on seed germination, shoot length of rice seedling and root length on rice seedlings

Fungi	Effect of culture filtrate of different stored fungi on seed germination (%)					Effect of culture filtrate of different stored fungi on seedling shoot length (mm)					Effect of culture filtrate of different stored fungi on seedling root length (mm)				
	Duration of treatment (hr)					Duration of treatment (hr)					Duration of treatment (hr)				
	0 hr	4 hr	8 hr	12 hr	24 hr	0 hr	4 hr	8 hr	12 hr	24 hr	0 hr	4 hr	8 hrs	12 hr	24 hr
Aspergillus flavus	93.333	11.324	11.324	11.324	11.324	11.324	91.00	87.667	82.333	76.00	12.216	12.333	12.896	13.125	13.552
A. niger	93.733	11.455	11.455	11.455	11.455	11.455	93.80	91.000	85.667	79.00	12.893	12.995	13.139	13.226	13.424
Penicillium sp.	94.667	11.651	11.651	11.651	11.651	11.651	94.33	92.000	87.667	81.00	12.975	12.995	13.329	13.417	13.654
Fusarium sp.	96.000	12.124	12.124	12.124	12.124	12.124	95.00	92.667	88.667	85.00	13.122	13.225	13.335	13.455	13.774
Control	96.667	12.285	12.285	12.285	12.285	12.285	96.08	94.333	90.000	87.33	13.345	13.456	13.558	13.700	13.835
LSD (P=0.05)	1.264	0.006	0.006	0.006	0.006	0.006	1.674	1.819	1.243	1.694	0.014	0.010	0.121	0.521	0.012

germination percentage was observed 93.3, 93.7, 94.7 and 96% in *Aspergillus flavus, A. niger, Penicillium* sp. and *Fusarium* sp. treated seeds, respectively after zero hour of treatment, whereas 76, 79, 81, 85% germination were observed after 24 hr of treatment. Germination % continued to decrease with extended treatment period, maximum loss of germination (12.9%) recorded in seed samples treated with culture filtrate of *A. flavus* for 24 hr and minimum in *Fusarium* sp. treated seeds (2.67%). Trend of loss in seed germination was comparatively higher to other cultures filtrates in *A. flavus* at all time duration (4, 8, 12 and 24 hr), respectively. Degree of inhibition in germination with culture filtrates was in sequence of *A. flavus*> *A. niger*> *Penicillium* sp.>*Fusarium* sp. In India, warm humid climate provides congenial atmosphere for the growth of fungi and production of mycotoxins (Saini *et al.* 2013).

Root and shoot length: Mean root and shoot length of 14 days old seedlings were observed as 13.3 and 12.3 mm respectively. Seed samples treated with culture filtrates showed considerable increase in root length per cent and decrease in shoot length per cent with some abnormal growth. Maximum abnormalities in seedlings root and shoot length was recorded in *A. flavus* followed by *A. niger, Penicillium* sp. and *Fusarium* sp. respectively (Table 1). Pathak and Zaidi (2013) reported that fungal infection reduced seed germination; increase in seedlings abnormalities and seed discoloration may be due to the invasion of storage fungi.

Total carbohydrate content: Total carbohydrate content of un-inoculated seeds slightly changed during storage, whereas all fungal culture filtrates treated seeds continuously changed with incubation time (Table 2). Highest net loss in carbohydrate content was recorded (47.9%) in sample inoculated with *A. flavus* after 21 days of incubation and lowest loss (9.3%) was recorded with *Fusarium* sp. Trend of carbohydrate loss in treated seed samples was *A. flavus*>*A. niger* > *Penicillium* sp.>*Fusarium* sp. Infestation fungi drastically reduced carbohydrate content and studies also showed varied in their capacity to degrade carbohydrate content of seed due to utilization of carbohydrate by fungi or due to enzyme activities (Fagbohum *et al.* 2011).

Total amylose content : Total amylose content decreased after inoculation with storage fungi culture filtrates. It was very less or slightly changed in all culture filtrates treated seeds however maximum deterioration was observed in *A. flavus* (3.04 %) treated seeds at 14 days of treatment and at par with *Fusarium* sp. treated seeds (Table 2). Storage fungi like *A. species* (*A. flavus* and *A. niger*) and *Penicillium* sp. produce a large variety of extracellular enzymes and amylases, which is reported to degrade extracellular starch and utilized as carbon source (Saranraj and Stella 2013).

Total protein content: Loss in total protein content in all treated seeds was highest (5.39%) in *A. flavus* treated seeds. Trend of loss was observed same as other quality parameters, viz. amylose and carbohydrate content. Minimum loss was observed in *Penicillium* sp. (1.63%). Oyeleke *et al.* (2010) isolated proteases by *A. flavus* and *A. fumigates* from local rice husk which are responsible for protein degradation.

Alpha-amylase activity: Alpha-amylase activity increased significantly during 21 days of incubation after culture filtrate treatment (Table 2). The maximum alpha amylase activity was recorded after 21 days in *A. flavus* culture treated seeds (1st time fold more, 107%) and lowest in *Fusarium* sp. culture treated seeds (half time fold more, 45.36%). Fungi, *viz. A.* sp and *Penicillium* sp. are known to produce extracellular amylases (Divakara *et al.* 2017).

Lipoxygenase (LOX) activity : Net greatest LOX activity was recorded in *A. flavus* (71.02%) and lowest in *Fusarium* sp. (47.8%) at 21 days of incubation after culture treatments (Table 2). Storage fungi induced enzyme and signalling pathways, which are responsible to mycotoxins production and ageing process. Experiments suggested under storage condition, lipids in rice seeds broken down by lipoxygenase into free fatty acids (FFA).

SDS-PAGE profiling of seed storage proteins: In

Table 2 Effect of culture filtrate of different stored fungi on total carbohydrate, amylose, total protein content, α amylase and lipoxygenase (LOX) activity in treated and untreated rice seeds

Fungi	Changes in total carbohydrate content (mg/g)			Changes in amylose content (%)			Changes in total protein content (mg/g)			Changes in α amylase activity (g/mg maltose in 30 min.)			Changes in lipoxygenase (LOX) activity (unit/mg protein)		
	Duration of treatment (days)			Duration of treatment (days)			Duration of treatment (days)			Duration of treatment (days)			Duration of treatment (days)		
	7	14	21	7	14	21	7	14	21	7	14	21	7	14	21
Aspergillus flavus	0.187	0.176	0.112	24.113	23.885	23.254	79.322	78.155	75.225	23.226	27.121	29.652	38.547	49.653	75.465
A. niger	0.203	0.185	0.172	24.223	24.009	23.684	80.363	79.109	77.433	21.184	25.322	27.585	33.327	46.554	70.256
Penicillium sp.	0.211	0.191	0.182	24.225	24.217	23.782	81.885	80.652	78.215	18.364	23.541	26.257	32.124	44.623	68.624
Fusarium sp.	0.221	0.209	0.195	24.225	24.521	23.886	82.124	81.685	78.015	15.684	18.642	20.824	29.657	41.672	65.226
Control	0.235	0.222	0.215	24.305	24.221	23.981	82.636	80.885	79.518	8.748	10.236	14.325	25.251	35.215	44.125
LSD (*P*=0.05)	0.004	0.004	0.006	0.004	0.049	0.005	0.006	0.941	0.052	0.107	0.006	0.005	1.407	0.012	0.006

electrophoretic study, specific protein band pattern was obtained under a particular fungal culture filtrate treated condition (control or treated). When total protein of treated seeds with different culture filtrates were electro-phoretically analysed on SDS-PAGE, it was found that 40±2 kDa and 66±2 kDa protein bands were disintegrated in low molecular mass polypeptide in *Aspergillus flavus* culture treated seeds, while 68±2 kDa polypeptide band induced and 50±2 kDa polypeptide band down regulated in *A. niger* after 21 days of treatment with culture filtrate. *Penicillium* sp. culture filtrate treated seeds exhibited that 68±2 kDa and 40±2 kDa protein band degenerated after 21 days of treatment whereas in *Fusarium* sp., high molecular weight polypeptide band of 97±2 kDa and 40±2 kDa disintegrated after 21 days of incubation respectively. Similarity coefficient of control and all fungal culture filtrates treated seeds also supported the results. Overall highest deterioration was observed in *A. flavus* which was due to its ability to produce aflatoxins and extracellular alpha amylases, proteases, and responding signalling pathways for ageing. Homology of control and treated seeds with all fungal culture filtrates for protein banding pattern varied from 0.9142 to 0.7359. Maximum similarity between treated and control as observed in *Fusarium* sp. (0.8750) treated seeds and minimum in *A. flavus* (0.7359) after 21 days of treatment. From study that stored fungi commonly infected rice and differ in their ability to deteriorate seeds under conditions which favours fungal growth. Fungal contamination of rice is one of the most valuable features that responsible for most of problems related to quality deterioration in storage.

ACKNOWLEDGEMENT

The first author is greatly thankful to the Director, Shri Ram Solvents Extractions Pvt Ltd for providing financial support to the presented research work.

REFERENCES

Basak S and Johari R P. 2002. Purification and characterization of lipoxygenase from aromatic and non-aromatic rice (*Oryza sativa* L.). *Indian Journal of Biochemistry and Biophysics* **39** (6): 401–405.

Das S, Nayak M, Patra BC, Ramakrishnan B and Krishnan P.2010. Characterization of seeds of selected wild species of rice (*Oryza*) stored under high temperature and humidity condition. *Indian Journal of Biochemistry and Biophysics* **47** (3):178–184.

Divakara M, Aiyaz ST, Chandra M, Nayaka S and Niranjana S R. 2017. Effect of toxigenic *Aspergillus flavus* and aflatoxins on seed quality parameters of *Sorghum bicolor* (L.). *Microbial Biosystems* **2** (1): 1–8.

Dobois M, Gilles K A, Hamilton J K, Rebers P A, and Smith F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28** (3): 350–356.

Fabohun E D and Lowel O U. 2011. Effect of storage on nutrient composition and mycoflora of sun-dried soybean (*Glycine max*). *African Journal of food Science* **5** (8): 473–477.

FAO.2004. Hybrid rice for food security: International year of rice, rice is life.www. rice2004.org.

Galani S, Naz F, Soomro F, Jamil I, Hassan Z U, Azhar A and Ashraf A. 2011. Seed storage protein polymorphism in ten elite rice (*Oryza sativa* L.) genotypes of Sindh. *African Journal of Biotechnology* **10**(7): 1106–1111.

Gilman J C. 2001. A manual of soil fungi, second edition, Biotech Books Publications, Delhi.

International Seed Testing Association (ISTA).1985. International rules for seed testing. *Seed Science and Technology* **13:** 299–573.

Islam W and Ahmed M. 2019. Identification of different fungi associated with grain discoloration complex disease of rice and management of infested grains through fungicides. *International Journal of Scientific Research in Agricultural Sciences* **4**(2): 30–35.

Kumar R, Mishra J S, Kumar S, Hans H, Bhatt B P, Srivastava A K and Singh S. 2019. Production potential, economics and energetic of rice (*Oryza sativa*) genotypes as influenced by varying levels of nitrogen. *Indian Journal of Agricultural Sciences* **89**(11) : 1846–1849.

Leelasuphakul W, Sivanunsakul O and Phonpaichit S. 2006. Purification, Characterization and synergistic activity of beta 1,3-glucanase and antibiotic extract from an antagonistic *Bacillus subtilis* NSRS 89-24 against rice blast and sheath blight. *Enzyme and Microbial Technology* **38:** 990–997.

Lowry O H, Rosenbrough N J, Farr A L and Randal R H. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**(1): 265–275.

Oyeleke S B, Egbin E C and Auta S H. 2010. Screening of *Aspergillus flavus* and *Aspergillus fumigates* strains for extracellular protease enzyme production. *Journal of Microbials and Antimicrobials* **2**(7): 83–87.

Pathak N and Zaidi R K.2013.Fungi associated with wheat seed discolouration and abnormalities *in vitro* study. *Agriculture Sciences* **4**(9): 516–520.

Sadasivam S and Manickam A. 2009. *Biochemical Methods*, 2nd edition. New Age International Pvt Ltd Publishers.

Saini K S M, Reddy S R and Reddy S M. 2013. Incidence of toxigenic fungi on paddy of Godavari Belt Region, A P, India. *Asian Journal of Experimental Biological Science* **4**(3): 426–430.

Saranraj P and Stella D. 2013. Fungal amylase-a review. *International Journal of Microbiological Research* **4**(2): 203–211.

Satheesh L P and Murugan K. 2011. Antimicrobial activity of protease inhibitor from leaves of *Coccinia grandis* (L) voight. *Indian Journal of Experimental Biology* **49**(5): 366–374.

Singh H N, Singh S, Singh U S, Singh J, Zaidi N W and Mohanty S. 2013. Economic evaluation of Pusa Rice Hybrid 10 cultivation: a micro-economic evidence of Uttarakhand, India. *Indian Journal of Traditional Knowledge* **12**(3): 472–477.

Sung J M and Chiu C C. 1995. Lipid peroxidation and peroxide-scavenging enzymes of naturally aged soybean seed. *Plant Science* **110**(1): 45–52.

Tappel A L.1953.The mechanism of the oxidation of unsaturated fatty acid catalyzed by hematin compounds. *Archives of Biochemistry and Biophysics* **44** (2): 378–395.