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Status of Seed Discoloration of Rice, Mycoflora Associated and its Impact on Seed Health and Quality of Farmer Saved Seeds

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ABSTRACT: Seed discoloration of rice due to infection by different fungi leads to problems in seed certification programme. This study was performed on seed samples of 20 cultivars collected from different districts of Punjab, Haryana and Rajasthan. Average disease incidence per cent from all districts was maximum on variety PR 124 (33.37%) followed by Pusa 44 (31.16) and PR 118 (28.18). Highest incidence of discoloration was 64% in PR 124 sample obtained from district Jalandhar followed by variety Pusa 44 from Moga and HKR 47 from Fatehgarh Sahib where the incidence was 41.41 and 40.08 % respectively. For testing germination and vigour of seedlings, the discolored seeds were separated and divided into four different categories based on percent area of discoloration. Seedling test was carried out using in-between blotter paper method. Seed mycoflora associated with discoloration was isolated from discolored grains. Among the field fungi, maximum of *Curvularia lunata* followed by *Alternaria alternata*, *Drechslera oryzae* and *Fusarium* species was recorded. Among the storage fungi, *Aspergillus flavus* dominated over other fungi; *A. niger*, *Penicillum* spp., *Chetomium* spp. and *Rhizopus* spp. Seed germination, root length, shoot length, dry weight of the seedlings and seedling vigour was significantly reduced by increase in percent area of discoloration. Seed germination was below seed certification standard (80%) when intensity of discoloration was >50%. Discolored seeds gave rise to seed rot, produced stunted and blighted seedlings. Therefore, it is suggested to avoid using discolored seeds for sowing purpose.

Keywords: Seed discoloration, Germination, Seedling blight, Seed rot

India has the largest area under rice (44.6 M ha) cultivation in the world. Rice is a staple food crop and provides food security for more than two-third population in India. Seed-borne diseases cause enormous losses to rice crop. The infected seed may fail to germinate, transmit pathogen from seed to seedlings and later seedling to growing plants [1]. Glume discoloration is a term for the alteration in colour of mature seed from its original colour due to infection by different pathogens and epiphytes that leads to obstructions during seed certification programme. Abnormalities are often caused by a number of mycoflora either singly or in association, invading seeds before or after harvest. All these maladies of rice are broadly termed as grain discoloration which appears externally on the glumes or internally in kernels or both. Due to weather changes, it is gaining importance in almost all rice growing areas of world in recent years [2]. Farmer saved seed is commercial grain that is cleaned by farmers for replanting. Saved seed does not require third party inspections to confirm the varietal purity, identity or quality. Saved seed can easily and inexpensively have germination and vigor tests performed to guarantee standard levels. As per the estimates of Directorate of Plant Protection, Quarantine and Storage, Faridabad, nationwide 70% requirement of seed is met from the farmer saved seeds most of which is sown without any treatment [3]. Therefore the study was planned to assess the status of emerging problem of grain discoloration, its impact

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on seed health as well as quality parameters of rice and the most common and frequent pathogens associated with it so as to develop its management strategies.

MATERIALS AND METHODS

Collection of seed samples and recording data on discoloration: Unprocessed farmer saved seed samples of rice were collected from districts of Punjab, Haryana and Rajasthan falling in different agro-climatic zones. Seed samples of twenty one popularly grown cultivars; Pusa 44, PR 108, PR 111, PR 112, PR 114, PR 116, PR 118, PR 121, PR 122, PR 123, PR 124, PR 126, MP 53, 4042, Super 666, Pusa 44, HKR 47, Pusa 1121, Pusa 1509, Basmati 386 and Sharbati were collected from various markets. A detailed questionnaire was used to collect information on seed selection and management practices and farmers' perception of seed quality. The study was performed during the years 2015 and 2016 at the Seed Pathology Laboratory of Punjab Agricultural University Ludhiana after a storage period of 8 months at room temperature in cotton bags. Discolored seeds were counted from a sample size of 2000 seeds separated and divided into five different categories based on the area of discoloration of seed [4]. Percent incidence and severity of discoloration was thus calculated from all the samples of each and every variety.

Detection of mycoflora: One hundred seeds of each category of discoloration were tested by standard blotter method [5]. Circular pieces of filter papers were soaked in sterilized water and then placed at the bottom of 9 cm Petri dishes. Seeds were surface sterilized with 2.5% sodium hypochlorite solution for 2 min. Twenty-five seeds were plated in each plate; four plates were used for each sample. The seeded petri dishes were incubated at 25±2°C under alternating cycles of 12h Near Ultraviolet (NUV) light and darkness for 7 days. After incubation, the seeds were examined under stero-binocular microscope in order to record the associated seed-borne mycoflora.

Isolation and identification of seed associated mycoflora: The mycoflora associated with discolored seeds were isolated by using agar plate method of detection. The seeds were surface sterilized with 2.5% sodium hypochlorite solution and plated on agar medium in Petri dishes aseptically. Hyphal tips from different fungal colonies developed at 25°C were transferred aseptically to the other petri dishes aseptically and pure culture growth of different fungi wasmaintained. The mycoflora was initially identified morphologically on the basis of colony color and shape. Later microscopic examinations were performed to confirm the pathogens on the basis of spore shape, size and mycelial characters as described in the technical bulletin on seed-borne diseases and seed health testing of rice [6, 7]. Pure cultures of the isolated fungi were maintained.

Seedlings test: Seedling test was carried out using between the blotter papers method [5]. Two sheets of square blotter paper (23x26.5 cm) were moistened in distilled water leaving adequate margins. One hundred seeds for each cultivar and discolored seeds were surface sterilized with 2.5% sodium hypochlorite solution for 2 min and placed evenly (25 seeds in 1 replicate) between blotters in four replicates. The sheets were folded along one edge and then rolled up. The rolls were placed up right inside a plastic bag to avoid drying during incubation. The rolled sheets were incubated was at 28°C, at 12h cycle of light and darkness using day light fluorescent tubes as the light source. After 14 days of incubation, the seedlings were evaluated for number of normal seedlings, seed rot and abnormal seedlings which were classified to rotten, stunted and blighted. Seedling length, shoot length and root length, fresh weight and dry weight of the healthy and diseased seedlings was recorded and vigour index was calculated [8].

RESULTS AND DISCUSSION

Status of grain discoloration of rice on popularly cultivated varieties of rice

Average disease incidence per cent from all districts was highest on variety PR 124 followed by Pusa 44 and PR 118. Highest incidence of discoloration was 64% in PR 124 in a sample obtained from district Jalandhar followed by variety Pusa 44 from Moga and HKR 47 from Fatehgarh Sahib where the incidence was 41.41 and 40.08 %, respectively. Severity of discoloration was highest on variety Pusa 44 followed by PR 124, PR 118

and HKR 47. Percent incidence and severity of discoloration was lowest in Basmati 386 (Table 1).

Seed mycoflora associated with different grades of discoloration

The seed samples were divided into 5 categories (0-5) based on seed area affected [4] by discoloration (Table 2). Seed mycoflora of all categories of discoloration was studied individually and it was observed that *Curvularia lunata* was the most dominant among other mycoflora and increase in frequency of each pathogen was noticed with increase in area of discoloration was noticed. Average incidence of *C. lunata* was maximum 22.66%. *Alternaria alternata* was next in abundance (5.4% average incidence), followed by *Drechslera oryzae* and *Fusarium* species. Among the storage fungi, incidence of *Aspergillus flavus* was maximum (27.6%) followed by *A. niger* (19.52%), *Penicillium* sp. (9.02%), *Chaetomium globosum* (5.16%) and *Rhizopus* sp. (2.44%).

Seedling test for seed health and quality

Seed health and quality studies of discolored and healthy seeds revealed that discoloration of rice seeds caused severe reduction in germination and vigour of seedlings. Seed germination was below Indian Minimum Seed Certification Standard (80%) when intensity of discoloration was >50%. Discoloured seeds resulted in seed rot, produced stunted and blighted seedlings. It was observed that number of normal seedlings was reduced with the increase in area of discoloration. On an average only 55.16% seedlings were normal where as 17.22% of seedlings were stunted, 18.05% were blighted and 9.15% seeds were rotten when seedling symptom test was performed under laboratory conditions (Table 3).

The study was conducted to assess the quality of seed produced and used by farmers for their own use. Understanding farmers' seed quality status will enable farmers to devise strategies to improve quality at the farm level. Various species of mycoflora have been isolated from discolored grains by various workers. The fungi that have been reported to be associated with discoloration of grains are; *Bipolaris oryzae, Alternaia alternate, Alternaria padwickii, Pyricularia oryzae, Fusarium moniliforme, F. graminearum, Nigrospora oryzae* and *Curvularia* spp. [2, 9-11]. Grain discoloration caused by fungi such as *B. oryzae, A. padwickii, M. grisea* (*P. oryzae*), *F. moniliforme, F. graminearum, S. oryzea* and *C. oryzae* caused reduction in seed viability and such seeds on planting usually exhibit pre-or post-emergence death of seedlings [8,12]. The present study indicated that *Curvularia lunata, Alternaria, Fusarium and Drechslera oryzae* are the most prevalent field fungi and *A. flavus* is the dominating storage fungi causing seed discoloration

Table 1. Incidence of seed discoloration on popular varieties of rice grown in north western part of India

Variety	Disease Incidence (%)	Severity	Variety	Disease Incidence (%)	Severity
PR 108	2.24	0.99	PR126	13.57	3.18
PR111	2.18	0.26	MP 53	2.09	0.42
PR112	6.99	1.76	4042	2.35	1.37
PR 114	9.41	1.63	Super 666	2.28	0.26
PR116	8.46	3.07	Pusa 44	31.16	7.28
PR118	28.18	6.14	HKR 47	30.65	5.94
PR121	18.35	3.13	Sharbati	2.03	0.37
PR122	27.07	5.35	PUSA 1121	12.55	1.85
PR123	12.27	2.55	PUSA 1509	7.94	1.57
PR124	33.37	6.42	Basmati 386	1.87	0.15

Table 2. Frequency of seed mycoflora associated with different grades of discoloration in rice

Severity of Discoloration	Incidence of Field Fungi (%)				Incidence of storage Fungi (%)				
	Fusarium spp.	*Alternaria alternata*	*Drechslera oryzae*	*Curvularia lunata*	*Aspergillus flavus*	*Aspergillus niger*	*Penicillium* spp.	*Rhizopus* spp.	*Chaetomium globosum*
Nil	0.8	1.8	1.2	15.2	28.2	25.2	18.8	2.8	6.0
25%	3.6	3.9	2.8	18.3	23.8	21.5	13.9	2.2	10
50%	3.9	4.7	4.8	22.5	25.3	23.8	6.7	2.6	5.7
75%	4.6	7.2	5.4	28.1	29.8	15.1	3.5	3.2	3.1
100%	7.1	9.4	6.8	29.2	30.9	12.0	2.2	1.4	1.0
Mean	4.0	5.4	4.2	22.66	27.6	19.52	9.02	2.44	5.16

Table 3. Impact of seed discoloration of rice on seed health and quality parameters of rice

Category of discoloration	Percent discolored area	Normal seedlings (%)	Seed rot (%)	Stunted seedlings (%)	Blighted seedlings (%)	Germination (%)	Shoot length (cm)	Root length (cm)	Dry weight (g)	Vigour index
0	Nil	78.95	5.15	8.90	5.62	92.0	9.4	11.0	0.073	6.72
1	25.0	74.58	6.58	10.25	8.26	87.0	7.4	9.6	0.068	5.92
2	50.0	68.45	8.75	12.45	10.25	68.0	6.1	8.1	0.065	4.42
3	75.0	46.25	10.05	15.25	28.24	62.0	5.3	7.2	0.062	3.84
4	100.0	7.60	15.25	39.25	37.90	41.0	5.2	7.6	0.061	2.50
Mean		55.17	9.16	17.22	18.05	70.0	6.7	8.7	0.066	4.68

of rice. These microorganisms have various effects on the seed health and quality. There was an indication of direct relationship between seed infection and seed rot or seedling rot in case of fungi *D. oryzae* and *Pyricularia oryzae* [13]. When infection was by field fungi, the main effects were; reduced viability, decrease in germination, coleoptiles or radicle decay, seedling blights, chaffy grain formation and ultimately low yield [14, 15]. Germination was below seed certification standard (80%) when intensity of discoloration was >50% in fresh seed and when >25% in stored seed [16]. Stored seed carried more fungal infection (33-55%) than fresh seed (25.5-33%). When infection was by storage fungi like *Aspergillus* and *Penicillium*, besides a reduction in viability and grain quality, there could also be production of toxins [17]. Keeping in view the adverse effects produced by these seed discoloration related fungi, management strategies should be worked out and devised to the farmers.

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