Madras Agric. J., 100 (1-3): 179-182, March 2013
https://doi.org/10.29321/MAJ.10.001267

Assessment of Weight Loss, Seed Germination and Association of Pathogenic Fungi with Discoloured Rice Seed

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The maximum seed weight loss (41.36 %) was recorded in rice cv. Gurjari (category D) followed by cv. Jaya (41.25). Seeds inoculated with pathogens reduced germination. The germination per cent inoculated seed with *S. oryzae* was 52.22 in Jaya and 54.98 in Gurjari. While, in case of seed inoculated with A. *niger*, germination was 77.16 per cent in cv.Jaya and 78.40 per cent in cv.Gurjari. The detected fungi were; *Sarocladium oryzae*, *Fusarium moniliforme*, *Curvularia lunata*, *Alternaria alternata*, and *Aspergillus niger.*

Key words: Seed, Rice, Weight loss, Grain Discolouration (GD).

Seed discolouration is one of the most important impediments and reported from all rice growing areas of the world (Dash and Avdesh Narain, 1988). Association of different fungi is considered to be the major cause for different types of discolouration. It reduces seed value and consumption quality of grains. The occurrence of grain discolouration has increased in South Gujarat in mid and late matured varieties for the last 10 years and has become major problem in rice cultivation as it reduces quality and quantity of rice production, thus causing economical losses to farmers, traders and consumer. Therefore, the present studies were carried out to identify the fungi associated with this and to quantify losses due to discolouration in paddy.

Materials and Methods

Weight loss

The seeds of two varieties of rice *viz*., Jaya and Gurjari were examined critically and grouped into different categories based on the external discoloured (colour stain) symptoms on husk (seed coat) and the abnormal appearance of seed as per categories described below, (Misra and Vir, 1991).

Category	Seed Characteristics
A	Apparently clean bold and healthy seed husk
B	Less than 25 per cent seed husk area discoloured.
C	More than 25 per cent but less than 50 percent husk area discoloured and shrivelled.
D	More than 50 per cent seed husk area discoloured and empty shrivelled.

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After gradation, 1000 seeds in five replicates in each category were weighed and the per cent loss in weight for the respective grades were calculated. The weight of apparently clean bold and healthy seed without any symptoms of discolouration for each variety was considered as standard healthy seed weight (Category A). The seed weight of each discoloured category was recorded and the average weight was calculated. The weight of respective cultivars of healthy seed was used to calculate weight loss in each category of seed discolouration.

Seed Germination

The effect of seed mycoflora on germination was evaluated by employing blotter paper method (ISTA, 1976) for *Saracladium oryzae, Fusarium moniliforme, Currularia lunata, Alternaria alternata,* and *Aspergillus niger*.

The fungi were grown on PDA (Potato Dextrose Agar) medium and spore suspensions were prepared from 10 days old culture, using sterile distilled water. After filtering through muslin cloth, the concentration of spores was adjusted to 1 x 10^5 spores /ml. This spore suspension was used as inoculum to inoculate the surface sterilized seed. Apparently healthy seeds of Jaya and Gurjari (seed lot without natural infection) were soaked in culture filtrate for 24 hours. Seeds soaked in sterile distilled water served as control.

Twenty five seeds of each treatment/pathogen were placed at equidistance (16 seeds at outer periphery, 8 seeds on inner periphery and one seed in the centre) on three layers of white sterile blotting paper. The blotter paper discs were presoaked in sterile distilled water. Each treatment was replicated 16 times.

All the seeded petriplates were maintained for germination at room temperature (27 ± 2°C) for five days in an incubator. Observations of seed germination were recorded five days after incubation.

Associated fungi

In order to understand the association of organisms with grain discolouration (GD), blighted panicles, rotted sheath tillers, abnormaly discoloured grains were collected during crop period and such marked diseased plants were critically observed for occurrence of GD up to harvest. Such diseased samples were periodically subjected to microscopy, isolation and simultaneously natural symptoms were recorded to confirm the association of fungi.

Results and Discussion

Weight Loss

There was significant loss in seed weight of categories B, C and D as compared to category A in both the cultivars (Table 1).

Table 1. Weight loss of discoloured rice seed

Category/ Characteristics of diseased seed	Jaya		Gurjari	
	Mean 1000 seed weight (g)	% wt. loss	Mean 1000 seed weight (g)	% wt. loss
A. Apparently clean, healthy seed husk	31.44	0	32.83	0
B. Seed husk having less than 25% area discoloured	27.31	13.13	29.14	11.23
C. Seed husk having more than 25% but less than 50% area discoloured	24.80	21.11	26.01	20.77
D. Seed husk having more than 50% area discoloured	18.47	41.25	19.25	41.36
S. Em.+	0.47		0.53	
C.D. at 5%	1.40		1.58	
C.V. %	4.09		4.41	

In cv. Jaya, the weight loss of discoloured seed was 13.13% in category B; in category C it was 21.11% and maximum loss 41.25% was recorded in category D as compared to category A (healthy seed).

In cv. Gurjari, the weight loss of discoloured seed weight was 11.23% in category B; in category C loss in seed weight was 20.77% and maximum loss of 41.36% in seed weight was in category D as compared to category A (healthy seed). The physical weight loss is a serious concern to farmers. It also reduced the quality of which millers are more concerned due to more broken kernel.

Negi *et al.* (2002) reported loss in seed weight due to discolouration by fungi. Misra and Vir (1991) reported significant loss in 1000 seed weight of discoloured rice seed in variety IRTP 06811 (31.2%) and in Aswath (50.2%). Whereas, loss in discoloured seed weight was 20 percent in Masuri and 45 percent in IR-8 (Roy, 1983). Periasamy *et al*. (2002) reported maximum weight reduction in rice seed 76-100% area discoloured.

Table 2. Effect of seed inoculation with pathogen on germination of rice seed.

Treatment	Germination percentage	
	Jaya	Gurjari
S. oryzae	46.27*****(52.22) ******	47.86*(54.98) **
F. moniliforme	54.33 (65.96)	53.29 (64.24)
C. lunata	56.21 (69.00)	58.47 (72.64)
A. alternata	59.86 (74.74)	61.03 (76.48)
A. niger	61.52 (77.16)	62.36 (78.40)
Control	90.00 (100.0)	90.00 (100.0)
SEm±	1.42	1.22
C.D. at 5%	4.15	3.56
CV%	4.35	3.67

* Figures are original values
** Figures in parenthesis are arcsine transformed values

Seed Germination

The culture filtrates of all test fungi showed pronounced inhibitory effect on seed germination to both cultivars. The data are presented in Table 2. The culture filtrate of *S. oryzae* had significantly less germination of rice seed, 52.22 per cent in Jaya and 54.98 per cent in Gurjari than control. The culture filtrate of *F. moniliforme* had poor seed germination to the extent of 65.96 per cent and *C. lunata* to an extent of 69.00 per cent in cv. Jaya. In cv. Gurjari, the seed germination in *F. moniliforme* inoculated seed was 64.24 per cent whereas, *C. lunata* inoculated seed, it was 72.64 per cent. The per cent germination in seed inoculation with *A. alternata* was 74.74 and 76.48 per cent in Jaya and Gurjari, respectively. While, in seed inoculated with *A. niger*, germination was 77.16 per cent in Jaya and 78.40 per cent in Gurjari.

In control, cent per cent germination was recorded in both cultivars. Germination was significantly reduced by seed inoculation than control irrespective to pathogen tested in both cultivars.

The similar results of effect on seed germination were reported by Imolehin (1983) and Jayaweera *et al*. (1988) in seed infection with *F. moniliforme*, *A. alternata* and *C. lunata*. Chung (1975), Velazhahan *et al*. (1989) and Reddy *et al*. (2000) reported reduced seed germination in rice seed inoculated with *S. oryzae*. Ranganathaiah (1985) reported reduced

Table 3. Cultural and morphological characters of vegetative and reproductive structures of fungal isolates.

Isolate	Cultural character	Morphological character
Isolate No. 1 *Sarocladium oryzae*	Produced white, pinkish mycelium and covers the entire petriplate (90mm) in seven to ten days (27 + 2°C) of incubation on PDA	Mycelium was white, sparsely branched and septate. Conidiophores were hyaline,smooth, once or twice branched with apical conidiogenous cells in groups of 2-5 monophialidic discrete, elongate and cylindrical. Conidiophores were 55 x 3 µm in diameter. Single individual intercalary phialides observed on conidiophores, which were flask shaped, elongated and narrow towards apex. Conidia hyaline, smooth, thin walled, cylindrical with rounded ends, one celled and measured 5.42-7.98 x 0.8-1.56 µm (Av. 6.56 x 1.18 µm).(Gams and Hawksworth, 2009).
Isolate No. 2 *Fusarium moniliforme*	Produced creamy white to light orange fungal hyphae, later on became pinkish and appeared as powdery growth on PDA.	Mycelial growth consisted of aggregated or loose mycelium with scattered chains of microconidia. Microconidia were hyaline, produced in monilial chain, mostly unicellular but rarely one septate and measuring 4.01-10.23 x 1.63-3.15 µm (Av. 7.12-2.39µm). They were oval to club shaped with flattened base. Macroconidia were produced on macroconidiophore. They were hyaline, 3-7 septate and measured 22.29-43.71 x 2.71-3.65 µm (Av. 33.0 x 3.18µm), slender, falcate to almost straight and tapered toward either ends. They were slightly hooked at the tip, thin walled with the apical cell slightly curved and tapering to both the end point. (Gangopadhyay, 1983).
Isolate No. 3 *Alternaria alternata*	Produced olive brown to dark brown mycelia growth on PDA.	Hyphae dark brown, thick and septate. Conidiophores were simple, erect and often clustered, produced darkly pigmented conidia in an acropetal succession of simple or branched chains. Conidia were septate, ellipsoidal, light to dark brown in color, variable in size and shape with a short cylindrical beak up to one third of length of conidium with 3 to 6 transverse and one or two longitudinal septa. They measured 19.09-36.11 x 9.19-16.31 µm (Av. 27.6 x 12.7 µm). (Bedi and Singh, 1972 and Sreekantiah *et al.*, 1973).
Isolate No. 4 *Curvularia lunata*	Produced dark greenish mycelial growth with irregular margin which later on became grayish black in color on PDA.	Conidiophores were solitary, straight, dark brown. Conidia mostly curved, curvature at the bigger cells, three septate and cells were often hyaline, central cell was bigger and dark brown, apical cell was rounded, smooth walled, without protuberant hilum and measured 17.43-23.97 x 10.03-14.87 µm (Av. 20.7 x 12.45 µm). (Rashid, 2001).
Isolate No. 5 *Aspergillus niger*	Produced hyaline to white to light yellow mycelium grew rapidly and turned into dirty white to black colony on PDA medium.	Hyphae were hyaline and septate. Conidiophores were erect, unbranched, straight, hyaline to light brown, long aseptate and darker near the vesicle. Vesicle was globose, thick walled and brown to black. Conidia produced in chain were globose, single celled, pale to dark brown on maturity. They measured 3-4.5 µm (Av.3.7 µm) in diameter. (Pariasamy *et al.*, 2006).

germination in rice seed infected with *A. alternata*. Periasamy et al (2002) reported that the infection of *F. moniliforme, C. lunata, A. alternata, A. flavus* and *A. niger* caused seedling rot and seedling mortality. Maximum seedling mortality was found in seeds having 76 - 100% discolouration.

Associated fungi

The different isolates obtained from discoloured and diseased rice seed lots were purified by single spore or hyphal tip isolation technique. The identification of each fungus isolated was done by studying the cultural and morphological characters given in Table 3 and by detail microscopic examination of vegetative, reproductive structures and spore of the each isolate and comparing their description in the literature and also confirm with the help of Indian Type Culture Collection (ITCC) at IARI, New Delhi and Agarkar Research Institute, Pune (No. 7818-22) and based on practical experience of scientists and professors in the Department of Plant Pathology, N.M. College of Agriculture and ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari.

Conclusion

The maximum loss in seed weight was in category D which was 41.25 per cent in Jaya, 41.36 per cent in Gurjari. The percentage reduction in weight of seed in category C was 20.77 per cent in Gurjari, 21.11 per cent in Jaya. Whereas, in category B loss in seed weight was 11.23 per cent in Gurjari and 13.13 per cent in Jaya. Germination was drastically reduced by seed inoculation in both the genotypes irrespective of pathogens. The percentage germination after inoculation was minimum in *S. oryzae* i.e. 52.22 (Jaya) and 54.68 (Gurjari) followed by *F. moniliforme* in which germination was 65.96 per cent (Jaya) and 64.24 per cent (Gurjari).The microscopic examination and repeated isolation from discolored seed samples revealed the presence of *Sarocladium* sp., *Fusarium*

sp., *Curvularia* sp., *Alternaria* sp. and *Aspergillus* sp.. After purification of each isolate, the cultural and morphological characters of vegetative and reproductive structures of each isolate were studied. The following fungi were identified; *S. oryzae*, *F. moniliforme*, *C. lunata*, *A. alternata* and *A. niger*.

Acknowledgement

The senior author is highly thankful to Professor, Department of Plant Pathology, N.M. College of Agriculture and ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat for providing the necessary facilities to carry out this research work.

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Received: August 10, 2012; Accepted: February 11, 2013