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Grain discolouration disease of rice : a review

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Grain discolouration caused by several microorganisms, is a minor disease, but it is gaining importance in almost all rice growing areas of the world in recent years, Recent findings on various aspects of the disease viz., occurrence, symptoms and effects of grain discolouration, environments and other factors affecting grain discolouration, mycoflora associated with discoloured grains, varietal resistance, inoculation technique, scoring system, detection of mycoflora in discoloured grains, its role in seed health and seed certification and management are reviewed.

Key words : Rice, grain discolouration, fungi, review

Grain discolouration (Gd) of rice, also known as glume discolouration, dirty panicle, peaky rice or grain spotting, is an early indication of poor seed/grain quality which is generally associated with microorganisms. Such grains are of poor market value and consumption appeal in addition to their abnormal performance as seed of source of nutrient value. Among the microorganisms involved with grains discolouration in rice, fungi predominate ; however, several bacterial species have also been reported. Besides the involvement of microorganisms some non-pathological conditions may also cause similar effects leading to abnormal colour changes in rice grains. Some of these factors are abnormal fertilization/anthesis, unfavourable weather conditions viz., temperature and / or moisture stress, and imbalanced soil nutrients. Besides these, premature harvested seeds in heaps, injury induced by strong winds or cyclone due to collision of panicles, attack by insect pests during grain formation.

Comprehsnsive reviews (Ou, 1985; Narain, 1992; Lee, 1992) have highlighted the importance of grain discolouration. In the present review, an attempt has been made to give an appraisal of recent advances of work done so far in India and abroad particularly on fungal organisms. An intensive study is an urgent need to reinvestigate the various aspects of the disease as it might become a possible future threat and constraint to rice production.

Occurrence

Grain discolouration disease occurs in most rice production regions of the world like Sierra Leone (Raymundo and Fomba, 1979), Nigeria (Ayotada and Salako, 1980 ; Navasero and Winslow, 1987), India (Upadhyay, 1985), Thailand (Arunyanart *et al.*, 1981), Colombia (Zeigler *et al.*, 1987), Venezuela (Rodriguez *et al.*, 1988), Indonesia (Zulfiki *et al.*, 1991, Castano *et al.*, 1991), Cameroon (Jones *et al.*, 1991), and Taiwan (Fang *et al.*, 1996).

In India, several workers reported this disease from different parts, namely, Haryana (Ahuja *et al.*, 1980 ; Singh and Chand, 1985), Assam (Roy, 1983), Bihar (Jha and Prasad, 1984), Karnataka (Pandurangegowda *et al.*, 1983 ; Ranganathaiah, 1985), Tamil Nadu (Subramanian *et al.*, 1986), Manipur (Singh, 1987), Madhya Pradesh (Murty *et al.*, 1987), Punjab (Sharma *et al.*, 1987 ; 1992), Orissa (Das and Naraian, 1988), and Himachal Pradesh (Vaid *et al.*, 1994).

Symptoms and effects of grain discolouration

The fungi associated externally or internally with rice seeds responsible for discolouration, have been reported by many workers (Raymundo and Fomba, 1979 ; Navasero and Winslow, 1987 ; Arunyanart *et al.*, 1981 ; Rodriguez *et al.*, 1988 ; Castano *et al.*, 1991 ; Zulfiki *et al.*, 1991 ; Jones *et al.*, 1991 ;

Ahuja, 1980 ; Singh and Chand, 1985; Roy, 1983 ; Jha and Prasad, 1984; Ranganathaiah, 1985; Singh, 1987; Murty *et al.,* 1987; Sharma *et al.,* 1987, 1992; Das and Narain, 1988; Padwick, 1950; Bora and Gogoi, 1992; Mittal and Sharma, 1978; Gonzalez and Hernandiz, 1982; Imolehin, 1983; Ngala, 1983; Soave *et al.,* 1983; Koroleva *et al.,* 1984; Mia *et al.,* 1979, 1985 ; Misra and Dharma Vir, 1988; Jin, 1989 ; Duraiswamy and Mariappan, 1983; Malavolta and Takada, 1997; Sharma and Vaid, 1997). Symptoms may be of one spot of any colour, lesions to bleached areas over glumes, or combinations of many discernible patches, lesions, or spots. The colour ranges from ash grey, light brown, light pink, dark brown, dark purple, purplish brown to black spots.

Discolouration reduced the germination of rice seeds. The loss in germinability was proportional to the severtiy of discolouration (Sharma *et al.,* 1987). The infected seeds weighed lesser than healthy seeds. The infected husk also weighed less (Murty *et al.,* 1987). In Nigeria, the discoloured grains had lower dry weights for rough and brown rice than healthy grain accentuated discolouration (Navasero and Winslow, 1987). Zulfiki *et al.,* (1991) categorised discoloured grains in five severity groups, ranging from 1-100%. Results showed, higher the disease severity, lower the germination and seedling height. The disease reduced germination as much as 40% and reduced seedling height by 3-20%. The higher the severtiy, the more the empty grains/panicle, with parallel weight reduction. Jones *et al.* (1991) recorded direct yield losses in discoloured glumes because infected panicles failed to fully exsert. Seeds collected from mature panicles were dehusked and the discoloured grains were classified as green, purple or brown by Duraiswamy and Mariappan (1983). Singh and Chand (1985) found that infected grains are covered with brown to dark brown lesions. Sachan and Agarwal (1994a) reported that fungi associated with all the eight types of seed discolourations ash grey, eye shape spot, light brown, light pink black, dark purple, dark brown and light to dark brown dot line spots. In rice resulted in loss of viability, germination and seedling vigour, in each case maximum loss in seed having discoloration on both embryo plus endosperm regions as compared to lower categories of discolourations.

Environmental and other factors affecting grain discolouration

Stage of crop maturity coupled with suitable environmental and other agronomic conditions are generally most important for the attack of most fungi which have been widely reported from different parts of the world to cause grain discolouration.

Ayotada and Salako (1980) reported that rainted wet land conditions, unbalanced NPK supply and timing or application of straw ash, Mg or Si with NPK affected grain discolouration of rice cultivars in Nigeria.

Grain discolouration was reduced when larger spacing was provided in the field. Higher discolouration was also recorded when older seedlings were transplanted as compared to the younger seedlings. In transplanted crop discolouration was more as compared to direct sown crop. Grain discolouration increased with the increasing level of nitrogen and phosphorus (Misra and Dharam Vir, 1992). Duraiswamy and Mariappan (1983) observed that rice panicles harvested from October to December had more discoloured grains. Low temperature, high humidity, high rainfall and more rainy days which prevailed during the three months appeared to cause higher percentage of grain discolouration. Acid soils of upland rice in Indonesia caused severe problem of grain discolouration (Castano *et al.,* 1991). Also, lodging of the rice plants, due to strong wind or any other means, which provide a wet or humid environment, increases grain discolouration (Lee, 1992; Misra *et al.,* 1994).

Of the four stages (boot, heading, milk and dough), the heading is the most susceptible stage to grain discolouration (Sharma and Vaid, 1997).

Mycoflora associated with discoloured grains

The major fungi associated with discoloured rice grains could be field fungi which are parasitic and infect the grains before harvest. These are generally : *Alternaria, alternata, A. longissima, Cercospora oryzae, Chaetomium* sp., *Cladosporium cladosporioides, Curvularia lunata, C. geniculata, C. pallescens, C. veruciformis,* *Epicoccum purpurasce, Fusarium moniliforme, F. equiseti, F. graminea-rum, F. pallidoroseum. F. semitectum, Helminthos-porium oryzae. H. rostrata, H. teramera, Nigros-pora oryzae, Pestalotia oryzae, Phoma sorghina, Pyricularia oryzae, Sarocladium oryzae, Sclerotium oryzae, Trichoconis padwickii, Trichothecium roseum* etc. Some fungi could also develop as saprophytes on harvested grains under storage when conditions are abnormal. These fungi are : *Aspergillus niger, A. parasitica, A flavus and other species, Penicillium* spp., *Mucor sp., Verticillium inter-textum* etc. (Narain, 1992 ; Arunyanart *et al.*, 1981; Castano *et al.*, 1991; Ahuja *et al.*, 1980; Roy 1983; Jha and Prasad 1984; Singh 1987; Murty *et al.*, 1987; Sharma *et al.*, 1987; Bora and Gogoi 1992; Koroleva *et al.*, 1984; Misra and Dharma Vir, 1988; Jin, 1989, Sharma and Vaid, 1997; Misra *et al.*, 1994; Duraiswamy, 1982; Saponaro *et al.*, 1986; Ray, 1993; Sachan and Agarwal 1994a, b, 1995).

The prevalence of these fungi varies greatly. Study of stored grain samples in Assam, India (Roy, 1983) showed that 22 fungi were associated with about 93% of spotted grains. *C. lunata* was the most common fungus associated with 37% of discoloured grains and *Fusarium* and *Chaetomium* spp. (6%). Misra and Dharam Vir (1988), also, found *C. lunata* as the most predominant fungus out of 28 fungi detected in 97 samples of discoloured rice seed from four agroclimatically different areas of India. Castano *et al.* (1991) identified 20 fungal genera from discoloured seeds of susceptible upland cultivar Tondano and panicles of local upland cultivar Arias and Simariti in West Sumatra, Indonesia. *H. oryzae* was the most common fungus isolated in agar plate test.

Location of inoculum and its influence on nutritional value in discoloured grains

Majority of fungi, namely *Alternaria alternata, Curvularia lunata, Fusarium moniliforme, Helminthosporium oryzae, Nigrospora oryzae, Sarocladium oryzae and Trichoconies padwickii* responsible for causing seed discolouration are reported to be seed borne in nature (Mohan and Subramanium, 1979). The fungi associated with discoloured seeds may result in deterioration of nutritional value of seed due to physical, physiological and biochemical changes in the seeds (Narain, 1992 ; Duraiswamy and Mariappan, 1983). The location of seed borne fungi associated with seed discolouration on nutritional value of seed have been studied by several workers.

The seedborne inoculum of *A. alternata* and *H. oryzae* were found in seed coat and endosperm of coloured seeds. The fungi namely *Curvularia geniculata, C. lunata, Fusarium equiseti, F. graminearum, F. moniliforme* and *S. oryzae* resulted in decrease in starch content as compared to healthy seeds. The reduction in strach content was higher in the seeds having discolouration on both embryo plus endosperm region as compared to lower categories of discolouration (Duraiswamy and Mariappan, 1983; Sachan and Agarwal, 1995; Mohan and Subramanium, 1979; Vidhyasekaran and Ramadoss, 1973). In seeds with *H. oryzae*, a higher amount of protein was detected as compared to healthy seeds (Duraiswamy and Mariappan, 1983; Sachan and Agarwal, 1995; Vidhyasekaran *et al.*, 1984) but in seeds with *A. alternata* and *S. oryzae*, a decrease in protein content was noted (Agarwal *et al.*, 1989; Sachan and Agarwal, 1995; Mohan and Subramanium, 1979, Vidhyasekaran *et al.*, 1984).

Biochemical analysis of discoloured rice grains (brown, purple due to *H. oryzae, T. padwickii* and *C. lunata*, green due to *H. oryzae* and healthy white grains by Duraiswamy and Mariappan (1983) revealed that brown grains had higher levels of phenol, reducing and non-reducing sugars, amylose and gel consistency and lower level of gelatinization than white grains ; purple grains also had high levels of phenol, reducing and non-reducing sugars and gel consistency, amylose and gelatinization which were, however, less than healthy grains ; results of green grains were similar to those of brown grains, but brown grains contained more amylose and had higher gel consistency than other discoloured grains. Discoloured grains contained glycine in addition to eight other amino acids present in wihte grains. Navasero and Winslow (1987), also, reported that amylose content slightly decreased in discoloured grains.

Varietal resistance

As far as varietal resistance is concerned many workers have screened rice cultivars for grain discolouration disease. Imporved Sona, PAU 1-608A, Sabarmati, R 36-2486, Pusa 2-21, Prasad rated as low score ($\leq$ 5) whereas Basmati 370, IR 8, Jaya, Ratna, PR 106 were susceptible to the disease in Haryana, India (Ahuja *et al.,* 1980). Kannagi is the susceptible cultivar in Tamil Nadu, India (Duraiswamy, 1982) whereas Jaya, IR 20, Rasi are resistant in Karnataka, India (Pandurangegowda *et al.,* 1983). Grain discolouration is increasingly destructive and attacks almost all high yielding cultivars like Phalguna, Surekha, Bangoli, PR 106, IR 8, Jaya, Co 40, Kranti, Ratna and Mahsuri in different states of India (Upadhyay, 1985). Thirty entries of International Rice Yield Nursery (IRYN) tested in 1984 *thaladi* (Sept.-Oct. to Jan.-Feb.) rice in Tamil Nadu, India, only two viz., IR 25604-99-1-3-2-2 and IR 27316-96-3-2-2 scored 1 (Subramanian *et al.,* 1986). Out of 18 stabilized lines and 18 cultivars of rice evaluated for grain discolouration at Karnataka, India during 1986, wet season, Mahsuri, Intan Gouri, and Prakash were resistant while Jaya, IR 20 and Jaya/Mahsuri derivatives were susceptible (Shashidhar *et al.,* 1988). IET 9790 (RAU 4045-2A; Fine Gora / IET 2832), a semi-dwarf, very short duration (80-85d) cultivar has resistance against grain discolouration in upland environment (Prasad and Tomar, 1989). CTH 1, 3, 4, IET 10131, 10626, 11220 and 11221 are promising for grain dicolouration in Karnataka, India (Saifulla *et al.,* 1996).

Ayotada and Salako (1980) found IR 8 and FARO-15 are least infected (resistant) while MAS 2401 and ITA 307 are susceptible to grain discolouration in Nigeria (Navasero and Windslow 1987). IAC 899, and lowland rice cultivar of Goias, Brazil is susceptible to grain discolouration (Prabhu and Santos, 1988).

Out of 53 rice hybrids screened at Assam, India during the *sali* (June / July-Nov./Dec.) seasons of 1996 and 1997, only five (PBCMS 2A x IR 50, PBCMS 2A x Ranjit, PBCMS 3A x Madhav, PBCMS 3A x Pankaj, and PBCMS 3A x Remi) were reacted as moderately resistant, none was resistant to the disease (Singha *et al.,* 2000).

Inoculation Technique

The panicles of the test cultivar are artificially inoculated with fungi by spraying of spore suspension (4 x 10^4 spores ml^{-1}) of each grain discolouring fungus at flowering stage in morning hours with an atomizer. After inoculation, the panicles are coverd with polythene bags for 48 h for inoculation. Observation are recorded after 15 days of inoculation (Duraiswamy and Mariappan, 1983; Ray, 1993).

Scoring system

The Standard Evaluation System (SES) designed by IRRI (1996) for grain discolouration disease is based on percentage of grains with severely discoloured glumes where 0 = highly resistant (HR) (no incidence); 1 = resistant (R) (less than 1%); 3 = moderately resistant (MR) (6-25%) ; 7 = susceptible (S) (26-50%), and 9 = highly susceptible (HS) (51-100%). Artificilly inoculated or naturally infected cultivars may be scored according to this scale. Severity of grain discolouration can be estimated by counting grains with more than 25% of glume surface affected.

Detection of mycoflora in discoloured grains, its role in seed health and seed certification

Blotter and/or agar plate methods detect most of the fungi. Some slow growing fungi are better seen on a blotter. Pathogenicity test are performed for confirmation of the fungus (Bora and Gogoi, 1992; Misra *et al.,* 1994) in seed health studies.

Discoloured seeds pose a serious problem in seed certification. Discoloured seeds, which may otherwise be viable with recommended germinability as per certification standards may not be acceptable as seed because of poor physical look and high expected incidence of seed borne fungi (Neergard, 1979).

Management of grain discolouration

Several workers have made successful attempts to manage grain discolouration by application of different chemical as seed treatment to increase germinability and spraying at different stages of flowering to combat the disease.

Seed treatment by fungicides like difolatan, benlate and bavistin alone or in combination with thiram, captan, meneb, brassicol (Rush and Gifford, 1972; Webster *et al.,* 1973), various organomercurials (Tandon and Dwivedi, 1977), captan, thiram, dithane M-45, dithane Z-78 and brassicol (Dharam Vir, 1976; Bedi and Sharma, 1975; Pawer *et al.,* 1985), thiram, emisan and derosal (Sharma *et al.,* 1987), bavistin and mancozeb (Laxman and Mohan, 1988), thiram (Pasha *et al.,* 1991), thiram and carbendazim combination (Sharma *et al.,* 1992), hexathirr in combination with derosal and bavistin, followed by hexathir, bavistin and emisan alone (Sharma *et al.,* 1993), bavistin, folpan and bayleton (Vaid *et al.,* 1994), bavistin and dithane M-45 combination or bavistin and thiram combination (Sachan and Agarwal 1994b), mancozeb and tricyclazole (Saifulla *et al.,* 1996), increased germinability and seedling vigour of discoloured seeds of rice and reduce seed borne inoculum. Dip seed treatment has been observed to be superior to that of dry seed dressing (Sharma *et al.,* 1993). Macedo *et al.,* (1996) found that herbicides like butachlor, propanil, oxadizon and lactofen at 0, 12.5, 25, 50 and 100 μg/ml decreased percentage occurrence of fungi on rice seeds, but to a different extent.

Spraying of fungicides like polyoxin Z, sisthane and edifenphos (Arunyanart *et al.,* 1981), guazatin, IBP and mancozeb (Duraiswamy, 1982), edifenphos or copper oxychloride (Govindarajan and Kannaiyan, 1982), edifenphos and mancozeb (Singh and Chand, 1985), iprodione (Rodriguez *et al.,* 1988), carbendazim and IBP (Ray, 1993b), benomyl (Misra *et al.,* 1994), bavistin or tilt (Vaid *et al.,* 1994), prochloraz (Malavolta and Takoda, 1997) were found to be very effective. Two or three sprays at 10 day interval starting at 50% panicle exsertion stage gave good control of the discase. Application of indofil M-45 (0.25%) at the boot leaf stage followed by spray with common salt (10%) 20 days after 50% flowering was found effective in controlling grain discolouration (Deka *et al.,* 1996).

Proper insect pest control and cultural practices that prevent lodging will also help to minimize grain discolouration. In addition, it is crucial that grain be harvested at proper moisture content. Post harvest operations to keep seeds clean, dry (13-14% moisture) to prevent invasion of microorganisms and free from rodents and insect pests during storage can help to prevent grain discolouration and consequent damage to seeds (Lee, 1992; Misra *et al.,* (1994).

Future lines of work

Most of the fungi that can infect rice grains in the field and cause discolouration are pathogens of other plant parts as well. Detail study of those fungi including control measures have been studied in individual disease. But very little work has been done in hybrid rice and its components. Future research work is needed on this line.

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