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Review Paper

GRAIN DISCOLORATION: AN EMERGING THREAT TO RICE CROP IN PAKISTAN

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ABSTRACT

In Pakistan, on an average 6 million tons of rice is produced each year that is about 30% of the world's paddy rice. Rice grain discoloration disease (a bacterial/fungal disease) is emerging as a major threat in Pakistan that deteriorates grain quality and texture. With abrupt changes in climatic conditions in the country, the disease severity may be minor to major across different ecological zones. Grain discoloration affects the grain morphology (size and shape of the grain) and ultimately significantly lower yield of the crop. Grain discoloration also affects the drying, shelling, milling and processing of the rice due to weight loss. In coming years huge loss is expected in Pakistan due to this disease. With the passage of time the disease is also spreading to the major rice growing countries and resulting in huge loss in yield. The complexity of the disease is very serious threat to the rice worldwide. To cope with this alarming disease we have to devise the strategies to better utilization of genetic resources through advanced molecular breeding approaches. In addition to breeding, precise identification of pathogen and improvement in agronomic practices would also help deal with the problem.

Keywords: Grain discoloration, rice, crop disease, breeding and genetics.

INTRODUCTION

Rice is not only a major food crop in Pakistan but also an important export commodity. In Pakistan, rice crop is subjected to various diseases which affect its quality as well as reduces the yield. In the recent years, a new yield reducing disease ‘rice grain discoloration’ is emerging as a potent threat to rice crops. Thus far, neither effective control measures nor rice varieties showing complete resistance to the disease are currently available. In past, various plant pathogens with high optimal temperatures have emerged or become prevalent worldwide for spreading various diseases (Schaad, 2008). In United States panicle blighting has been sporadic problem in the major rice production area for many years. Ear blight, grain discoloration and other similar diseases have been attributed to fungal causal agents (Atkins, 1974; Ou, 1985; Lee, 1992 a, b). It is usually due to discoloration of grain, whole panicle, distinct lesions, panicle blight, brown/black spots on grain, discoloration of florets and a number of viral/bacterial/fungal agents that are responsible for developing the disease (LSU Agricultural Center, 1987; Groth *et al*., 1991; Rush, 1998; Shahjahan, 1998; LSU Agricultural Center, 1999; Shahjahan, 2000 a, b). Rush and Shahjahan reported that *Burkholderia glumae* (formerly *Pseudomonas glumae*) was the main causal agent in 60 % area of Louisiana field (Rush, 1998; Shahjahan, 2000b). Bacterial sheath rot and grain blighting was first reported by Klement (1955) in Hungary and caused by *Pseudomonas oryzicola.* On the other hand, grain rot, seedling blight, seedling rot caused by bacterial pathogens including *Pseudomonas glumae* (*Burkholderia glumae*), but only *P. glumae* caused seedling blight on inoculated plants (Tanii *et al*., 1974; Uematsu, *et al*., 1976; Goto *et al*., 1987). In this sense, the grain discoloration may be considered as a potential threat to the rice producing countries and number of reports from various parts of the rice world about this disease strongly supports this concept.

Rice grain discoloration was reported as independent disease in the literature causing significant yield losses (Mew *et al*., 2004; Arshad *et al*., 2009; Prabhu *et al*., 2012; Ashfaq *et al*., 2013; Chandramani and Awadhiya 2014). Since 1980’s, the disease has been reported from different countries in the world, including Latin America (Zeigler and Alvarez, 1987; 1989 a,b, c,1990), Vietnam (Trung *et al*., 1993), Korea (Jeong *et al*., 2003), Taiwan (Chien and Chang, 1987), Philippines (Cottyn *et al*., 1996 a,b) and the Gulf of Mexico rice production area in the U.S. (Rush *et al*., 1998; Shahjahan *et al*., 1998; 2000 a, b). Rice grain discoloration is becoming a serious problem in Pakistan as well as in other parts of Asia for reduction in rice yield (Phat *et al*., 2005; Arshad *et al*., 2009). The threat is increasing year after year by decreasing the yield potential of rice crop up to 6% (Savary *et al*., 2000). Rice yield losses rate is increasing with emerging threat of rice diseases in Asia and all over the World (Table 1). Very high yield losses caused by bakanae disease of rice ranging from 3-9.5 %. On the other hand, rice blast majorly affects the rice yield in Pakistan and other parts of the World and its losses range from 11.9 to 37.8 % (Charles *et al*., 2015; Gupta *et al*., 2015; Duku *et al*., 2016; Mizobuchi *et al*., 2016). Rice grain discoloration is a major threat with respect to the future concerns. To cope with the disease survey of rice fields should be conducted to assess disease prevalence, incidences and collection of diseased samples. After the confirmation of pathogen through isolations and pathogenicity test, molecular studies should be done to determine the nature of pathogen and its role in causing the disease. Genetic resistance against the disease should be identified in Pakistani rice germplasm and utilized for breeding rice cultivars.

Causes of grain discoloration: Grain discoloration caused by the involvement of many biotic and abiotic factors including microorganisms attack (fungal, bacterial and viral), high humidity, high moisture, panicle emergence stage, grain filling stage, high temperature, high wind pressure during pollination, weak plant defense system, nutrient deficiency, less plant population, immature grain filling, lack of proper pollination/fertilization, chemicals/fungicides, rainfall at maturity stage and grain lesion. In some cases rusty, water-soaked lesions appear on the lemma or palea, brown immature lighter grains panicle, glumes discoloration, kernel discoloration, grain rot, grain discoloration by insect pest and diseases (Yan *et al*., 2010; Ashfaq *et al*., 2013).

Symptoms of grain discoloration: The symptoms of rice discoloration are brown or black spots on grain, hollow light weight panicle, blackish brown stripes on grain and infected panicle with unfilled grains. Grain discoloration affects the grain morphology in term of grain size and shape (Figure 1).

Overview of the researches conducted to address rice grain discoloration: Different types of pathogens (bacterial, fungal, viral and other biotic/abiotic factors) are responsible for causing the several diseases of rice crop and ultimately resulting in grain discoloration of rice in many tropical countries of the world i.e. grain rot, sheath rot complex, panicle blight, grain discoloration, seedling rot and other emerging diseases reported by many scientists (Uematsu *et al*., 1976; Kadota and Ohuchi, 1983; Zeigler and Alvarez 1987, 1989a; Cottyn *et al*., 1996 a, b; Shahjahan *et al*., 1998; Cottyn 2001). Various pathogen species has a drastic effect on rice crop in relation with changing environment. *Burkholderia glumae* at rice plant growth stages is responsible for causing of grain rot, sheath rot, seedling rot, discoloration and decreasing yield as described in Japan (Goto and Ohata, 1956; Kurita and Tabei 1967; Uematsu *et al*., 1976; Goto *et al*., 1987). In East Asian countries it has also been reported as a rice pathogen (Chien and Chang, 1987; Cottyn *et al*., 1996a, b; Jeong *et al*., 2003; Luo *et al*., 2007) and in Latin America.

Rice Grain discoloration is becoming a serious threat to rice crop in Pakistan (Phat *et al*., 2005; Arshad *et al*., 2009). Rice grain discoloration affects the qualitative and quantitative traits (Sumangata *et al*., 2009, Tariq *et al*., 2012) that ultimately result in yield penalty. Rice yield also affected by many biotic factors i.e. infected brown spot grain disease, insects and other predominant diseases (Hajano *et al*., 2012; Jabeen *et al*., 2012; Tariq *et al*., 2012) and losses due to brown spot infected grains have been recorded in the range of 16% to 43% (Datnoff *et al*., 1997). These diseases are also considered to affect the grain quality, breaking of rice grains during milling, weight loss, exports, post-harvest losses, crop yield and ultimately badly affect the economy of Pakistan (Ghazanfar *et al*., 2013). The pathogens associated with discolored rice grain disease have also been reported by many researchers (Khan *et al*., 2000; Javid *et al*., 2002). Rice yield reduction is caused by many rice diseases worldwide estimated about 14-18% (Mew and Gonzales, 2002; Mew *et al*., 2004) and some areas resulting heavy yield losses ranging from 50 to 90% (Agrios, 2005). For example, in Tamil Nadu yield losses were up to 39% (Shanmugam *et al*., 2006). Rice grain discoloration is also a major limiting factor for rice yield (Rajappan *et al*., 2001). Rice molecular markers play a very important role for screening, selection and identifying the new resistant rice lines against diseases and other biotic and abiotic stresses (Choudhary *et al*., 2013; Pinta *et al*., 2013). Molecular markers used as a helping tool for new genes identification and selection of resistant rice lines along with conventional breeding techniques and ultimately leads to the development of new resistant genetic material (Yu *et al*., 2008; Mizobuchi *et al*., 2013). Molecular markers were also found associated with the screening and identification of plant pathogens (Mannan and Hameed, 2013). In addition, the *B. glumae* major source of inoculums to emerging panicles because its cells present on flag leaf sheaths and their infection primarily occurs at the heading stage/booting stage (Tsuchima and Naito, 1991; Tsushima, 1996; Yuan 2005). Phat *et al*., (2005) reported that rice yield loss due to pests and diseases has been noticed more and more seriously. Grain discoloration is considered as one of popular problems in Mekong Delta.

Rice discoloration break out due to conditions of high temperatures at night and high rains (Tsushima *et al*., 1985; Zeigler and Alvarez, 1990; Mew, 1992;), disease spikelet production by inoculation during pollination gives the highest rates of floret infection (Shahjahan *et al*., 1998a), high humidity during panicle emergence and causes the yield losses of the crop (Tsushima *et al*., 1995; Shahjahan 2000b). Rice pathogens (fungi and bacteria) associated with discolored grains affect germination ability, seed health; seed quality, seed morphology and yield potential of the crop (Ou, 1985; Misra *et al.*, 1990). Bacteria are found associated with 28-32% of discolored seed (Baldacci and Corbetta, 1964; Misra *et al.*, 1990). During the growing season rice pathogens exist on the phylloplane of rice plants, stored seeds at room temperature in winter, weeds in the field, previous rice crop tissues buried in the soil, improper cultivation of soil and climate change (Sogou and Tsuzaki, 1983; Matsuda and Sato 1987; Tsushima *et al.*, 1987; Otofuji *et al.*, 1988; Tsushima *et al.*, 1989; Hikichi 1993 a,b; Tsuchima *et al.*, 1996). An effective control measure for plant diseases is to eradicate sources of contamination that are associated with pathogen, suggesting that low temperature treatment may kill the pathogen. Grain discoloration badly affects the crop every year and its effect is very high in all major rice growing areas of Pakistan (Table 2).

Table 1. Involvement of pathogen causing rice grain discoloration disease

S. No	Rice pathogens/ Other effects	% of pathogens involvement in grain discoloration	Yield Losses	References
1	Bacteria	18-65%	Severe	Cottyn *et al*., (1996a); Saberi *et al*., (2013).
2	Fungi	1-80%	Severe	Sharma *et al*., (1997); Mew *et al*., (2004).
3	Virus	25-50%	Moderately severe	Jennings, (1963); Lamey and Everett, (1967); Vargas, (1985).
4	Insect pest	2-12%	Low	Lee *et al*., (1986); Salim *et al*., (2001); Mew *et al*., (2004).
5	Environmental effects	Less than 10 %	Low	Ou (1985); Lee *et al*., (1986); Mew *et al*., (2004).

Table 2. Basmati and non basmati rice growing area in Pakistan and grain discoloration hot spot location.

S. No	Province	Sowing Time	Variety Name	Districts	Disease incidence rate/hot spot location
1	Punjab	May 20- June 30	Basmati 370, Basmati Pak, Basmati 385, Super Basmati, Basmati 2000, Shaheen Basmati, Basmati 515, KS-282, KSK-133, NIAB IR-9, Basmati 198, Super Basmati, KS-282, KSK-133, and rice hybrids	Lahore, Gujranwala, Sheikhupura, Sialkot, Narowal, Hafizabad, Gujrat, Sahiwal, Mandi Bahauddin, Nankana Sahib, Jahng, Chiniot	High/Booting stage/High humidity/central Punjab, rice belt
2	Sindh	April 25- June 30	IR-6, DR-82, DR-83, DR-92, Sada Hayat, Sarshar, Shahkar and rice hybrids IR-6, Shadab, Shua-92, Khushboo-95 and hybrids	Larkana, Dadu, Shikarpur, Qambar-Shahdadkot, Jacobabad, Kashomore Thatta, Badin, and Tando Muhammad Khan	High/Maturity stage/Humidity/Grain ripening stage/upper Sindh
3	KPK	May1- May 20	IR-6, DR-83, Sarshar, Sada Hayat, Shahkar and rice hybrids	Swat, Dera Ismail Khan, Malakand, Batgram, Kohistan, Mansehra, Mingora, Barikot, Kabal, Matta and Khwazakhela	High/High humidity/Panicle emergence stage/common in swat area
4	Balochistan	May 15- June 30	IR-6, KSK-282, KSK-133, JP-5, KashmirNafees, Swat-I, Swat-II, Dilrosh-97, Fakher-e-Malakund	Nasirabad	High/High temperature/High humidity/

Table 3. Impact of disease on production of rice crop.

Disease	Pathogen	Prevailing stage	Favorable Environment	Losses	References
Grain Discoloration/kernel spotting/Grain rot/Seedling rot	*Burkholderia glumae Pseudomonas glumae/ Fuscovaginae*, *Curvularia* spp, *Fusarium* spp, *Sarocladium oryzae*	Panicle emergence stage/maturity stage	Wet/ Humid	Severe	Uematsu *et al*., (1976); Ou, (1985); Imbe *et al*., (1986); Lee *et al*., (1986); Mishra and Dharam, (1992); Wasano and Okuda, (1994); Cottyn *et al.*, (1996 a, b); Shahjahan *et al*., (2000); Mew *et al.*, (2004); Nandakumar *et al*., (2009); Ham *et al*., (2011a,b); Zhou *et al*., (2011); Mizobuchi *et al*., (2013).
Bacterial blight	*Xanthomonas oryzae* pv. *Oryzae*	Seedling stage/Early growth stages of crop	Wet/High Humid	Severe	Srinivasan *et al*., (1959); Dye, (1980).
Blast	*Pyricularia grisea*	Early stage/Tillering/Maturity stage	Temperate Humid	Severe	Suzuki, (1934); Padmanabhan, (1965); Ou, (1985).
Brown spot	*Cochliobolus miyabeanus*	Near maturity stage	Temperate	Severe	Fazil and Schroeder, (1966); Gangopadyay and Chakrabarti, (1982); Roy, (1993).
Sheath blight	*Rhizoctonia solani And Thanatephorus cucumeris*	Nursery stage/Seedling stage	High Humidity	Severe	Savary *et al*., (1995); Willocquet *et al.*, (2000); Singh, (2005).
Stem rot	*Magnaporthe salvinii*	Later growth stages of rice	Wet/High Humidity	Moderate	Ou, (1985).
False smut	*Ustilaginoidea virens*	Flowering stage	Temperate Humid	Moderate	Mehrotra, (1990); Rush *etal*., (2000); Atia, (2004).
Sheath rot	*Sarocladium oryzae*	Early panicle emergence stage	Wet /Humid	Moderate	Sawada, (1922). Kawamura, (1940).
Seedling blight	*Cochliobolus miyabeanus/ Pseudomonas* sp	Early stage	Temperate/Humid	Moderate	Azegami *et al*., (1985); Azegami *et al*., (1988).
Bacterial panicle blight	*Burkholderia glumae And Burkholderia gladioli*	Later stage/Panicle emergence stage	Temperate/Humid	Severe	Nandakumar *et al*., (2009).
Bakanae	*Gibberella fujikuroi*	Seedling stage	Temperate/Humid	Severe	Hemmi *et al*., (1931).
Tungro virus	*RTBV/RTSV*	At any stage	Temperate	Moderate	Khush and Ling, (1974).
Bacterial leaf streak	*Xanthornonas oryzicola*	Tillering stage	Wet/temperate	Moderate	Fang *et al*., (1957).
Black kernel	*Curvularia lunata*	At maturity stage	High humidity	Moderate	Boedijn, (1933); Martin, (1939).

Figure 1. Rice grain discoloration samples collected from major rice growing areas of Pakistan.

Various rice varieties have different levels of susceptibility, tolerance and resistance to panicle blight and discoloration in accordance with specific pathogen (Sayler *et al*., 2006; Saichuk, 2009). Some varieties with high level of disease resistance (Lemont, Jupiter) and some with susceptible (LM-1) or lower level of resistance (Sayler *et al*., 2006; Sha *et al*., 2006; Groth *et al*., 2007). Such rice varieties are being used to study the genes and molecular mechanisms of various rice diseases (Nandakumar *et al*., 2007; Nandakumar and Rush, 2008). Pakistan has an agro based economy. More than 50 % of population of Pakistan depends on agriculture for their livelihood. Rice is the 2nd most important cash crop and export commodity after cotton covering 11% of total cropped area. Basmati is premium rice that fetches about US$ 1150 per ton as compared to US$ 550 per ton of coarse rice from the international market. The value and quantity wise share of Pakistan in total world rice trade is increasing with rice export earned foreign exchange of US$ 1.667 billion. According to the Pakistan Economic Survey (Anonymous, 2014) rice accounts 3.1 percent of the value added to agriculture and 0.7 percent to GDP. However, to meet the consumption rates of the increasing population, an annual increase of 0.6-0.9% in rice production is needed (Carriger and Vallee, 2007). These statistical values emphasizes on the importance of rice crop production and management. Rice diseases and grain discoloration lowering the rice production and yield potential of the crop (Table 3).

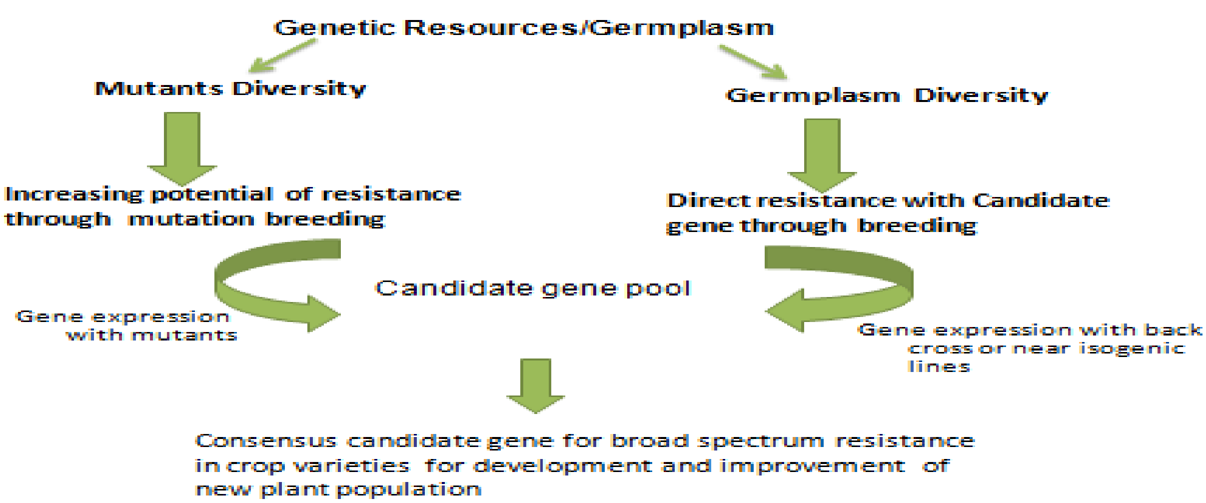

Figure 2. Utilization of framework for increasing the resistance against diseases

To overcome the yield loss of rice due to rice grain discoloration and other rice diseases following main objectives must be included for all future research programs for controlling the rice diseases.

- Screening of wide rice germplasm leading to the identification of resistant variety.
- The identification of real causal agent of rice grain discoloration/rice diseases.
- Exploring the epidemiology of diseases and favorable environmental conditions for the development and spread of diseases.
- Devising recommendation of best cultural practices and management strategies to overcome the spread of this epidemic disease.
- Development of disease resistant homozygous population/lines of rice.
- Development of Potential disease resistant DNA markers associated with rice discoloration disease
- Improvement of the grain quality/increasing the yield of rice.

Utilization of modern techniques for the improvement of rice crop: Conventional plant breeding and molecular techniques have contributed greatly towards the yield improvement of rice by controlling rice diseases and other abiotic factors. In this era rice molecular markers, rice biotechnology, rice proteomics, genomics, and other advanced techniques offer opportunity to scientist for precision breeding. These can be the more useful for desirable combination of genes efficiently (Raghuvanshi *et al*., 2009). DNA markers were converted into PCR based markers and used for many crops including rice and other cereals for their improvement (Collard *et al*., 2008; Collard and Mackil, 2008). For marker assisted selection, DNA sequencing and molecular mapping of rice helped in mining a number of rice markers especially SSR markers suitable for selection, screening and development of new rice varieties (Gupta and Varshney, 2000; IRGSP, 2005).

The comparison of *Oryza sativa* L. and *Oryza glabberimma* L. varieties has led to identification of new, strong reproducible capability and potential single nucleotide polymorphism (SNPs) markers (Shen *et al*., 2004). SNPs markers were also identified by many scientist generating partial sequences of defined region of entire set of related rice genotypes (Monna *et al*., 2006; Shirasawa *et al*., 2007). New molecular breeding tools are very reliable for selection, screening and identification of new rice varieties especially against diseases and other factors that involved in rice crop production. MAS breeding have many applications for the improvement of rice crop i.e. genetic diversity, screening and selection of genotypes, identification of genotypes, specific gene identification, marker assisted back crossing, gene pyramiding, mapping populations and development of new rice varieties (Jain *et al*., 2004; Collard and Mackill, 2008; Perez *et al*., 2008). Likewise realizing the importance of rice disease especially the rice blast and bacterial leaf blight, several efforts have been made for increasing the resistance against these diseases. On the other hand, rice grain discoloration a new emerging threat for rice crop for the last decade and its losses increasingly every year. To overcome these losses such types of techniques should be utilized for its improvement.

Rice genome has also been used to clone the various genes against biotic and abiotic factors for the improvement of tillering capacity, salt tolerance, submergence tolerance, disease resistance, heading date, shattering, yield and grain quality through marker assisted map based approach (Sakamoto and Matsuoka, 2008; Fitzgerald *et al*., 2009; Haung *et al*., 2009). Such types of genes and QTLs are of great value for the improvement and development new rice lines through breeding and other molecular techniques for future utilization and enhancement of the germplasm.

Prospects of managing rice grain discoloration: Modern molecular techniques, diverse genetic germplasm resources, resistant rice varieties and wild species will be very helpful for the improvement of rice crop against diseases (Figure 2). One of the main objectives of ongoing research in the area of rice genomics is to understand the gene function and their regulatory networks for controlling the biotic and abiotic factors. To overcome such type of limitations multi target mutation and gene silencing would be helpful. To determine the relationship of insertion mutants and flanking sequence tags (FSTs) are available in rice to target the genes. This could be possible by the adoption of new approaches to genome sequencing and DNA pooling. On the other hand, TILLING and site specific gene silencing could also be helpful to reach inaccessible genes. The knowledge about the rice genes have a great research impact on syntenic genomes of other crop species and used as a model experimental crop for other cereals.

The genomic diversity, epigenomics and allelic variation of *oryza* species needs to be incorporated in molecular plant breeding programs for the improvement of rice phenotypes with better quantitative and qualitative traits. Screening of genetic resources i.e. land races, wild species, cultivated species, elite breeding lines and high yielding strains, generation of potential DNA markers (SNPs, SSRs) and their close association with breeding efforts is required for production of high yielding genotypes resistant against disease and other factors of environment. The efforts are required for generating, analyzing, enabling tools/resources, sharing genetic resources, functional annotation, bioinformatics, DNA hybridization and marker assisted breeding for the improvement and investigation of new genes in this crop.

We hope the genomic research and advanced techniques will bring a significant change for crop improvement, functional gene investigation, marker trait association, gene mapping potential and identification of new QTLs specifically responsible for high yielding and disease resistance (Collard *et al*., 2008; Zhang *et al*., 2008).

Conclusion: It is clear that epidemiological surveys and accurate identification of the pathogens are essential before proposing practical control schemes. We have to adopt good management practices and develop disease resistant varieties to meet the food requirement of our fast growing population and to support our economy through export of better rice grain quality. On the other hand, genetic germplasm resources and advanced molecular techniques are key to the improvement in rice yield production and protection of the crop against diseases.

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