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An over View of Bakanae Disease of Rice

¹Muhammad Naeem, ¹Muhammad Iqbal, ²Nasira Parveen, ³Sami-Ul-Allh, ¹Qamar Abbas, ¹Abdul Rehman and ¹Muhammad Saad Shauket

¹Department of Plant Breeding and Genetics, University College of Agriculture and Environmental Sciences, TheIslamia University of Bahawalpur, Pakistan ²Departmet of Zoology, BahauddinZakariya University Multan, Pakistan ³College of Agriculture, Bahaddar Sub-Campus Layyah, BahauddinZakariya University Multan, Pakistan

Abstract: The bakanae disease is known to be discovered in 1828 but was named in 1898. This disease is believed to be caused by Fusarium moniliformin but other Fusarium species are also found to be involved in causing this disease. There is hardly any comprehensive study that describes the detailed information about the disease. This review was conducted to analyze the i) historical existence of disease, ii) epidemiological conditions and iii) possible ways to control the disease. The disease is reported both soil and seed borne. Pathogen actually is reported as seed borne and had a long life. As some seeds also remained in the field that become source of the disease in the next year, due to which this disease is also reported as soil borne by some researchers. The pathogen responsible for the disease has a number of races due to which breeding for the disease resistance faces problems. The pathogen enhances the production of secondary metabolites and gibbrellic acid (GA) which in turn cause the symptoms. It is suggested that the introduction of genes inhibiting the production of GA can be a way to control this disease instead of introducing many genes for different races of the pathogen. The most common way to control the disease is the use of fungicides which are harmful for health and even for beneficial microorganisms. Use of coarse varieties is another way to control this disease but in some areas, like in Pakistan and India, fine type varieties are preferred over course type, so this approach is also not feasible. Some QTLs (qBK1, qB1 and qB2) have been identified in fine rice varieties for this disease, which may be proved beneficial, if used, in the breeding program. As there are many races of pathogen responsible for this disease, use of horizontal resistance may be more practical than vertical resistance.

Key words: Bakanae disease • Rice • Fusarium species • Biotic stress

INTRODUCTION

Word Bakanae disease is derived from Japanese language which mean "foolish seedling". Bakanae disease of Rice in known to be existent in almost all rice growing areas of the world. This disease was first time observed in 1828 but in 1898 it was certified by Shotaro Hori that this disease is caused by fungi. In United States it was observed in 1999 [1]. Upto 1980's it was believed that this disease is caused by *F. moniliformin* [2, 3, 4], while later on it was observed that this disease is caused by *F. moniliformin* [5, 6] is the core specie responsible for its symptoms. It's very interesting to note that some *Fusarium* species are associated with disease plant but do not contribute in the

development of the disease symptoms [4]. *Fusarium fujikori* has number of host plants and is widely distributed throughout the world [7, 8]. It is a seed as well as soil borne disease which perpetuates from remains of infected plants from one season to next. Healthy seed sown in infected soil results in the infected seedlings. High humidity and temperature upto 35°C along with Nitrogen application fortifies the chances of disease incidence. Fungal spores can easily transfer from one plant to other with air. Incidence of disease is more pronounced in seeds harvestedduring wet season as compared to dry season regardless of variety and intensity of pathogen population [9]. Pathogen's recovery percentage increased from lower to higher level in endosperm, embryo, basal glumes, palea and lemma [9].

Corresponding Author: Muhammad Naeem, Department of Plant Breeding and Genetics, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan. E-mail: sami llh@yahoo.com. Am-Euras. J. Agric. & Environ. Sci., 16 (2): 270-277, 2016



Fig. 1a & b: Elongated seedlings as a result of Bakanae disease of rice

Pathogen can survive upto 26 months when stored at 10°C and 40% relative humidity [9]. Homology of disease and healthy seedling is so high that it is very challenging even for an expert to distinguish between the two. Bakaini disease perpetuates from seed as spore are attached with seed. Therefore if seed from the infected field is used in the next year it can cause huge losses. So it is very important to save seeds from the diseases. Normally seed it treated with fungicides to control the disease but use of fungicides in restricted only to the conventional farming whereas in organic farms this issue become a major problem. Best way to control this disease is to find the way that is acceptable to all farming system i.e. incorporation of resistant genes from the related species by conventional breeding methods. This review is made to critically analyze the disease, its causal organism, potential hazards and possible ways to control the disease that are acceptable to all farming communities.

Disease Epidemiology: Rice pathogenic fungus *Fusarium fujikuroi* is component of *Gibberella fujikori* species complex (GFSC) which causes Bakanae disease [10] resulting in hyper or hypoelongation of stem, pale green flag leaf (Figure 1a & b), yellowish green leaves, limited and dried leaves at later stages of infection, lesions on roots of infected plants, development of rigid or sinewy adventitious roots on first or second nodes and death of infected plants before maturity [5, 7, 11]. Usually white mycelium and occasionally pinkish sporodochia are produced on stem just above water level in case of severe infection. Appearance of disease symptoms in susceptible varieties inoculated with pathogen takes 5 days and severity reaches the peak about 70 days after inoculation [5]. If an infected plant is able to survive first three critical days of germination due to the production of amino acids, sugars acting as rich energy substrate for pathogen, it will remain alive up to 42 days [5, 7] Agarwal et al., 1989, Zainudin et al., 2008. Infected plant surviving beyond 40 days will produce sterile, empty and discolored seeds. This notorious pathogen is known to produce wide range of metabolites such as pigments Bikaverin, Fusarubins, Mycotoxins, Fusarins, Fusaric acid, Phytohormones, Gibberellic acid [12, 13] GA(3) and its precursors GA(4) and GA(7) [14] along with many unidentified metabolites [13, 15] as summarized in Table 1. Mycotoxins produced in cereals are injurious to human and animals. Gibberelic Acidalong with exudates such as amino acids and sugar are responsible for excessive height [16] solely produced by F. fujikuroi while remaining metabolites by rest of the Bakanae disease's pathogenic species [6]. Fumonisins cause browning of stalk, leaf and ear of rice at flowering period [17]. All of the Fusarium species are known to hamper seed germination percentage, grain discoloration with F. fujikori more pathogenic than other [6]. Infection rate of Bakanae disease ranges from 0.25% to 20% caused by F. fujikuroi, F. proliferatum, F. andiyazi and F. verticilliodes [18] having F. fujikuroi, limited to Asia while remaining three invaded to Asia & Africa [6]. F. fujikori not only damages plant but is parasitic as well [19]. Due to multiple causing agents there are some difference in symptoms which may cause problems in disease identification.

Genetic Studies: Genomic analysis of *F. fujikori* revealed clusters of genetic loci responsible for production of putative still unidentified secondary metabolites [13]. Gibberelic acid was first time studied in 1954 but its effect on rice is still equally important in Agricultural world [20]. Gibberelic acid is synthesized by genes encoding bifunctionalentcopolydiphosphate/kaurene synthase and cytochromemonooxygenases [14]. Expression of gene geranyldiphosphate synthesae-2 (ggs) responsible for exclusive GA (3) synthesis is unaffected due to environmental factors, is present in single copy in

	Glbberellic Acid $GA(3)$	F1.4. 0.03
	Gibbeleine Held GH(5)	[14, 20]
Stem elongation	Precursors of GA, i.e., GA(4), GA(7)	[14]
Hyper elongation	Gibberellic Acids	[12, 13]
	Bikaverin, Fusarubins, fusarins, fusaric acid,	[12, 13]
	Fumonisins FB(1), FB(2), Gibberellin (A3)	[6]
	Fumonisins	[17]
	FB(1), FB(2)	[6]
	FB(1), FB(2)	[6]
	Hyper elongation	Hyper elongationGibberellic AcidsBikaverin, Fusarubins, fusaric acid, Fumonisins FB(1), FB(2), Gibberellin (A3) Fumonisins FB(1), FB(2) FB(1), FB(2)

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Table 1: Symptoms of Bakanae disease formed by various *fusarium* species

Fusarium fujikori genome and is key enzyme in isoprenoid biosynthesis [14]. Gene ggsencompases 418 amino acids, lackintron and is similar to Neurospora crassa (al-3) gene lacking nitrogen & carbon catabolite repression [14]. From above discussion, it can be concluded that the casual organism enhances the production of GA which in return produce symptoms. One way to control this disease may be the introduction of genes which suppress the enzymes responsible for the production of Gibberelic acid and this disease can be controlled without introducing many resistant genes for different strains.

Gibberella fujikori species complex (GFSC) is comprised of 34 strains and nine mating populations named as Mating Population A-Jshowing chromosome n=12 differing only due to electrophoretic karyotypes, secondary metabolite production and sensitivity against antifungal agentshowever intercrossing between mating population results in sterility [6, 21, 22]. Population A, B and D encompasses a genome size of 45-50 Mbp while C & E 50-55 Mbp [23]. On the basis of mycotoxin production pathogen is graded as low (<100mug/g) moderate (100-500mug/g) and highly toxic (>500mug/g). In rice mating population C of G. fujikuroi produces moderate to high concentration of Fusaric acid [24].

Disease Management: Currently the most common control method being used in chemical control. However it is expensive, pollutes ecosystem and increasing resistance in host against fungicideshas been observed as well [1, 25].

Biological Control: Fungi from *Trichoderma* genus and bacteria form *Pseudomonas* and *Bacillus* genus are principal biological control agents [26, 27, 28]. Eight Bacterial strains of *Pseudomonas fluorescens* and five strains of *Bacillus cereus* [29] have been identified as biological control agents against *F. fujikori*. Among these *Pseudomonas fluorescens* (Strain 15) suggested by

Kazempour *et al.* [30] produces broad-spectrum antibiotic phenazine-corboxylic acid (PCA) and *Bacillius cereus* strain 35 [29] have best inhibitory effect on the growth of *F. moniliforme* having inhibition percentage of 69 and 66 percent respectively, although all bacterial strains showed inhibitory effect on *F. fujikuroi* growth. *Talaromyces* sp. isolate KNB-422 possesses mycoparasitic ability against *Gibberella fujikori* [31] therefore can be used for the control of this pathogen.

Cultural Control: Experiments have shown that pathogen *Gibberrlla fujikouri* is mostly found in the soil. But it is not spreadive. This microorganism have long survival time, when the rice growth is consecutive whereas the residues has small effect. Infested seeds are the primary source of inoculums. The pathogens are found abundantly in the harvested plants. About 10-20% of the seed is naturally infected with this fungus [19]. Reducing the moisture level up to 8% and storage at proper and hygienic place minimizes the chances of disease infestation in subsequent generations. Increased sowing of coarse varieties as compared to fine varieties reduces risk of the disease [32, 33] so in the area where epidemic conditions prevail, sowing of coarse rice should be preferred to control the disease.

Chemical Control: Apart from cultural and biological control chemical control results in abrupt and complete control for the disease. Various fungicides are used worldwide for the control of the disease among these Deconil, Dithane-M, Derosil are the best fungicides for the disease control [33, 34]. In addition to these lot of new and completely effective chemicals have been synthesized for the commercial control of Bakanae disease, Ethynyl Phenyl amide [35], amide compound [36], Pyrazole-4-Carboxamide [37], Sodium hypochlorite [38].

Transgenic Approaches: Transgenic approach can improve rice crop contrary to conventional methods by

adding targeted gene in its genome [39]. To overcome the limitations and discrepancies of conventional breeding methods molecular and transgenic techniques present quick, perfect, accurate and reliable method for genetic improvement of disease resistance. Many genes and mechanisms such as pathogen recognition, signal transduction, downstream defense related proteins and crosstalk have been recognized and elucidated for creating resistance in rice varieties. Similarly molecular techniques including the use of promoter sequences in target genome and modification of target protein structures have been studied and proposed to improve the effectiveness of disease resistance. Therefore it's very important to consider potential effect of the transgenes on rice yield, tolerance to abiotic stresses and defense against pathogens for genetic improvement in rice [40].

In last 10 years, improvement in rice transformation methods such as Agrobacterium mediated transformation, gene gun method as well as by protoplast intake for the production of transgenic rice have been accomplished. Genes have been added in rice to improve resistance against Biotic and A-biotic stress. In Basmati rice application of tissue culture protocols and transformation methods, gene stacking and gene pyramiding for obtaining useful transgenic plants are extremely useful. Transformation technology have been used in creating resistance against insect pests (stem borer, leaf folder, gall midge), bacterial diseases (bacterial leaf blight), fungal diseases (blast and Bakanae), abiotic stresses and improvement of nutritional quality in Basmati rice [39]. Rice diseases drastically limit yield and production throughout the world. Owing to this threat viral resistant transgenic lines having genes encoding viral coat protein, enzymatic expression of RNA interference and suppression of insect vectors have been developed. Expression of cloned resistance genes, antimicrobial protein gene, defense and signaling gene can be utilized for the generation of improved fungal resistant rice varieties [41].

The species specific primer pairs Fuji1F/TEF1R specific to *F. fujikori* and Proli1F/TEF1R specific to *F. proliferatum* gave a product of 179 and 188 bp in PCR reation. These primers plays important role in the detection of fungal germs in seeds and tissues as the pathogenic contamination [42]. Phenotypic and Molecular Marker gives information about Genetic structure of fungal pathogen. DNA markers commonly used areAFLP, RAPD, RFLP, SSR support and validate data along with phenotypic observations. VCG is important marker in asexually reproducing F-oxysporium as in relation with aggressiveness [43]. By using sequence specific primers

5'having base pair sequence (Forward) TGAGACTTGTGTCTGAACT-3' and (Reverse) 5'-GATAACATCATAACTCCTTGCG-3', TEF-1 forward primer 5'-ATGGGTAAGGARGACAAGAC-3' TEF-2 reverse primer: 5'-GGARTACCAGTSATCATGTT-3' [6] and SEQ ID NO 1: 5'-TGAGACTTGTGTCTGAGAGCT-3', SEQ ID NO 2: 5'-GATAACATCATAACTCCTGCG-3' (Patent # KR2012045917-A) is found to be very easy method for the detection of F. fujikori. This method is rapid, accurate and convenient [44]. Using two primer AA2M2 and L45 primer 20 isolates of F. fujikuroi have been characterized by (UP-PCR) in Philippines [45].

Three QTLs qBK1, qB1 and qB2 have been detected for resistance to Bakanae disease on chromosome 1 and 10 of Oryza sativa genome [46, 47]. An SSR marker closely linked with the qBK1 OTL is a wonderful opportunity for marker assisted selection against this disease. The genetically acquired resistance supersede all other strategies and approaches of attaining resistance against Bakanae disease [48]. Fusarium database is online available which can be used to generate new primers for identification of particular fungus from a mixture of pathogens by deriving sequence specific primers [49]. Development of transgenic rice having Phenazine Carboxylic acid genes taken from Pseudomonas flouresence could be an optimum opportunity for the genetic control of F. fujikorias has been reported by Maesomeh Anvari [50].

Conclusion and Recommendations: This disease although identified long time ago but remained neglected in the scientific community of world probably because of difficulty in the identification of the diseased plant, lack of information for resistant source in the rice germplasm and probably due tolimited impact on yield. As far as resistant varieties are concerned literature search revealed fruitless results up to present. Resistant varieties reported in the present article are those which have been evaluated to be resistant for the said disease (Table 3). None of these varieties were deliberately breed for Bakanae disease. This situation should not be continued in future because a sudden outbreak in the commercial rice cultivated areas will cause severe yield losses because once entered in the field it cannot be controlled by any other mean such as chemical control. Therefore care should be taken to develop genetically resistant varieties to ensure maximum vield. A breeding population derived from cross of susceptible variety verses resistant variety should be created so as to map resistance Genes/QTLs for Bakanae disease in coarse as well as fine varieties. Blood of coarse varieties must be incorporated in the breeding program

Country	Province	Disease Incidence %		Vield Losses	
Pakistan	Jammu Kashmir	10.50	[51]	Tield E05505	
1 akistali	Maianaiaa anaaa an Daamati 285	10-50	[51]		
	Major rice areas, on Basmati-385	10-70	[52]		
India http://www.rkmp.co.in/content/	Utterpardesh	1.2-11.7	[53]	15-25%	[54, 55, 56]
introduction-and-history-of-bakanae-disease	Andrapardesh				
	Tamil Nadu,				
	Manipur				
	WesstBangal	3-95.5			
	Haryana	2.1-2.8,	[53]		Sowing of Untreated
	Punjab	10.5-40	[53]		seed and susceptible
					variety
	Assam,				
	Bihar	1.8-8.7	[53]		
	Rajastan	2.4-13.6	[53]		
Indonesia and Malysia	Rompin	12.5	[5]		
	Sungia Leman	5.3			
	Sekinchan	2.5			
	East Java	5			
	Padang	12			
USA	California				
Bangladesh				26.7%	[57]
Korea					
Thailand	Northern and Central Thailand			3.7-14.7%	[58]

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Table 3: List of Rice varieties with known behavior against Bakanae disease

Name of Variety	Reaction	Origin	Reference	
IR50	Resistant	IRRI Philippine	[9]	
IR43	Moderately Resistant	IRRI Philippine	[9]	
IR841	Susceptible	IRRI Philippine	[9]	
Basmati-385	Susceptible	Pakistan	[59]	

because these were less prone to the disease as compared to the fine varieties. Disease infestation from China, Japan, Kore, Pakistan and India has shown that there is no limitation of species or sub-species of rice. All of the genotypes are vulnerable to the disease if they lack the resistant gene in their blood.

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