Current Studies on Bakanae Disease in Rice: Host Range, Molecular Identification, and Disease Management

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ABSTRACT

The seed borne disease such as bakanae is difficult to control. Crop yield loss caused by bakanae depending on the regions and varieties grown, ranging from 3.0% to 95.4%. Bakanae is an important disease of rice worldwide and the pathogen was identified as Fusarium fujikuroi Nirenberg (teleomorph: *Gibberella fujikuroi* Sawada). Currently, four Fusaria (*F. fujikuroi*, *F. proliferatum*, *F. verticillioides* and *F. andiyazi*) belonging to *F. fujikuroi* species complex are generally known as the pathogens of bakanae. The infection occurs through both seed and soil-borne transmission. When infection occurs during the heading stage, rice seeds become contaminated. Molecular detection of pathogens of bakanae is important because identification based on morphological and biological characters could lead to incorrect species designation and time-consuming. Seed disinfection has been studied for a long time in Korea for the management of the bakanae disease of rice. As seed disinfectants have been studied to control bakanae, resistance studies to chemicals have been also conducted. Presently biological control and resistant varieties are not widely used. The detection of this pathogen is critical for seed certification and for preventing field infections. In South Korea, bakanae is designated as a regulated pathogen. To provide highly qualified rice seeds to farms, Korea Seed & Variety Service (KSVS) has been producing and distributing certified rice seeds for producing healthy rice in fields. Therefore, the objective of the study is to summarize the recent progress in molecular identification, fungicide resistance, and the management strategy of bakanae.

1. Introduction

The staple food crop of Korea is rice (*Oryza sativa* L.), on top of that a model monocotyledons crop species for scientific research. According to previous study, rice production will increase by above 40% by 2030 to encounter the increasing demands of a rising human population and increasing livestock production [1]. However, several challenges have to be met to achieve the goal of increasing rice production sustainably. According to the "2020 Rice Production Survey Results" released by Statistics Korea, rice production in 2020 is estimated to be 3,507,000 tons. But, it decreased by 6.4% from last year (3,744,000 tons) and was the lowest level since 1968 (3,200,000 tons).

Moreover, 90% of the world's rice is grown in Asia. However, there are some parts of the countries do not have enough supply for food resources for the increasing population. Rice productivity is affected by both biotic and abiotic stresses. The main constraint is due to rice diseases [2,3]. Among the microorganisms, phytopathogenic fungi play the major role and leads to significant yield losses. Among lots of rice diseases, the important seed-borne diseases such as rice blast, narrow brown spot, brown spot, rice bunt, false smut, and bakanae, are the important fungal diseases. In addition, bacteria also cause seed-borne diseases such as bacterial leaf blight and bacterial leaf streak [4,5]. Among fungal diseases, bakanae (foolish seedling), is one of the most severe threats to rice production in various rice-growing countries worldwide. Korea Seed & Variety Service (KSVS) produces and distributes government-certified rice seeds for a stable supply of high-quality rice. Because bakanae is difficult to control, KSVS designates and manages bakanae as a specific disease in the process of producing certified rice seeds.

Furthermore, it has been shown that the bakanae disease of rice is caused by one or more seed-borne *Fusarium* species, mainly *Fusarium fujikuroi* a

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member of the *Gibberella fujikuroi* species complex [6–8]. Yield loss caused by bakanae disease depends on the regions and varieties grown, ranging from 3.0 to 95.4% [9]. Previous study showed that in 2003, 2.9% of rice seedlings in the seed boxes were infected by *F. fujikuroi* and it increased to 28.8% a in 2006 [10]. According to Rural Development Administration (RDA), bakanae disease incidence decreased from 28.8% in 2006 to 20.0% in 2012 but it dramatically increased to 30.9% in 2013 in the fields [11,12]. Choi et al. [11] reported that bakanae was increasing in Korea as the decline of seed disinfection efficacy, expansion of cultivation area of early maturing and bakanae susceptible varieties, and change of cultivation methods.

There have been many studies on bakanae disease so far, but the information is scattered and difficult to grasp at a glance. This review covers various aspects of bakanae disease that will be useful for researchers regarding the recent progress in molecular identification of the pathogen, fungicide resistance, and management strategy.

2. Pathogen and its identification

2.1. Pathogen

Bakanae disease was first identified in 1828 in Japan and is currently identified in Asia, Europe, Africa, and America. The pathogen was demonstrated as *Fusarium heterosporium* Nees by Shotaro Hori [13]. It was later named *G. fujikuroi* Sawada by Ito and Kimura [14]. Wollenweber and Reinking [15] described the anamorph of the fungus as *Fusarium moniliforme* Sheldon and the teleomorph as *G. fujikuroi* Sawada. However, due to controversy over the species concept of *F. moniliforme*, the name "*F. moniliforme*" is no longer used [16]. Afterward, it was re-identified as *F. fujikuroi* Nirenberg [17], the teleomorph of *G. fujikuroi* Sawada (Figure 1). *F. fujikuroi* comes under hemibiotrophic fungi, i.e., primary infection depends on living host (biotrophic) whereas further infection and survival encompasses destruction and consumption of host cells (necrotroph) [18].

Currently, on rice, *F. fujikuroi*, *F. proliferatum*, *F. verticillioides,* and *F. andiyazi* belonging to *F. fujikuroi* species complex are responsible for bakanae disease [8]. It shows that more than one species of *Fusarium* can cause bakanae on rice. The strains of *F. fujikuroi* were more pathogenic than other isolates of three *Fusarium* species. Mohd Zainuddin et al. [19] reported that *F. fujikuroi* isolates were highly pathogenic and the only species which produced gibberellin acid (GA$_3$) causing abnormal elongation symptoms of bakanae. However, out of 16 strains of *F. fujikuroi*, only one strain did not
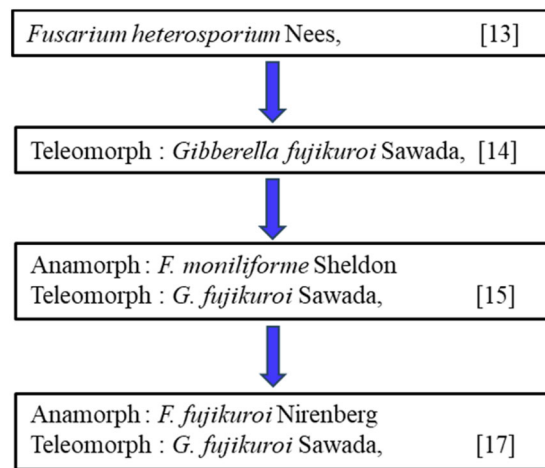
produce GA$_3$ and it was nonpathogenic. Whereas, *F. proliferatum* and *F. verticillioides* isolates did not produce GA$_3$ and not showing bakanae disease symptoms. Amatulli et al. [20] studies showed that *F. fujikuroi* was the most prevalent *Fusarium* species isolates derived from bakanae diseased rice plants and seeds. *F. fujikuroi* isolates were able to cause bakanae but *F. proliferatum* and *F. verticillioides* isolates were not virulent. On the contrary to the study of Amatulli et al. [20], Quazi et al. [21] collected 12 isolates of *F. proliferatum* which were derived from bakanae infected rice plants and proved that all isolates were pathogenic. In addition, there are other reports that *F. proliferatum* were associated with bakanae of rice [6,8,22], suggesting that *F. proliferatum* and *F. verticillioides* isolates reported by Amatulli et al. [20] may vary in pathogenicity depending on the isolates from different crops and cultivar.

Recent studies showed that *F. andiyazi* was also found to be responsible for bakanae on rice [8,23,24]. Moreover, *F. fujikuroi* was known to attack a wide variety of important crops such as rice, maize, cotton, and sugarcane [25,26]. According to Mohd Zainuddin et al. [8] and Choi et al. [12], not all isolates of *F. fujikuroi* caused bakanae symptoms.

2.2. Pathogen identification

Early detection of bakanae pathogen is requisite to stop the occurrence and spread of the rice disease. In general, conventional diagnosis methods such as isolation, pure culturing, followed by microscopically inspecting the morphological characteristics of pathogen, *F. fujikori* from infected plants of [19,27]. However, species identification through morphological and microbiological characteristics found to be time consuming and difficult, it needs well trained expertise [7,28]. Therefore, molecular identification based on DNA analysis have been extensively used to identify and distinguish the isolates/strains in a species [29]. In particular, species-specific PCR primers are a powerful tool for differentiation of fungi at species level [30,31].

Species-specific primers to detect some *Fusarium* species have been generated from sequences of several housekeeping genes such as transcription elongation factor 1 alpha (*TEF-1α*) and calmodulin or internal transcribed spacer (*ITS*) region of ribosomal RNA [32–36]. In the case of bakanae on rice, a specific primer made from nucleotide deletion of the *TEF* gene was developed to detect *F. proliferatum* which is a similar species to *F. fujikuroi* in Italy [29]. Although the two species have differences in the *TEF* gene sequences, the pathogenicity on rice is

Fusarium heterosporium Nees,	[13]

↓

| Teleomorph : Gibberella fujikuroi Sawada, [14] | |

↓

| Anamorph : F. moniliforme Sheldon
Teleomorph : G. fujikuroi Sawada, | [15] |

↓

| Anamorph : F. fujikuroi Nirenberg
Teleomorph : G. fujikuroi Sawada, | [17] |

Figure 1. The history of the identification of bakanae pathogen.

not always different between the two species. In general, bakanae of rice is caused by *F. fujikuroi*. However, there have been several studies that reported that bakanae of rice is also caused by more than one species of *Fusarium* particularly such as *F. proliferatum* other than *F. fujikuroi* [8,21].

Furthermore, various PCR detection assays have been developed for the identification of mycotoxigenic *Fusarium* species such as *F. graminearum, F. culmorum, F. poae, F. sporotrichioides*, and *F. verticilioides*, based on mycotoxin biosynthesis [37–41]. *F. proliferatum* belongs to *G. fujikuroi* species complex and has very similar characteristics to *F. fujikuroi* both morphologically and phylogenetically [37]. Thus, to detect *F. fujikuroi* species from rice seed and plant samples, the conventional and Real-Time PCR primer sets are developed based on the difference in translation elongation factor gene sequences between *F. fujikuroi* and *F. proliferatum* [29]. Nonpathogenic *F. fujikuroi* has been reported in Malaysia and Indonesia [19]. However, there are not yet specific primers developed to detect *F. fujikuroi* species complex regardless of pathogenic species or not [19]. Hence, advanced technique like loop-mediated isothermal amplification (LAMP) is substituting for seed health analysis, due to its sensitivity, specificity, on-site application, and low-cost technology over molecular technology and are extensively used as an analytic tool [42].

3. Symptoms and secondary metabolites

Bakanae disease symptoms vary depending on its disease severity. It includes root and crown rot, seedling blight, stunting, etiolation, abnormally elongated seedlings, yellowing with empty panicles, and in severe cases, dead and sterile seeds [43–45].

Besides, diseased plants finally die, although panicles on remaining plants don't grow any grains, thus reduction in rice production [7,46]. The most classic symptom of bakanae is the abnormal elongation of seedlings induced by fungal-secreted gibberellic acid (GA_3) [9,47,48]. GA_3 is a significant plant hormone produced by the fungus causing bakanae [49]. Although GA_3 is considered a secondary metabolite in *Fusarium* species (because they are not important for fungal growth and development), they are thought to contribute to the virulence mechanism of fungi [50]. *Fusarium* species of *G. fujikuroi* species complex are also able to produce mycotoxins [51,52] which can affect human and animal health following ingestion of contaminated grains [53,54]. Strains of mating type population C (anamorph, *F. fujikuroi*) produced moniliformin, beauvericin, and GA_3, but little or no fumonisin, and strains of mating type population D (anamorph, *F. proliferatum*) produced moniliformin, beauvericin, and fumonisin but no GA_3 [7]. Wulff et al. reported that most strains of *F. proliferatum* and *F. verticillioides* produced fumonisins (FB_1 and FB_2), but not GA_3. Thus, *F. fujikuroi*, the only *Fusarium* species capable of GA3 synthesis, by regulation and expression of jasmonic acid-responsive gene and jasmonic acid-mediated plant immune responses [55,56]. Furthermore, production of GA was also linked with the fungicide sensitivity of different *F. fujikuroi* isolates [57,58].

4. Survival, dispersal, and disease cycle

The pathogen, *F. fujikuroi* species complex causing bakanae is primarily seed-borne but it also transmitted *via* soil and the debris of plants [59,60]. Seed-borne inoculum is a more important source because

soil-borne inoculum is reduced rapidly over time [7,61–63]. According to Volante et al. [50], contaminated seeds with fungal species provide space for initial entry of infection. The conidia are easily transmitted *via* water and wind and initiate new infections. Under favorable conditions, infected plants are capable of producing lots of conidia that subsequently infect neighboring plants [62,64,65]. According to Kanjanson [61], at room temperature survival rate of bakanae pathogen in seeds and infected plants around 4–10 months. The pathogen is dispersed primarily with infested seeds and it survives under harsh conditions [59,60]. Watanabe [59] reported that infected plant residues from the previous season also could be able to occur the disease in the field. When infection occurs during the heading stage, it makes rice seeds contaminate [9]. This will be the next year's new inoculum. Disease occurrence of bakanae according to the seed maturation period was reported in Korea [66]. The average occurrence rates were 49%, 50%, 36%, and 29% at the heading stage, milk stage, dough ripe stage, and yellow ripe stage, respectively. It showed that rice seeds were infected by the bakanae pathogen later stages of plants, the more decreased bakanae occurrence. To decrease the incidence of bakanae, it was important to reduce infections in the early stages of rice plant growth.

The survival rate of the fungi is correlated to the soil temperature (optimum temperature is 35 °C), at high temperature the level of infection rate can increase particularly during flowering stage [67]. The level of disease incidence is also related to genetic nature of the cultivars, for example, the aromatic cultivars were more sensitive than non-aromatic ones [68], coarse varieties were more resistant [69] and level of the susceptibility may change throughout the crop stages [70]. According to Sunani et al. [71] the fungus infects seeds in three different ways. In pathogen, *F. verticillioides* the microconidia act as the source of infection [72] and conidia of *F. fujikuroi* also systemically infect the seeds [71]. This data is vital because it helps the choice of the most effective chemical treatment for disease control [7].

5. Host range of the pathogen

The pathogen responsible for bakanae is present not only in rice but also in other crops or weeds (Table 1). According to Carter et al. [73], water grass (*Echinochloa* spp.) plants which were showing typical symptoms of bakanae, were observed and collected from the field in California, in 2002. Moreover, isolates of *F. fujikuroi* collected from water grass (early water grass (*Echinochloa*

oryzoides), and barnyard grass (*E. crus-galli*)) produced bakanae disease symptoms on rice. They reported many monocotyledonous weeds in California rice fields should be evaluated as potential hosts of the bakanae pathogen in further study. Bakanae symptoms were observed in barnyard grass in a paddy field in Korea, in 2009 [74]. *Fusarium* species were isolated from the infected stem of barnyard grass and they were identified as *F. fujikuroi*. The isolates performed the pathogenicity test on barnyard grass and rice by artificial inoculation, and as a result, both barnyard grass and rice showed virulence. They reported that the time at which the spore formation of bakanae was observed on the stem of barnyard grass, coincided with the heading stage of rice, which means barnyard grass can play an important role as a source of transmission for bakanae in rice. It indicated that gramineous weeds control should be fulfilled for effective bakanae management.

6. Molecular identification

Molecular detection methods of pathogens causing bakanae are becoming important because based on morphological and biological characteristics could lead to incorrect species designation and be time-consuming [20]. Previous studies revealed great diversity among *Fusarium* species, underestimated by earlier morphological criteria [95–98]. Different techniques such as RAPD (random amplification of polymorphic DNA), AFLP (amplified fragment length polymorphism), and RFLP (restriction fragment length polymorphism) analysis have been used to characterize and identify the different mating populations in the *G. fujikuroi* complex [99–101], as well as multiple gene phylogenies [96]. In general, the *ITS* gene is the most widely used for sequencing the DNA region of universal fungi [102]. Further, Geiser [103] found that intron-rich portion of protein coding genes are excellent markers for identification of species level of fungi. Due to high evolution rate in gene introns found to be better than the *ITS* regions of the nuclear ribosomal RNA gene repeat [95,97]. The translation elongation factor 1-alpha (*TEF*) gene, the RNA polymerase largest subunit gene (*RPB1*), and the RNA polymerase second largest subunit gene (*RPB2*) commonly have been used for analyzing molecular characters of *Fusarium* isolates [95,104,105]. In particular, the *TEF* gene has been generally used for species identification in *Fusarium* species because has high phylogenetic utility [8,20–23,26,95,103,106–109].

In addition to *TEF* gene, *FUM1* gene for fumonisin synthesis has been utilized for the species identification of *Fusarium fujikuroi* species complex

Table 1. Host range of *Fusarium fujikuroi*.

Plant species	Scientific name	Disease	Country	Reference
Water Grass	*Echinochloa oryzoides, E. crus-galli*	Bakane disease	United States of America	[73]
Barnyard grass	*Echinochloa crus-galli*	Bakane disease	South Korea	[74]
Rice, Maize, and Soybean	*Oryza sativa, Zea mays,* and *Glycine max*	Mycotoxins	China	[75]
Soybean	*Glycine max*	Fusarium wilt	South Korea	[76]
Lettuce	*Lactuca serriola*	Fusarium wilt	South Korea	[77]
Onion	*Allium cepa*	Anthracnose-Twister	Philippines	[78]
Peanut	*Arachis hypogaea*	Root rot	China	[79]
Pima Cotton	*Gossypium barbadense*	Fusarium wilt	United States of America	[80]
Conifer	*Torreya grandis*	Root rot	China	[81]
Kiwifruit	*Actinidia* spp.	Brown Leaf Spot	China	[82]
Maize	*Zea mays*	Ear and root rot	Mexico	[83]
Lilly	*Lilium lancifolium*	Bulb rot	China	[84]
Iron walnut	*Juglans sigillata*	Stem Rot Disease	China	[85]
Kohila	*Lasia spinosa*	Leaf Spot	China	[86]
Rice, Sugarcane and Maize	*Oryza sativa, Saccharum officinarum* and *Zea mays*	Mycotoxins	Malaysia	[26]
Soybean	*Glycine max*	Root Rot	China	[87]
Canna lily	*Canna edulis Ker.*	Stem Wilt	China	[88]
Maize	*Zea mays L.*	Ear Rot	China	[89]
Pineapple	*Ananas comosus*	Fusariosis	Malaysia	[90]
Soybean	*Glycine max*	Root rot	Indiana	[91]
Rattan pepper	*Zanthoxylum armatum*	Round Spot Disease	China	[92]
Soybean	*Glycine max*	Damping-off	United States of America	[93]
Wine grapes	*Vitis vinifera*	Mycotoxins	United States of America	[94]

associated with rice, maize and soybean [110,111]. Choi et al. [112] reported that rice samples in Korea were contaminated predominantly with *F. fujikuroi* (47.4%), *F. proliferatum* (27.3%), and *F. concentricum* (15.1%) that were confirmed with PCR with the primer sets of *TEF-1α* and *RPB2* gene. In contrast, maize samples were contaminated by *F. verticillioides* (33.9%), *F. fujikuroi* (25.3%), and *F. proliferatum* (21.1%) [112]. Their study reported that the species diversity and frequency of *Fusarium fujikuroi* species complexes from maize and rice are like previous study conducted for Korean cereals [112–114]. Also, in their study fewer *F. fujikuroi* species complex were isolated from barley and soybean [112]. Also direct PCR without extraction of DNA could identify *F. fujikuroi* complexes using the primer set from the sequence of ITS 4 and ITS 5 [114] and *TEF-1α* region [95,114].

O'Donnell et al. [95] first developed the sequencing primers of the *TEF* gene in fungi, *F. oxysporum* complex. These primers were ef1 (forward primer; 5′-ATGGGTAAGGA(A/G) GACAAGAC-3′) and ef2 (reverse primer; 5′-GGA(G/A) GTACCAGT(G/C) ATCATGTT-3′), and they amplified about *TEF* region of 750 bp. For the correct identification and publicly available database, Geiser et al. [103] created "FUSARIUM – ID v. 1.0" which is a *TEF* DNA sequence database for identifying *Fusarium* species. It has been developed from v. 1.0 to v. 3.0 so far and has been widely used for identifying *Fusarium* spp. [8,20,103,106,108].

Mulè et al. [34] investigated a species-specific PCR assay based on a partial calmodulin gene sequence for the identification of *F. verticillioides* and *F. proliferatum*. The primer pair sets, VER1/2 and PRO1/2 gave a PCR product of 578 and 585 bp for *F. verticillioides* and *F. proliferatum,* respectively.

Kang et al. [110] developed the *F. verticillioides* specific primers, RVER1/2 derived from the nucleotide sequences of *RPB2* gene and amplified a 208 bp DNA fragment of *F. verticillioides*. Hwang et al. [111] studied the *F. fujikuroi* specific primer set, FfPNG1_232F/FfPNG1_355R, which was designed on the fumonisin biosynthesis gene cluster. This primer set amplified the PCR product of 124 bp for *F. fujikuroi*. Lin et al. [115] reported two species-specific primer sets which were based on the *ITS* region. The primers Pgx1-F/R were specific to *F. verticillioides* and Pgx2-F/R were specific to *F. proliferatum*, which amplified 439 and 400 bp of PCR product, respectively.

The molecular technique is useful but it has its limitations in that nonpathogenic *F. fujikuroi* species complex causing bakanae also detected [116]. Choi [116] developed gibberellic acid (GA$_3$) production-specific primers because abnormal elongation of seedlings due to the production of GA$_3$ by the pathogen is the typical symptom of bakanae. The primers *Bfsp* F/R were designed based on the gibberellic acid biosynthesize gene cluster, and their PCR amplification size was about 550 bp. Based on the *TEF-1α* gene, 35 strains of *F. fujikuroi* and 8 strains of other species belonging to the genus *Fusarium* spp. were used to validate a real-time PCR with Taqman probe as a diagnostic method on rice seeds [116]. Further, they designed specific primers and a Taqman probe for quantitative PCR (qPCR), but useful only for the amplifications of DNA fragments from different strains of *F. fujikuroi* repeatedly and reliably, but not from the other eight *Fusarium* species. The method was applied *in vivo* for the diagnosis of infected tissues and the detection and quantification of the pathogen in batches of naturally contaminated rice seeds of different rice

Table 2. Primers used for molecular identification of *Fusarium fujikuroi*.

Primer	Primer Sequence (5′→3′)	Amplicon size	Reference
ef1	ATGGGTAAGGA (A/G) GACAAGAC	750 bp	[20]
ef2	GGA (G/A) GTACCAGT (G/C) ATCATGTT		
TEF1F	ACGTGTCAAACTAAACATTCGA	179 bp	[29]
TEF1R	GCGACAACATACCAATGACG		
Fum1F1	CACATCTGTGGGCGATCC	682 bp	[75]
Fum1R2	ATATGGCCCCAGCTGCATA-		
EF1T	ATGGGTAAGGAGGACAAGAC	600 bp	[75]
EF2T	GGAAGTACCAGTGATCATGTT		
TEF-1	ATGGGTAAGGARGACAAGAC	600 bp	[95]
TEF-2	GGARGTACCAGTSATCATGTT		
B20_12133For1	CCTCTGGCAATTATCCGTCTCAT	260 bp	[112]
B20_12133Rev1	GTCGCTTGGTATCTCCTCGTGTGA		
B20_12133For2	GCAATCGAGGCATCAAAGGAATA	300 bp	[112]
B20_12133Rev2	CACCAGACATGAATGCCCGTAAA		
B20_12133For3	ACTCCTCGATGTCTTGCCGTTTCA	525 bp	[112]
B20_12133For3	TGCAGCATTTCCCTACCACAGC		
B14_6372For1	CGAACGACCTAGCAGCCCTTTTAC	282 bp	[112]
B14_6372Rev1	TCGCCTTGTTGGTAGTGTATGTCT		
B14_6372For2	AATTCCGTCGGCTGTTCCCTCCTC	257 bp	[112]
B14_6372Rev2	GTCATCACCTGCCGCATCATACAA		
B14_6372For3	CTCCAGAGAACAGCACCTTTGATA	175 bp	[112]
B14_6372Rev3	AGGCGATAGGGATACGGCGAATAC		
B14_J06375F2	AGAAAGATCGTGCGACTATGAAGA	382 bp	[112]
B14_J06375R2	TTGGTGCGTACGACTGGGTGAAC		
B20_J12141F2	TACGAGAGGGAAACCAAAGAACAC	434 bp	[112]
B20_J12141R2	CATGTGAACGCAAGCGAGAAATAG		
TqF2	GGCGCGTTTTGCCCTTTCCT	84 bp	[117]
TqR	AGCGGCTTCCTATTGTCGAA		
TqF1	CGAGTGATGGGCGCGTTTTG	93 bp	[117]
TqR	AGCGGCTTCCTATTGTCGAA		
FfujiFq	CACGTGTCAAACTAAACATTCG	116 bp	[117]
FfujiRq	GATGGTGATACCACGCTCAC		
GFmat1a	GTTCATCAAAGGGCAAGCG	200 bp	[118]
GFmat1b	TAAGCGCCCTCTTAACGCCTTC		
Gfmat2c	AGCGTCATTATTCGATCAAG	800 bp	[118]
GFmat2d	CTACGTTGAGAGCTGTACAG		
BFsp F	TGAGACTTGTGTCTGAGAGCT	547 bp	[119]
BFsp R	GATAACATCATAACTCCTGCG		
tef-1aF	CATCGAGAAGTTCGAGAAGG	230 bp	[119]
tef-1aR	ATGGTTAGTTTGACACGTGAC		
FfPNG1_232F	CTG CGA CAT CTC CCC AAG ATC	124 bp	[120]
FfPNG1_355R	CAA CAG ACC GGG GTT CTC		
B14J06375F2	AGAAAGATCGTGCGACTATGAAGA	382 bp	[121]
B14J06375R2	TTGGTGCGTACGACTGGGTGAAC		
B20J12141F2	TACGAGAGGGAAACCAAAGAACAC	434 bp	[121]
B20J12141R2	CATGTGAACGCAAGCGAGAAATAG		
VER 1	CTTCCTGCGATGTTTCTCC	578 bp	[122]
VER 2	AATTGGCCATTGGTATTATATATCTA		

cultivars. Primers used and developed so far for diagnosis of *F. fujikuroi* mentioned in Table 2.

6.1. Loop-mediated isothermal amplification (LAMP)

Several methods for the examination of bakanae disease, including fungal isolation and characterization, microbiological observations, and molecular identification, are the most commonly used methods for disease diagnosis. Furthermore, real-time PCR quantification results of *F. fujikuroi* showed different values among the different seed lines, it depends on their origin, susceptibility of the cultivar, soil and environmental conditions. Hence, these methods are inappropriate for field studies, as they need technical expertise, specialized equipment and time-consuming. Pleasing such weaknesses into account, a new technique known as LAMP has been

established for the identification of bakanae disease pathogens.

LAMP represents a rapid, specific, sensitive, and efficient molecular diagnostic technique that amplifies DNA sequences at a single temperature [42,123]. Franco Ortega et al. [124] proven LAMP protocol validated to detect *F. fujikuroi* on rice. Rong et al. [125] developed the *F. fujikuroi* specific LAMP-based detection assay using the intergenic spacer (*IGS*) region of nuclear ribosomal operon and successfully diagnosed suspected seed samples and diseased plants, where the sensitivity of the primers was 67 pg/μl of DNA. Whereas, the sensitivity of the LAMP assay developed by Sunani et al. [71] based on *NRPS31* was very high making it possible to detect *F. fujikuroi* inoculum as low as 10 pg/μl of DNA. Moreover, the sensitivity of the assay involves the design of suitable primers and the optimization of reaction conditions, while the specificity of the LAMP assay depends on the uniqueness of the

target marker. For the detection of *F. fujikuroi*, although the *TEF1* gene and *IGS* region are used as markers, successful LAMP amplification needs relatively strict reaction conditions due to the high similarity in the marker sequences. To overcome these difficulties, Jiang et al. [126] recently found the promoter sequence of desaturase in the GA$_3$ biosynthesis pathway was used as the target marker (*DesM231*) due to the difference of larger bases between *F. fujikuroi* and other species of Fusarium. Six primers, including two externals (F3 and B3), two internal (FIP and BIP), and two loop primers (F-loop and B-loop), were designed for *tef-1α* target gene amplification, and the reaction was performed for 45 min at 65 °C. Hence, this method has the potential to be used directly in the field, as a screening tool for seed batches.

7. Disease management

For the management of the bakanae disease of rice, four methods were tested in Bangladesh [127]. First, roguing was evaluated as an ineffective method in a field. Second, 15 fungicides were proved effective in various degrees *in vitro*. Third, foliar spray of the best effective 3 fungicides (folicur, knowin, and protaf) was demonstrated ineffective to control bakanae in the field. Lastly, looking for resistant varieties, 87 varieties and landraces were screened for resistance to bakanae *in vitro*. One landrace, A23 was suitable for use in the development of resistant varieties to bakanae.

7.1. Seed disinfection

Seed disinfection for the management of bakanae has been studied for a long time in Korea. The water temperature was proved to be a significant factor in controlling bakanae by treating seed disinfectants [128]. The control effect of the seed disinfectants increased as the water temperature increased. However, soaking periods of rice seeds in the suspension of disinfectants and the used amount of chemicals had no high relation to control bakanae. Hwang et al. [129] reported the seed disinfection methods of *F. fujikuroi* in rice seeds. The effect was the highest when the temperature of the disinfectant solution was 30 °C and the soaking period was 48 h. Seed soaking into the mixed solution of prochloraz and fludioxonil was studied for control of Bakanae disease of rice [130]. The mycelial growth of *F. fujikuroi* was inhibited by 100.0% at 5 μg/ml of prochloraz and 5 μg/ml of fludioxonil. The spore germination of *F. fujikuroi* was inhibited at 99.2% at 10 μg/ml of each fungicide. Heavily infected rice seeds immersed in a mixed solution

composed of 125 μg/ml of prochloraz and 50 μg/ml of fludioxonil showed a high control effect as 2.1% of disease symptoms. Moreover, it did not affect the seedling stand rate but higher concentrations of prochloraz reduced the seedling stand rate. With the longer seed soaking time and the higher temperature, the control effect on bakanae was improved. However, the seedling stand rate was lower around 80% over 35 °C. When soaked in a mixed solution of 125 μg/ml of prochloraz and 50 μg/ml of fludioxonil at 30 °C for 48 h, the control value was 96.7% and the seedling stand rate was 88.0%. It was effective in controlling bakanae and showed a stable control effect on plants.

Sodium hypochlorite (NaOCl) has been widely used as a disinfectant in various fields. Chun et al. [131] studied the influence of the pH of household bleach solutions on bacterial and fungal-contaminated rice seeds and rice seedling growth. Bacteria were eliminated from rice seeds which were soaked into household bleach solutions (50% bleach and 2.6% NaOCl) and adjusted to pH 7.0 in 0.5 M potassium phosphate but fungi were removed at pH 5.0 and below. NaOCl stimulated rice seedling growth directly, as well. Shin et al. [132] used NaOCl for the control of bakanae in rice seeds. When the infected rice seeds were immersed for 12 h in 0.5% and 0.3% NaOCl solutions, the disease incidences of rice seedlings were significantly reduced to 4.3% and 4.7% in each solution, compared to 97.3% of the control. Moreover, seedling stand rates were 29.1% and 26.9%, respectively, which were higher than that of the control. When the infected seeds were immersed in NaOCl before or after prochloraz, the rate of seed contamination rate and disease incidence was lower than prochloraz alone. Especially, the seed disinfection effect of treatment with prochloraz after NaOCl was higher than that of treatment with prochloraz before NaOCl.

7.2. Chemicals resistance

As seed disinfectants have been studied to control bakanae disease, resistance studies to chemicals have been also conducted. Kim et al. [10] studied the degradation of prochloraz by 2 strains of *F. fujikuroi* with differing sensitivity. A strain, CF106 was sensitive to the fungicide, and another strain, CF245 was resistant. CF245 completely degraded 1.0 mg/L of prochloraz 5 days later but no degradation was observed by CF106. A major degradation product of prochloraz was *N*-(2-(2,4,6-trichlorophenoxy) ethyl) propan-1-amine and it was detected from the culture filtrates of CF245. Shin et al. [133] investigated the resistance of *Fusarium* species to prochloraz. The effective concentration inhibiting mycelial

growth by 50% (EC_{50}) values of *Fusarium* species 36 isolates was an average of 0.25 µg·mL^{-1}, of which *F. fujikuroi* were confirmed to be 0.31 µg·mL^{-1}. To decide whether the isolate was resistant to prochloraz or not, the resistant baseline of EC_{50} was set at 0.5 g·mL^{-1}, and the ratio of resistant isolates was 13.8%. There was no correlation between the pathogenicity of *Fusarium* species on rice seedlings and the resistance to prochloraz. The resistant isolates of *F. Fujikuroi* had no cross-resistance to other sterol biosynthesis-inhibiting fungicides such as hexaconazole, triflumizole, tebuconazole, and difenoconazole. Lee et al. [134] reported fungicide resistance of *F. fujikuroi* isolates isolated in Korea. One hundred and eighteen isolates were collected from various places of rice fields in Korea from 2006 to 2009 and tested resistance assay to prochloraz, tebuconazole, and benomyl. The number of resistant isolates to each fungicide was 17, 19, and 43, respectively. Four isolates indicated double resistance to both prochloraz and tebuconazole, 6 isolates to prochloraz and benomyl, and 11 isolates to tebuconazole and benomyl. Two isolates isolated from Gyeongbuk province were resistant to all three fungicides. They suggested the effect of seed disinfection can be lowered by resistant strains at any time. This is a potential factor that can increase the occurrence of bakanae. To study the changes in the resistance to prochloraz of *Fusarium* spp. related to bakanae, *Fusarium* isolates were collected from various places in Korea between 2006–2006 and 2013–2014 years [11,12]. The high-frequency distribution of minimum inhibitory concentration (MIC) values of prochloraz, as opposed to isolates collected in 2006–2007 and 2013–2014 years, was 3.125–6.25 µg/ml and 6.25–12.5 µg/ml, respectively. The mean EC_{50} values increased from 0.3142 µg/ml in 2006–2007 to 0.8124 µg/ml in 2013–2014. The ratio of resistant isolates was 6.5% in 2006–2007 years and 41.6% in 2013–2014 years, indicating that the frequency of resistant isolates increased significantly.

7.3. Biological control

Since chemical control has problems such as polluting the environment and generating resistant strains, biological control of bakanae using antagonistic bacteria has been studied. Rosales and Mew [135] investigated that 10 strains of bacteria collected from paddy water, sclerotia, rhizosphere soils, and rice plant, reduced bakanae for the 3 years of field trials. Five strains steadily reduced bakanae but the others showed various effects among trials. Kazempour and Anvary [136] isolated 238 bacteria from the rhizosphere and found the antagonistic ability of 12 of them. Eight of these isolates were identified as *Pseudomonas fluorescens* which produced a broad-spectrum antibiotic phenazine-carboxylic acid (PCA), and they inhibited the growth of *F. Fujikuroi* in vitro. Hossain et al. [137] reported that an endophytic bacterium strain, *Bacillus oryzicola* YC7007 had considerable biocontrol activity against bakanae. The suspension of *B. oryzicola* YC7007 (2.0×10^7 cfu/ml) was treated to the rice rhizosphere and bakanae severity was reduced to 46–78% in pots and nursery box tests. Moreover, they investigated the efficiency comparison test between *B. oryzicola* YC7007 and chemical fungicides, against bakanae in rice nursery boxes. The seed treatment with dual chemical fungicides, prochloraz, and fludioxonil, considerably reduced the disease, and there was no significant difference between dual fungicides treatment and bacterial root drenching, with 55.6%–66.7% disease reduction, respectively. Biological control is environmental and can be an alternative to agricultural pesticides in the management of plant disease. However, microbial disinfectants have not been developed yet for practical use at commercial farms to control bakanae.

7.4. Resistant varieties

The last management for rice bakanae is the development of resistant varieties and it is important to select a resistant resource through a resistance assay. Traditional breeding requires a lot of time and effort to develop resistant varieties because a large number of genetic resources should be biologically assayed to determine whether they are resistant or not. Therefore, molecular breeding using molecular markers is required to compensate for this problem, but there are not many genetic studies on bakanae. Yang et al. [138] reported that two Quantitative Trait Loci (QTLs), *qB1* and *qB10* were detected for bakanae resistance on chromosome 1 and chromosome 10 in the Chunjiang 06/TN1 double haploid population. Hur et al. [139] identified a major QTL, *qBK1* on chromosome 1 derived from the Korean rice variety Shingwang, and reported RM9 which was an SSR marker, tightly linked the bakanae resistant QTL, *qBK1*. However, Hur et al. [140] reported that the discrepancy between the results of the bioassay for bakanae and the genotype using the molecular marker RM9 was 38% among 254 rice germplasm and noted that it could not be used for a broad range of rice germplasm. Fiyaz et al. [141,142] found three QTLs (*qBK1.1*, *qBK1.2*, and *qBK1.3*) on chromosome 1 using inclusive composite interval mapping (ICIM) in the recombinant inbred line (RILs) population derived from two rice parents, Pusa1342 (a resistant variety) and PB1121 (a susceptible variety). The main QTL, designated as

qBK1.2, showed 24.74% of phenotype variation for seedling mortality rate among 168 F_{14} RILs, and the other QTLs, qBK1.1, and qBK1.3 showed 4.76 and 6.49% of phenotype variation, respectively. Volante et al. [50] identified two genomic regions related to phenotypic variation for response to bakanae infection on rice chromosome 1 (named qBK1_628091) and chromosome 4 (named qBK4_31750955). Choi et al. [143] reported that random mutagenesis resulted in the identification of 2 genes responsible for prochloraz resistance in F. fujikuroi. They found that survival factor 1 and F-box/WD-repeat protein may positively and negatively regulate prochloraz resistance in F. fujikuroi. Lee et al. [144] reported that qBK1 from BC_6F_4 of Shingwang (a resistant variety) and Ilpum (a susceptible variety) crosses, was delimited to a 35-kb interval by the markers InDel 18 and InDel 19-14. Moreover, they found four candidate genes in a 35-kb region. Kang et al. [145] studies showed that a novel major QTL for bakanae disease resistance, qFfR9, on rice chromosome 9 with an F2:F3 population derived from a cross between a bakanae disease-resistant variety, "Samgwang", and a bakanae disease-susceptible variety, "Junam". Moreover, they suggest that QTL and its closest marker, KJ_09024, will be useful for bakanae disease-resistant rice variety breeding programs, and will help reducing yield loss caused by bakanae disease. Jo et al. [146] developed a cultivar "MY299BK" which is carrying the qBK1 gene derived from Shingwang (a resistant variety) and it was evaluated as having moderate resistance to bakanae. Recently, Lee et al. [147] identified $qBK4^T$ at chromosome 2 as a novel locus for bakanae disease resistance using QTL mapping–Genome-Wide Association Study (GWAS).

8. Regulations for the production of certified rice seeds in Korea

Since 1976, KSVS (Korea Seed and Variety Service) has been producing and distributing certified seeds of major crops such as rice, wheat, barley, and soybean. Highly qualified seeds produced by KSVS have contributed to agricultural productivity and the improvement of farmers' income. The seeds are inspected critically before distribution. There is a regulations "Seed inspection manual" (2021) by KSVS and it contains the inspection criteria for producing fields and produced seeds of crops, and the inspection criteria for rice are as follows.

The inspection of production fields is conducted once between the milk stage and the dough ripe stage. The production fields of foundation rice seed (wonwonjong in Korean) and registered rice seed (wonjong in Korean) should be isolated at least 3

Table 3. The inspection criteria for producing fields and produced seeds of rice by Korea seed & variety Service.

	Diseased plants (maximum limit, %)	
	Specific disease[a]	Other disease[b]
The inspection criteria for producing fields		
Fields of foundation rice seed	0.01	10.00
Fields of registered rice seed	0.01	15.00
Fields of certified rice seed	0.02	20.00
	Diseased seeds (maximum limit, %)	
	Specific disease[a]	Other disease[c]
The inspection criteria for produced seeds		
Foundation rice seeds	2.0	5.0
Registered rice seeds	5.0	10.0
Certified rice seeds	5.0	10.0

[a]Specific disease: bakanae, white tip disease.
[b]Other diseases: rice blast, brown spot, bacterial blight, sheath blight, stripe, dwarf, false smut, bacterial grain rot.
[c]Other diseases: rice blast, brown spot, false smut.

meters from different rice variety fields. The production fields of certified rice seed (bogeubjong in Korean) should be isolated at least 1 m from different rice variety fields. However, the isolation distance is not required if rice production fields and different rice variety fields are separated into the ridge between rice paddies, etc.

The inspection criteria for producing fields of rice include the varietal purity, weeds rate, diseased plants rate, etc. Among weeds, only Echinochloa species, a host plant of bakanae, is classified as a specific weed. A diseased plant refers to a plant infected with the blast, brown spot, bacterial blight, sheath blight, stripe, dwarf, false smut, bacterial grain rot, white tip, and bakanae. Especially bakanae is classified as a specific rice disease. The inspection criteria for producing fields of rice regulate the maximum limit of infection rate of bakanae on rice plants depending on the production stage. It is 0.01%, 0.01%, and 0.02% on the producing fields of foundation rice seed, registered rice seed and certified rice seed, respectively (Table 3).

The inspection criteria for produced seeds of rice include the varietal purity, germination rate, moisture content, weed seeds rate, damaged seeds rate and diseased seeds rate, etc. As with the inspection criteria of producing fields of rice, Echinochloa species and bakanae is classified as specific weed seeds and diseased seeds, respectively. The inspection criteria for rice seeds regulate the maximum limit of infection rate of bakanae on rice seeds as 2.0%, 5.0%, and 5.0% on the foundation rice seeds, registered rice seeds, and certified rice seeds, respectively (Table 3). In other words, bakanae is one of the very important diseases enough to be regulated by the government in Korea.

9. Conclusions

Bakanae is a seed-borne disease caused by pathogens, F. fujikuroi, F. proliferatum, F. verticillioides, and F.

andiyazi belonging to the *Fusarium fujikuroi* species complex. Seed infection occurs when rice plants are infected with a pathogen during the heading stage. Therefore, if bakanae diseased rice plants or host plants such as water grass and barnyard grass were observed during the heading stage, they must be removed to prevent seed infection. Various disease management methods have been studied for bakanae control and seed treatment with fungicides is the most widely used among them. One of the most important factors when disinfecting seeds is the temperature of the water. The control effect was the highest when the temperature of the disinfectant solution was 30 °C and the soaking period was 48 h. As fungicides began to be used to control bakanae, strains resistant to fungicides also appeared. The ratio of resistant isolates was 6 times higher in 2013–2014 years than in 2006–2007 years, showing that the frequency of resistant isolates increased significantly. Since chemical control has problems such as polluting the environment and generating resistant strains, biological control of bakanae using antagonistic bacteria has been studied. However, microbial disinfectants have not been developed yet for practical use at commercial farms. The development of resistant varieties is also one of the control management for bakanae. Currently, molecular breeding is widely used to develop resistant varieties, but there are not many genetic studies on bakanae. In Korea, a moderate resistance cultivar "MY299BK" carrying the resistance gene *qBK1* was developed in 2020. To provide highly qualified rice seeds to farms, KSVS has been producing and distributing certified rice seeds. Under the "Seed inspection manual", the seeds are inspected critically before distribution, and bakanae is designated as a specific disease supervised by the government. Bakanae is a serious problem in rice-growing regions of the world. For the control of bakanae which has an important effect on rice production, further studies on the occurrence of bakanae in domestic rice seeds and the development of species-specific primers for detecting pathogens need to be prioritized.

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