Various Methods for Controlling the *Bakanae* Disease in Rice

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ABSTRACT

Bakanae disease, caused by *Fusarium fujikuroi*, is a serious problem in rice production. This disease is widespread across the world and leads to substantial yield losses. *F. fujikuroi* is known to produce various secondary metabolites, including the plant hormone gibberellin, which induces typical *bakanae* symptoms. In this article, authors overviewed the methods for controlling *bakanae* disease, including the use of host resistance, chemical compounds, biocontrol agents, natural products, and physical methods. Although various strategies have been applied to control *bakanae* disease, the disease is not yet completely prevented. Authors discuss the advantages and disadvantages of these various methods. In addition, the mode of action of major fungicides and the resistance mechanisms to these fungicides were outlined. These information contribute to the development of more effective methods for controlling *bakanae* disease.

Keywords

bakanae disease, biological control, fungicide resistance, *Fusarium fujikuroi*, host resistance

1. Introduction

Bakanae is one of the most serious disease in rice. Diseased plants exhibit leaf discoloration, abnormal stem elongation and seedling rot. In addition, infected rice is infertile and produces fewer grains [1]. *Bakanae* disease was first described in 1898 in Japan [2] and currently occurs in most rice-producing regions worldwide. In addition to Asia, this disease has emerged in European and American countries, such as Italy [3], Macedonia [4], Russia [5], and America [6]. Substantial yield losses caused by *bakanae* disease pose a threat to staple food supply. It has been reported that *bakanae* disease can cause yield losses of 3.0–95.4%, depending on the rice cultivars and geographic regions [7].

Fusarium moniliforme was formerly known as the pathogen of rice *bakanae* disease [8]. However, it was subsequently revealed that *F. moniliforme* included some distinct species and *F. moniliforme* was thus renamed as the *Fusarium fujikuroi* species complex (FFSC), with other species in the section *Liseola* [9]. FFSC members cause diverse diseases in many agricultural crops, including rice, maize, pitch, fig, sugarcane, and mango. Morphological, phylogenetical, and biological species concepts have been applied in the taxonomy of the FFSC [10]. According to the phylogenetic analyses, the FFSC includes at least 50 distinct species and is delineated into three clades: the Asian, African, and American clades [11]. Of these, 34 morphospecies and 12 biological species, mating populations (i.e. MP-A to MP-L), were recognized in the FFSC [10, 12].

The FFSC members are also well known as phytohormone producers: gibberellins (GAs) [13], cytokinin [14], and auxins [14]. GAs contribute to the development of the typical symptoms of *bakanae* disease in rice [15].

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Mycotoxins produced by the FFSC members include fumonisin (FUM) [16], fusarins [17], fusaric acid [18], moniliformin [19], and beauvericin [20]. These pose health risk to mankind and animals [21].

Among the members of the Asian clade, *F. fujikuroi* has been intensively studied as the causal agent of rice *bakanae* disease. *F. fujikuroi* has been subclassified into gibberellin (G)-group/*bakanae* type and fumonisin (F)-group/stunt type [22, 23]. G-group/*bakanae* type strains produce GAs but no or low FUM, while F-group/stunt type strains produce FUM but no or low levels of GAs [22, 23]. Interestingly, different sensitivities to the fungicides thiophanate-methyl [22] and ipconazole [24] were observed between the G-group and F-group strains. Despite the development of management strategies, *F. fujikuroi* still causes *bakanae* disease worldwide. In this article, we review the various methods for controlling *bakanae* disease.

2. Host resistance

Application of resistant host plants is an environmentally friendly and cost-effective method. Screening is important to find practical resistant cultivars. Khan *et al.* [25] and Iqbal *et al.* [26] conducted field screening to identify *bakanae* resistant cultivars from Pakistan rice varieties and found that DM-15-1-95, DR-82, DR-83, KS-133, KS-282, IR-6, and IR-8 have the resistance. Hur *et al.* [27] inoculated *F. fujikuroi* conidia by tissue embedding method and selected the resistant varieties Hawn, Gwangmyeongbyeon, Erguailai, and Wonseadaesoo. Matić *et al.* [28] identified Selnio and Dorella as *bakanae* resistant and sensitive cultivars, respectively, by comparative transcriptome analyses.

Identification of resistance genes is necessary in understanding the resistance mechanisms and for marker-assisted rice breeding. Quantitative trait loci (QTL) associated with *bakanae* disease resistance in rice have been reported. Based on the genetic map and bioassay, *qB1* and *qFfR1* on chromosome 1 [29, 30], *qB4* on chromosome 4 [31], and *qB10* on chromosome 10 [29] were detected as QTL conferring resistance to *bakanae* disease. Hur *et al.* [32] identified *qBK1* as a major QTL by crossing the sensitive *japonica* variety Ilpum and the resistant *indica* variety Shingwang. Subsequently, Fiyaz *et al.* [33] identified *qBK1.1*, *qBK1.2*, and *qBK1.3* as QTL for *bakanae* disease resistance. However, *qBK1.1* and *qBK1* might be the same, as they were detected in the same genomic region [33]. Furthermore, *qBK1^{WD}* in the *japonica* variety Wonseadaesoo [34] and *qBK1^Z* of the Zenith variety [35] were identified to confer resistance to *bakanae* disease. Recently, a late maturing cultivar MY299BK which is *bakanae* resistant was developed by introducing the *qBK1* gene from Shingwang [36].

3. Chemical control

3.1 Benzimidazoles

Currently, application of chemical compounds is the most common method to control *bakanae* disease. Benzimidazoles, broad-spectrum fungicides, have been used to control *bakanae* disease for decades [37] (Table 1). Benzimidazoles disrupt mitosis and meiosis in fungal cells and impair cellular processes, such as cytoskeleton formation, cell division, and intracellular trafficking [38]. The representative benzimidazole fungicides are benomyl, carbendazim, thiophanate-methyl, thiabendazole, and fuberidazole. Seed treatment with a 0.3% suspension of fungicides, including 50% carbendazim or 80% thiophanate-methyl reduced the *bakanae* incidence by *in vitro* tests [39], while soaking the infected seeds in 10 g L⁻¹ benomyl for 10 min completely inhibited *bakanae* disease [40]. Effective control of *bakanae* disease was also achieved by seed treatment with thiabendazole [41].

However, resistant strains to benzimidazoles have been detected in *F. fujikuroi* under excessive use of the benzimidazole fungicide. Possible resistance mechanisms in fungi to fungicides are summarized in Fig. 1, including (I) change in the cell wall structure, (II) alterations of the fungicide target molecule, (III) overproduction of the

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fungicide target molecule, (IV) fungicide excretion, (V) fungicide degradation, and (VI) synthesis of a complementary molecule of the fungicide target. The D50Y mutation in β -tubulin was reported to confer benomyl resistance in *F. moniliforme* [42] (Table 1). Subsequently, Chen *et al.* [43] reported that the E198V and the F200Y mutations in β_2 -tubulin caused carbendazim resistance in Chinese *F. fujikuroi* (Table 1).

Figure 1: Possible mechanisms underlying the resistance of fungi to fungicides. The solid line indicates the normal way for the fungicide bind to the target molecule essential for fungal growth. The dotted line indicates a possible way to prevent fungicide action. (I) The fungal cell wall structure is changed, and the entry of the fungicide is prevented. (II) The structure of the fungicide target molecule is altered, and thus, the fungicide is unable to bind to it. (III) The fungicide cannot completely inhibit the function of the target molecule due to overproduction of the target molecule. (IV) The fungicide is degraded so that the fungicide loses its function. (V) The fungicide is excreted from the fungal cell by efflux pumps. (VI) The function of the fungicide target molecule is complemented by an alternative molecule.

3.2 Sterol demethylase inhibitors

Sterol demethylase inhibitors (DMIs), which are site-specific fungicides, have been used as a substitute of benzimidazoles to control of *bakanae* disease (Table 1). The target of DMIs is a cytochrome P450 sterol 14 α -demethylase (CYP51 enzyme). This enzyme is required for biosynthesis of ergosterol, a component of the fungal cell membrane that is essential for fungal growth. *F. fujikuroi* has three *CYP51* genes (*CYP51A*, *B*, and *C*) [44]. DMIs include chemical classes such as imidazole, piperazine, pyridine, pyrimidine, and triazole. Hossain *et al.* [45] showed the antifungal activity of triazole fungicides (viz. 2.5% difenoconazole emulsifiable concentrate (EC), 25% propiconazole EC, and 25% tebuconazole EC) against *F. moniliforme*. Effective inhibition of the growth of *F. moniliforme* was demonstrated by the triazole fungicides ipconazole [46, 47], propiconazole [48], and imidazole fungicide prochloraz [49]. Seed dipping in a 200-fold diluted solution of 6% ipconazole wettable powder (WP) for

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24 h [46], or in 0.0472 $\mu\text{g mL}^{-1}$ ipconazole [47] were highly effective for control of *bakanae* disease. Seedling treatment with 0.05% propiconazole EC was also effective at controlling *bakanae* disease, although phytotoxicity was observed, along with reductions in plant height and grain yield [48]. Park *et al.* [49] reported that 10 $\mu\text{g mL}^{-1}$ prochloraz completely inhibited mycelial growth of *F. fujikuroi*. Suzuki *et al.* [50] indicated that seed treatment with triflumizole EC showed a higher efficacy to control *bakanae* disease than triflumizole WP. Seed treatment with imidazole fungicide pefurazoate at 500 $\mu\text{g mL}^{-1}$ resulted in a 90% disease reduction [51]. Difenconazole and penconazole showed high effectiveness against *F. moniliforme* by *in vitro* evaluation [52]. Furthermore, soil drenching with difenconazole were also effective at managing *bakanae* disease in greenhouse tests [52].

Strains resistant to DMIs have been detected in *F. fujikuroi* under wide use of DMI fungicides. These resistance mechanisms are summarized in Table 1. Kim *et al.* [53] found that degradation of prochloraz in Korean *F. fujikuroi* strain CF245 was resistant to prochloraz. Subsequently, it was reported that an efflux transporter was also involved in the prochloraz resistance of this strain [54]. Furthermore, in case of another Korean strain CF337, structure changes in the fungal cell wall and an ATP binding cassette (ABC) transporter were reported to confer prochloraz resistance [55]. Whereas, Zhang *et al.* [44] reported that the S312T mutation in CYP51B and overexpression of the *CYP51A/CYP51B* genes caused prochloraz resistance in Chinese *F. fujikuroi*. Choi *et al.* [56] found an association between survival factor 1 and F-box/WD-repeat protein with prochloraz sensitivity of *F. fujikuroi*. Gene disruption of survival factor 1 decreased prochloraz resistance while gene disruption of F-box/WD-repeat protein increased prochloraz resistance [56].

3.3 Other chemical compounds

Chemical compounds other than benzimidazoles and DMIs have also been used as fungicides for *bakanae* disease control (Table 1). Phenamacril, former experimental code JS399-19, is a novel cyanoacrylate fungicide; this drug inhibits the ATPase activity of class I myosin [57] and 0.1544 $\mu\text{g mL}^{-1}$ phenamacril effectively reduced the growth of *F. fujikuroi* [47]. However, resistant strains to phenamacril were found in Chinese *F. fujikuroi* [58]. It has been reported that the S219P, S219L and K218T mutations in myosin-5 were correlated with high resistance to phenamacril in *F. fujikuroi* [58, 59] (Table 1). Fluazinam classified in arylaminopyridine [60], and a novel succinate dehydrogenase inhibitor pydiflumetofen [61] showed significant antifungal activity against *F. fujikuroi*. Seed treatment with 0.1 or 0.2 g available ingredient/kg pydiflumetofen resulted in a 90% reduction in *bakanae* disease [61]. Chelating agents, ethylenediaminetetraacetic acid and chitosan oligosaccharides, exhibited strong antifungal activities against *F. fujikuroi* [62]. Furthermore, the efficacy of combined use of fungicides has also been investigated. Hossain *et al.* [63] reported that 12% carbendazim + 63% mancozeb, 55% metiram + 5% pyraclostrobin, 2.5% fludioxonil + 2.5% celest extra 5 EC, and 200 g L⁻¹ tebuconazole + 100 g L⁻¹ trifloxystrobin completely inhibited the growth of *F. moniliforme in vitro*.

Table 1: Chemical compounds used for control of rice *bakanae* disease

	Chemical compound ^{a)}	Reference	Resistance mechanism ^{b)}
Benzimidazole	Benomyl (Benlate)	[40]	Mutation D50Y in β -tubulin [42]
	Carbendazim (Derosal; Bavistin; Knowin; Haydazin)	[39]	Mutations E198V and F200Y in β -tubulin [43]
	Thiabendazole (Mintezol; Tresaderm; Arbotect)	[41]	-
	Thiophanate-methyl (Topsin M)	[39, 41]	-
Demethylation inhibitor	Difenconazole (Score)	[45, 52]	-
	Ipconazole (Techlead-C Flowable)	[46, 47]	-

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	Pefurazoate (Healthied)	[51]	-
	Penconazole (Topas)	[52]	-
	Prochloraz (Sportak)	[49]	Prochloraz degradation [53], Efflux transport [54], Mutation S312T in CYP51B and overexpression of CYP51A/CYP51B [44], Changes of cell wall [55]
	Propiconazole (Protaf)	[45]	-
	Tebuconazole (Folicur)	[45]	-
	Triflumizole (Trifmine)	[50]	-
Others	Chitosan oligosaccharides	[62]	-
	Ethylenediaminetetraacetic acid	[62]	-
	Fluazinam	[60]	-
	Fludioxonil (Cannonball WG)	[63]	-
	Mancozeb (Dithan)	[63]	-
	Metiram (Arbatene)	[63]	-
	Phenamacril	[47]	Mutations S219P, S219L, and K218T in Myosin-5 [58, 59]
	Pydiflumetofen	[61]	-
	Pyraclostrobin	[63]	-
	Trifloxystrobin (Flint fungicide)	[63]	-

a) The brand name of chemical compounds is indicated in brackets.

b) Resistance mechanism revealed in *Fusarium fujikuroi*. "-" indicates that report was not found.

4. Biological control

4.1 Bacterial agents

Biocontrol agents for *bakanae* disease are summarized in Table 2. A growth reduction in *F. moniliforme* was observed with the *Pseudomonas fluorescens* strains PF-2, PF-9, and PF-13 [64]. Zhang *et al.* [65] further demonstrated that the antagonistic endophytic bacterium *Paenibacillus polymyxa* inhibited the growth of *F. moniliforme*. Strains of *Bacillus* spp., such as *B. cereus* [66], *B. megaterium* [67], *B. subtilis* [64], *B. circulans* [68], and *B. oryzzicola* [69] antagonised the growth of *F. fujikuroi*. Kumar *et al.* [64] showed that the *B. subtilis* strain B-44 was effective at reducing *bakanae* incidence in greenhouse experiments. *B. subtilis* strain QST 713 which has been commercialized as Serenade is a biofungicide for control *bakanae* disease [70]. Hossain *et al.* [69] demonstrated that treatment with the *B. oryzzicola* strain YC7007 reduced *bakanae* severity to 46–78% in pots and nursery box tests.

4.2 Fungal agents

Trichoderma spp. previously reported as biocontrol agents for *bakanae* are shown in Table 2. Parasitic activity on phytopathogenic fungus has been observed in *Trichoderma* spp. *T. asperellum* strain SKT-1, which has been commercialized as Eco-hope, was confirmed to penetrate the hyphae and cause cell wall degradation of *F. fujikuroi* [71]. Application of *T. harzianum* or *T. virens* reduced *bakanae* disease, although their efficacies were lower than that with thiophanate-methyl 80% WP at 2 g L⁻¹ [68]. *Bakanae* disease incidence was significantly reduced by the application of *Trichoderma* sp. strains selected by Ng *et al.* [72].

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The antagonistic yeasts *Metschnikowia pulcherrima*, *Pichia guilliermondii*, and *Sporidiobolus pararoseus* decreased *bakanae* disease severity under greenhouse conditions [70]. *M. pulcherrima* strain R23 or *P. guilliermondii* strain R9 reduced the disease index less than 5% by combinational use with rice seed thermotherapy at 60 °C for 10 min [70]. *Penicillium chrysogenum*, *Penicillium thomii*, and *Stachybotrys atra* were reported to be antagonistic to *F. moniliforme* [73]. Furthermore, endophytic fungi *Chaetomium globosum* strains NR-R688 and NR-SH321, *Penicillium* sp. strain NR-L243, and *Fusarium* sp. strain NR-L645 reduced *bakanae* disease incidence to 2–6% [74]. Recently, a non-pathogenic *Fusarium commune* strain W5 was reported as an effective biocontrol agent for *bakanae* disease [75]. Hyphal extension of *F. fujikuroi* was inhibited on/in rice seedlings and flowers by spray application of strain W5, which survived on/in rice seeds for at least 6 months [75].

Table 2: Biocontrol agents for rice *bakanae* disease

	Species	Reference
Bacterial agent	<i>Bacillus cereas</i>	[66]
	<i>Bacillus circulans</i>	[68]
	<i>Bacillus megaterium</i>	[67]
	<i>Bacillus oryzicola</i>	[69]
	<i>Bacillus subtilis</i> (strain QST 713: Serenade) ^{a)}	[64]
	<i>Paenibacillus polymyxa</i>	[65]
	<i>Pseudomonas fluorescens</i>	[64]
Fungal agent	<i>Metschnikowia pulcherrima</i> ^{b)}	[70]
	<i>Pichia guilliermondii</i> ^{b)}	[70]
	<i>Sporidiobolus pararoseus</i>	[70]
	<i>Chaetomium globosum</i>	[74]
	<i>Fusarium commune</i>	[75]
	<i>Fusarium</i> sp. (strain NR-L645)	[74]
	<i>Penicillium chrysogenum</i>	[73]
	<i>Penicillium thomii</i>	[73]
	<i>Penicillium</i> sp. (strain NR-L243)	[74]
	<i>Stachybotrys atra</i>	[73]
	<i>Trichoderma asperellum</i> (strain SKT-1: Eco-hope) ^{a)}	[71]
	<i>Trichoderma harzianum</i>	[68]
	<i>Trichoderma virens</i>	[68]

a) The strain and its brand name are indicated in brackets.

b) The agent was tested by alone or combinational use with thermotherapy of rice seeds at 60°C for 10 min.

5. Natural products

Extracts from microbes and plants are also potential substances for disease control. It has been found that surfactin A extracted from *Bacillus* sp. reduced the biomass of *F. moniliforme* to 16% at 2000 µg mL⁻¹ [76]. In addition, crude protein extracts from *Paenibacillus polymyxa* inhibited mycelial growth and spore germination of *F. moniliforme* [65]. Distortion and tumescence of germinated spores were observed following treatment with the crude protein extract [65]. Extracts of *Eucalyptus globulus*, *Artemisia judaica*, *Ammi visnaga*, and *Coriandrum sativum* decreased the growth of *F. fujikuroi* [77]. Baruah *et al.* [78] demonstrated that essential oils of *Eucalyptus citriodora*, *Cinnamomum tamala*, *Mentha piperita*, and *Cymbopogon martini* var. *motia* were effective at inhibiting the growth of *F. moniliforme*, among which the oil of *C. martini* var. *motia* had the highest antifungal activity. Control methods

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are generally conducted before the disease occurrence. However, in case of silica nanoparticles produced from rice husk, foliar application after the appearance of *bakanae* symptoms significantly reduced disease incidence [79].

6. Physical control

6.1 Seed treatments

Use of clean (non-infected) rice seeds is especially important to prevent *bakanae* disease as *bakanae* is a seed borne disease. The hot water immersion method has been used for seed disinfection; immersion of infected seeds in hot water at 58 °C or 60 °C for 10 to 20 min showed a similar disinfection efficacy to the conventional chemical treatment [80]. However, it is known that successful disinfection by hot water requires precise control of water temperature. Healthy rice seeds can be selected using salt water [81]. Hot water immersion and seed selection with salt water allow reductions in the use of chemical fungicides, although it may be difficult to achieve complete elimination of infected seeds with these methods. Recently, Ochi *et al.* [82] demonstrated that irradiation with atmospheric plasma was effective for rice seed disinfection. *Bakanae* disease severity index of infected seeds with irradiation was decreased to 18.1% compared with that of non-irradiation control.

6.2 Agronomic practices

Conventional agronomic practices are environmentally-friendly methods. Agronomic practices, such as the use of organic fertilizer, crop rotation, reasonable cultivation practices can help to control of *bakanae* disease. Burning the infected plants and crop residues can reduce the infection source [83]. A support vector machine classifier was developed to distinguish the healthy and infected seedlings to avoid *bakanae* disease [84]. Bagga *et al.* [85] reported that late planting of rice seedlings minimized the incidence of *bakanae* disease. In Australia, rotating rice cultivation with pasture grass and postponing the sowing date from December to late January are recommended to avoid *bakanae* disease in early maturing varieties [86].

7. Overview

Bakanae is a major rice disease widely distributed across the world. Considerable progress has been made in the research on management of this disease in the past. Various resistant genotypes in the host have been screened and some agronomic practices were proposed to control *bakanae* disease. However, satisfactory resistant cultivars are rare and agronomic practices are only partly successful, largely because they are highly dependent on environmental conditions. Seed treatment with fungicides is an effective method to control *bakanae* disease, although continuous use of the same fungicide raises the risk of developing resistant *F. fujikuroi* strains. Biological control is a sustainable method to control of *bakanae* disease. However, it is generally inefficient compared to fungicide control. In addition, the efficacy of biological control might be unstable, because most agents are living microorganisms which are more sensitive to environmental conditions such as soil, humidity, temperature, and so on. Although various effective biocontrol agents were found under greenhouse or *in vitro* tests so far, agents effective under field conditions are rare. In addition, the phytotoxicity of chemical compounds and the environmental safety of application of biocontrol agents need to be considered before starting use. Research on the elucidation of molecular mechanisms of disease resistance in rice plant and pathogenesis of *F. fujikuroi* can contribute to develop *bakanae* control method. Recently, RNA interference was demonstrated as a potential control method for barley scab (*Hordeum vulgare*), caused by *Fusarium graminearum* [87]. However, more efficient and sustainable control would probably be difficult to attain with a single control method, and therefore, integrated disease management based on various studies is important for control of *bakanae* disease.

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