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Review Article

RICE PANICLE BLIGHT: INSIGHTS INTO PATHOGENESIS, PATHOGENS, INFECTION DYNAMICS, AND SUSTAINABLE MANAGEMENT STRATEGIES

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ABSTRACT: Bacterial panicle blight of rice (B.P.B.) is one of the most serious diseases hampering the yield of rice around the world. B.P.B. is a highly destructive disease caused by Bacteria *Burkholderia glumae*. It has been reported to cause up to 75% yield losses, alongside quality reduction such as grain weight and total amylose content. Despite the adverse effect of the B.P.B., comprehensive information about this disease and its pathogenicity remains scarce. This paper focuses on a critical review of the causative pathogen, pathogenesis, the disease cycle, and epidemiology. Besides, the novel control measures such as the exclusion technique, resistance approach, use of chemicals, biocontrol, and cultural practices for the B.P.B. management and their limitations were discussed in this report. More so, the integration of control measures showed a positive result in reducing the damage caused by the B.P.B. disease. The most efficient and long-term approach to mitigating the damage caused by B.P.B. to rice yield is the development and use of resistant cultivars. Though, the use of this technology is yet to be fully explored.

Keywords: bacterial panicle blight, Burkholderia *glumae,* control measures, pathogenesis, genetic resistance, .

INTRODUCTION

Rice bacterial panicle blight (B.P.B.) was first reported in Japan in 1956, and the disease has since become serious threat to global rice production (Faizal Azizi et al., 2023; Honsho et al., 2017). At present, in many rice-growing countries across Asia, Africa, and South and Central America, the disease has been reported (Mondal & Mani, 2015; Nandakumar et al., 2007; Quesada-González , 2014; Zhou et al., 2011,) The disease is capable of causing several types of damage, such as seedling blight, sheath rot, panicle blighting, floret sterility, unfilled or aborted grains, and decreased milling quality, which may result in a yield reduction of up to 75% (Mishra et al., 2021; Nandakumar et al., 2009; Zhou XG, 2014). On a general note, more than one pathogen has been reported as the causative agent of B.P.B. with almost similar symptoms and damage to the rice plant (Maeda et al., 2006).

B.P.B. disease is caused by bacteria, the most noticeable of which include *B.glumae*, *B.gladioli*, and *B.plantarii* (Mulaw et al., 2018). All three species of the genus *Burkholderia* were collectively reported as the causative pathogens of the B.P.B. disease (Ham et al., 2011; Nandakumar et al., 2009). *Burkholderia glumae* was identified as the major causal agent of B.P.B. among these three species (Nandakumar et al., 2009). Ham et al., 2011 reported that *B.gladioli* and *B.plantarii* are also capable of causing comparable symptoms on rice as *B. glumea*, particularly under analogous climatic conditions. Thus, these species were less frequently isolated and showed less

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virulence than *B.glumae* (Ham et al., 2011). The bacteria's virulence properties depend on the production of toxoflavin and lipase, currently the two known pathogenic determinants of *B.glumae* (Devescovi et al., 2007; Kim et al., 2004). Similarly, Devescovi et al., identified avirulent mutant strains of *B.glumae* that are defective in toxoflavin and lipase production and are not capable of inducing B.P.B. infection in rice. The B.P.B. disease has strong correlations with several factors such a s climatic conditions and cultural practices, in depending on the rice varieties (Shew et al., 2019).

Researchers have substantiated that the climate, cultivation system, fertilizer application, water management, and substitution of rice varieties/combinations strongly influence the severity of the B.P.B. (Honsho et al., 2017; Li et al., 2016; Pinson et al., 2010). A combination of elevated temperature with high relative humidity or regular rainfall, especially during heading-flowering stages, favors the B.P.B. infection and advances more damages on the rice crop(Ham et al., 2011). A temperature range of (35-40 ^{o}C) was reported to be optimal for the growth of *B glumae* (Nandakumar et al., 2009). On the contrary, Pinson et al., 2010 reported that the temperature range between (30 to 35) favors the optimal growth of *B. glumea* and its proliferation process. As a result, tropical and semi-tropical countries, alongside other countries with high average temperatures, will frequently experience the disease (Ham et al., 2011). More so, the prevailing global warming has the potential to escalate the damage caused by the B.P.B. disease (Protic et al., 2024; Shew et al., 2019)**.**

Hence, checking the damage level is indispensable to maximize rice yield. Researchers have attempted to develop several management options comprising exclusion techniques, resistant cultivar development, use of chemicals, biocontrol, and cultural practices (Honsho et al., 2017). The plant quarantine procedures and the use of certified seeds have been reported to be an effective strategy in preventing the introduction of B.P.B. pathogens into countries that are free of pathogens (Zhou-qi et al., 2016). Globally, significant research efforts have been carried out to develop resistant cultivars as an important and sustainable rice B.P.B. management strategy. Regrettably, no single genes or quantitative trait loci (QTLs) for full resistance were identified (Mizobuchi

et al., 2016; Pinson et al., 2010). However, some partial resistant cultivars are available commercially. Nonetheless, the development and the use of resistance cultivars as a long-term measure for curtailing the B.P.B. disease remains underexplored. Pinson et al., 2010 presented the first study of QTLs associated with B.P.B. partial resistance, where they identified twelve QTLs on seven chromosomes. Similarly, Mizobuchi et al., 2013 reported two QTLs, RGB1 and RGB2, on chromosomes 10 and 1, respectively. However, none of the identified QTLs could account for up to 30% of the total phenotypic variance observed. This infers the need for more germplasms to be screened for B.P.B. resistance and to identify additional QTLs associated with B.P.B. resistance.

The application of oxolinic acid at the boot to the heading stage of rice in multi-year field trials conducted in Louisiana in Texas has been shown to reduce B.P.B. severity by up to 88% (Groth et al., 2001; Zhou et al., 2011). This chemical is not registered in other rice-producing countries (Honsho et al., 2017). The excessive use of the chemical has led to the unfortunate development of resistant strains of the *B. glumea* (Hikichi et al., 1998). The other control measures attempted include biocontrol and cultural practices (Adachi et al., 2012; Wamishe et al., 2014). The most perceptible limitation of biocontrol is the need for high skills and lack of commercial availability (Honsho et al., 2017), while the control practices involve the application of fertilizer (Nitrogen) and the excessive use of which often increase the severity of B.P.B. disease (Wamishe et al., 2014). The existing control measures for managing the B.P.B. disease have attributed limitations that undermine their optimal use in controlling the disease.

Considering the existing knowledge gap and lack of comprehensive information, this paper reported a critical review on breeding for genetic resistance and other novel control measures. The recent information on pathogens responsible for the B.P.B., its detection, and the sign and symptoms of the disease were diligently studied. More so, the contemporary studies on the disease cycle and epidemiology of the B.P.B. were included. The geographical distribution, economic implication, and control measures observed to curtail the B.P.B. were all studied and reported. Besides, the limitations of the B.P.B. management strategies were highlighted.

2. Bacterial Panicle Blight Disease

Bacterial panicle blight disease is one of the important diseases impeding the average yield of rice in most countries (Ham et.al., 2011; Luo et al., 2007; Mirghasempour et al., 2018) Majorly, researchers considered B.P.B. as a seed-borne disease in which the infested seeds serve as the primary inoculum source (Cottyn et al., 2001; Nandakumar et al., 2009; Sayler et al., 2006; Zhou-qi et al., 2016). However, soil and irrigation water have also been reported to serve as source of inoculum for the disease. Lu and Allen 2012 reported a considerable amount of both soil and irrigation water analyzed to have tested positive for *B.glumae* and *B.gladioli*, which indicates that the source of the inoculum could as well be from soil and irrigation water. More so, Karki et al., 2012 concur that B.P.B. disease could be borne from the soil and the irrigation water applied. This essentially depends on the environmental conditions, particularly, temperature and relative humidity. Regardless of the source of inoculum, the symptoms, disease cycle, and epidemiology of the disease are identical (Mulaw et al., 2018).

2.1. Bacterial Panicle Blight (B.P.B.) Disease Symptoms

Symptoms of the disease include seedling blight, sheath rot, and panicle blight (Nandakumar et al., 2009). Besides, the lesion is observed on the flag leaf sheath and leaf margin which extends downwards linearly as the infection progresses. This linear lesion is characterized by a reddish-brown edge with a grey center which eventually became darker and necrotic in appearance (Ham et al., 2011). Researchers reported Panicles having one or more of their florets blighted with grains that do not fill or abort. The basal third of the florets may start as a white or light grey color divided from the rest of the floret by a reddish-brown margin, which subsequently becomes straw in color (Ham et al., 2011; Nandakumar et al., 2009; Wamishe et al., 2014). The tiller is the first part of the crop to exhibit symptoms of B.P.B. infection with the flag leaf sheath developing dark brown lesion. At this stage, such tillers may have the entire panicles damaged, with some or all the grains being aborted if adequate management is not taken to curtail the B.P.B. disease.

2.1.1. Symptoms on tillers and leaves:

Rice tillers infected with panicle blight disease exhibit a developed dark brown lesion on the flag leaf sheath; such tillers may bear panicles that are severely damaged, as shown in figure 1. Similarly, figure 1B shows a typical infected flag leaf, clearly presenting a linear lesion extending downwards from the blade collar to the initial part of the panicle. Nandakumar et al., (2009) reported such a feature indicating the B.P.B. infection. The secreted substances such as the toxoflavin or lipase may eventually spread to the adjacent parts, particularly the panicles (Kim et al., 2014). Similarly, Mizobuchi et al., (2016), reported that infected flag leaves could serve as a source of inoculum to the emerging panicles. The tendencies of the disease to get spread swiftly are due to the bacteria's extrinsic features and the presence of flagella, which aids its movement (Bashir et al., 2024; J. Kim et al., 2007). The necrotic brown lesion with a distinct margin on the flag leaf, which easily binge all over the essential parts of the crop, is another index showing that the possession of flagella by *B. glumae* aids in its mobility and is also an essential part of its pathogenesis (HAM et al., 2011; Ortega et al., 2020).

2.1.2. Symptoms on Panicles:

The specific symptom of B.P.B. is a blight on the panicles accompanied by the color change. The infected panicles eventually have blighted kernels that first look white to light gray with a dark brown margin on the bottommost third of the developing grains, which may be (Rangaraj Nandakumar et al., 2009; Pedraza, Bautista, & Uribe-Vélez, 2018). Figure 2A, B, and C is a typical illustration displaying the B.P.B. symptoms on the panicle at the early growth stage, rachis branches stage, and harvesting stage. The prevailing brownish color of panicles, as indicated in Fig 2a, resulted from the B.P.B. attack, which hinders the entire booting, flowering alongside pollination processes required for normal crop (rice) development (Wamishe et al., 2014). As the crop grows older to the rachis branches stage, the aborted dark brownish panicles become more prominent, as depicted in Fig 2b. From these figures (2 aand b), the infected brownish panicles in the latter are comparably more. This implies that early B.P.B. infection without adequate management could deteriorate the entire panicles of the rice crops. Also, the adverse effect of the B.P.B. on the rice at the harvesting stage is analogous to the symptoms at the rachis branches stages, only that the level of the aborted brownish panicles is more severe (Nandakumar et al., 2009). For instance, Figure 2c

presents the infected rice panicles at the harvesting stage, most of which contain no rice grains, and this is due to the pollen abortion at the earlier stages of development. More so, the growth of secondary fungi is often observed, though it may not necessarily contribute to the panicle's damages (Honsho et al., 2017). Thus, this could eventually reduce the gross yield of the rice crop.

Figure 1. Panicle blight symptoms on rice tiller (A), flag leaf sheath lesion (B), caused by bacterial panicle blight disease. The photo was taken seven days after inoculation. (Adapted from Bashir et al., 2024)

Figure 2. Bacterial panicle blight symptoms on rice panicles (A) early symptoms on panicles (B) at the early infection stage, the panicles turn reddish-brown (C) Infected kernels bear aborted seeds. The photo was taken seven days, 14 days, and 21 days after inoculation. **(Bashir *et al*., 2024).**

2.1.3. Symptoms on the grains:

The manifestation of the B.P.B. disease is often seen finally on the grains. Nandakumar et al.(2009), reported grain rot, unfilled or aborted grains, and reduction in milling quality as some of the damages caused by the B.P.B., resulting in the final yield

reduction (Li et al., 2016). Figure 3 compares the developmental symptoms between healthy and infected kernels of rice. Similarly, Wamishe et al., (2014) reported infected panicles having most of their kernels blighted due to B.P.B., which appears first as white to light gray with a dark-brown margin on the bottommost third of the developing grains (figure 3). Thus, Jeong et al.,(2003), report toxoflavin to be an indispensable factor in inducing the developmental symptoms on the seedlings and grains of the rice plant.

Figure 3. Comparison between healthy kernels (A), the early infected kernels (B), and Kernels with aborted seeds due to B.P.B. infection (C). **(Bashir *et al*., 2024)**

2.2. Disease Cycle and Epidemiology

Several researchers have reported scientific works on the B.P.B. disease cycle and its epidemiology (Cottyn et al., 2001; Kumar, Meshram, & Sinha, 2017; Zhou-qi et al., 2016). Consensus reached by the previous researchers revealed *B. glumae* and *B.gladioli* as the two bacterial pathogens responsible for B.P.B. infection in rice (Cottyn et al., 2001; Sayler et al., 2006; Syahri et al., 2019; Zhou-qi et al., 2016). *B. glumae* is a seed-borne pathogen detected in different sections of naturally infected plants. Comprising the epidermis, parenchyma, and sclerenchyma(Pedraza et al., 2018). Similarly, bashir et al., (2024) reported B.P.B. disease mainly as a seed-borne disease, but sometimes inoculum can thrive in soil. Studies conducted in Japan, China, the United States, and the Philippines revealed the presence of *B.glumae* and *B.gladioli* in a varied range of seed lots (Cottyn et al., 2001; Sayler et al., 2006; Zhou-qi et al., 2016). This suggests that the source of primary inoculum is from infected seeds. Mizobuchi et al., (2016), reported *B. glumea* cells present on leaf sheaths which may serve as a major source of inoculum to emerging panicles. It has also been observed that when the B.P.B. pathogen is injected into rice boots, visible symptoms appear first on the flag leaf sheath and subsequently progress onto the panicles(Bashir et al., 2024; Yuan, 2004). The primary infection site for *B. glumae* is through the plumules, and the bacterial invasion site is the lemma and paleae. The pathogen enters through the stomata, where it multiplies in parenchymatous intercellular spaces(Kumar et al., 2017), after which surrounding healthy tissues of the host are then infected. The bacterium utilizes the intermediate sugars in developing grains soon after colonizing and multiplying in the spikelets(Pedraza et al., 2018). The infection process of *B. glumae* was studied using real-time fluorescence quantitative PCR, revealing the pathogen capable of infecting the rice plant. It is directly through colonization of the vascular bundle of lateral roots, after which it spreads to the upper tissues such as leaf sheaths and leaf blade as an epiphyte through the vascular system(Li et al., 2016). In combination with high relative humidity and frequent rainfall, the warm night temperature has been identified as the predisposing environmental conditions that lead to higher chances of disease occurrence(Shew et al., 2019). Minimum average temperature $> 23^{o}C$ and moderate rainfall (<300mm/day) occurring during heading triggers the disease development (Syahri et al., 2019). Similarly, Lee et al., (2015), established that B.P.B. occurred

commonly when relative humidity was more than 95% for 24 hours during the rice flowering stage. Nandakumar et al. (2009) reported a temperature range of 35 to 40°C as the optimum for the growth of *B.gladioli* and *B.glumae*. In addition to rice crops, *B. glumae* has also been reported to affect other plant species, including tomato, sesame, eggplant, and hot pepper (Jeong et al., 2003).

3. Bacterial Panicle Blight Pathogens

Substantial literature has been published on the pathogens responsible for bacterial panicle blight disease in rice. In the 1950s, *Burkholderia glumae* (formerly *Pseudomonas glumae*) was first discovered in Japan and confirmed as the pathogen responsible for the grain rot, sheath rot, and seedling rot (Goto K, 1956; Kurita T, 1967). *B. glumae* has been reported as the major rice pathogen in many rice-producing countries cutting across Asia, Africa, North America, and Latin America. Researchers have characterized *B. glumae* as anaerobic, gram-negative, and motile bacteria with two to four flagella (Bashir et al., 2024; Singh Deepali and Vishunavat Karuna, 2015). It has a rod shape with major dimensions of 0.5-0.7 x 1.5 -2.5 µm in width and length, respectively (Figure 4). As depicted in Figure 4, the number of flagella present depends on the strain, and they thrive optimally at a temperature between 30 to 35 ^{o}C. A typical *B. glumea* strain has a G.C. composition of over 68.2% of the D.N.A. sequence(Schaad, 2008). Similarly, Hussain et al. (2020) reported 68.2 % G.C. content for the *B.glumae* strain (AU6208) genome sequence. Though, Seo et al. (Seo et al., 2015) reported a lower percentage composition with a magnitude of 67. 31% G.C. content for the BGR1 genome sequence. Essentially, the degree of similarity in genome size, the number of the coding sequence, and percentage G.C. content are the key index for determining the similarity between two or more bacteria strains (Hussain et al., 2020). Also, studies have substantiated that the *B. glumae* can be characterized morphologically on different media types such as semi selective medium and sucrose-phosphate-glutamate (S-PG). The pathogen can be characterized using biochemical method through cellular fatty acid analysis and molecularly through polymerase chain reaction(Jungkhun et al., 2022). Nandakumar et al., isolated and characterized strains in the pathogen complex by PCR in the Southern United States. They found 76 and 5 percent of the bacterial strain sampled

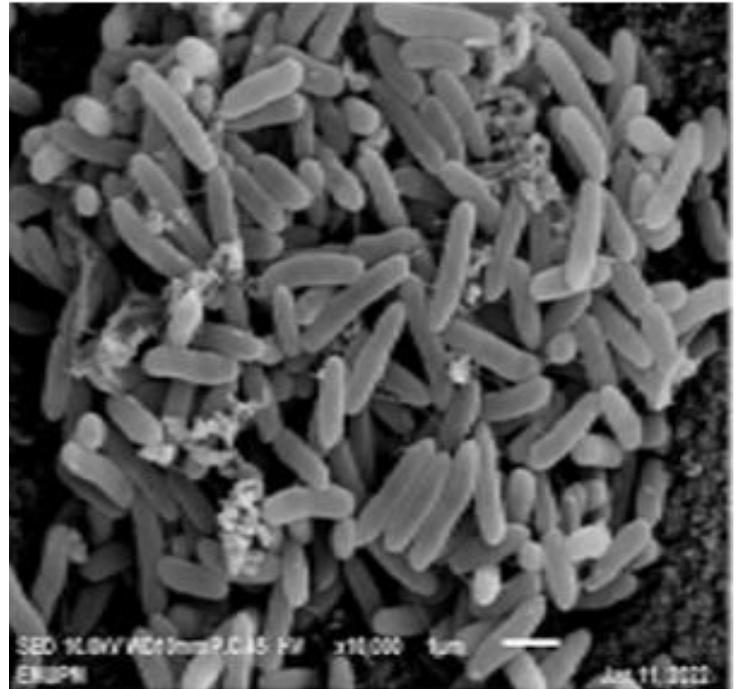to be *B.glumae* and *B.gladioli*, respectively (Nandakumar et al., 2009).

Figure 4: Rod-Shaped Structure of Bacterial Strain Viewed Under the Scanning Electron Microscope (SEM) (Bashir *et al*., 2024).

Their result demonstrated that the two strains were the major pathogens responsible for causing B.P.B. disease in the Southern United States. Thus, both *B. glumae* and *B. gladioli* were reported to produce similar symptoms on infected plants(Nandakumar et al., 2009), but the former is more frequently isolated and tends to be more virulent (Ham et al., 2011;Nandakumar et al., 2009). In addition to *B. glumae* and *B. gladioli*, Mulaw et al.(2018), reported *Burkholderia plantarii* as another causal pathogen of the B.P.B. disease.

3.1. Pathogenesis of B. glumae

B.glumae pathogenesis is a complex process involving several virulence factors (Zhou-qi et al., 2016). The main pathogenic determining factors of *B.glumae* were identified through several molecular genetic studies(Devescovi et al., 2007; S. Kim et al., 2014; Melanson et al., 2017). Zhou-qi et al. (2016) reported Pytotoxins and lipases as the fundamental factors influencing the severity of the pathogenesis of *B.glumae*. Collectively, researchers have shown that Pytotoxins exhibited a comparable higher influence

on the pathogenesis process compared to the lipases (Hussain et al., 2020; Hari and Ham, 2014; Ramachandran et al., 2021)

3.1.1. **Phytotoxins**

Kim et al. (2004) and Melanson et al. (Melanson et al., 2017) reported toxoflavin and fervenulin as the most perceptible Pytotoxins substances generated by the *B. glumae*, characterized to be isomerides. More so, the toxin substances are bright yellow, and their production rate depends on the growth temperature and evolutionary potential of *B. glumae* (Hussain et al., 2020). Researchers reported that *B.glumae* produces toxoflavin at a temperature of 30-38°C, which delays the growth of rice leaves and roots and induces chlorosis on the panicles(Jeong et al., 2003; Suzuki et al., 2004). However, at a temperature between 25 to 28 °C, the virulence activities of the bacterial are completely inhibited(Karki et al., 2012). This is because, at this temperature, the bacteria cannot generate the toxoflavin, which is the major factor responsible for the pathogenesis process. Similarly, Jeong et al., (2003), reported that fervenulin and toxoflavin are responsible for the pathogenicity of rice seedling grain rot due to reduced growth of leaves and roots in rice seedlings which may eventually contribute to chlorotic symptoms on rice panicles. Nonetheless, Devescovi et al., (2007), substantiated that *B. glumea* strains that at no time produce toxoflavin are incapable of causing an infection on the rice crop, particularly if the environmental condition does not favor the mutation process, which could revolutionize the pathogen to become virulent (Kim et al., 2004).

Studies have shown that the virulence properties of the pathogen are closely associated with the regulatory substances regulating toxoflavin biosynthesis and transport (Kim et al., 2004; Suzuki et al., 2004; Yoshinori, 2004). The tox operon responsible for the biosynthesis of toxoflavin is polycistronic and consists of five genes (toxA – tox E) and four genes (toxF to toxI), respectively. Also, toxR, which encodes a LysR-type transcriptional activator, is required for the biosynthesis of toxoflavin because mutant strains of *B.glumae* with disrupted toxR gene could not produce a significant amount of toxoflavin (Suzuki et., 2004). A common biosynthetic pathway is proposed for riboflavin synthesis to synthesize toxoflavin starting with the guanosine 5' – triphosphate (G.T.P.). Kim et al. (

2004) reported an additional gene, toxJ, coding for another transcriptional activator of toxoflavin biosynthesis, all regulated by quorum sensing (Q.S.). More so, the importance of the B. *glumae* quorum-sensing system in regulating toxoflavin biosynthesis was particularly demonstrated by Kim et al. (2004). They identified the quorum sensing system genes, tofl, and tofR, which encode the A.H.L. synthase for N-hexanoyl homoserine lactone (C6-HSL) C8-HSL and a cognate receptor for C8-HSL, respectively. However, it is noteworthy that very little is known about how *B. glumae* transport toxoflavin and protect themselves against it (Zhou-qi et al., 2016).

3.1.2. Lipases

Kang et al., (2008) reported lipases to be involved in *B. glumae* pathogenicity, with Lip A being the most important virulent-relative lipase. Lip B is another important lipase involved in Lip A synthesis and has a profound effect on the stability of the proteins for proteolytic degradation (Frenken et al., 1993; Khattabi et al., 2000). Similarly, Devescovi et al., (2007), reported that Ca2+ plays an important structural role in stabilizing the lipase of *B. glumae* under adverse conditions. This made it to be more destructive, as well as ensured resistance of the pathogen under detrimental conditions. Conversely, another study has also found that lipase production, like toxoflavin biosynthesis, by *B.glumae* is dependent on the Tofl/TofR quorum-sensing mechanism mediated by C8-HSL(Devescovi et al., 2007).
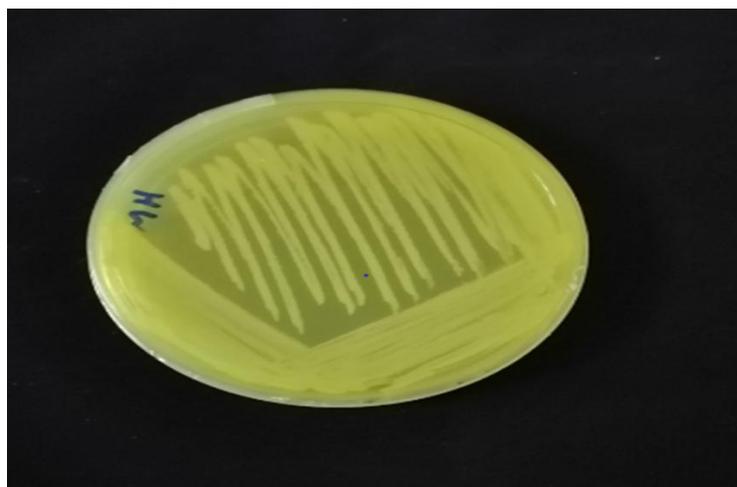

Figure 5. Production toxin (toxoflavin) by *B. glumae* evident by the yellow pigment on a King's B agar plate. The photo was taken 48 hours after inoculation at 37⁰ C. (**Bashir *et al*., 2024).**

3.2. Detection of B.P.B. Pathogen

Researchers have employed several methods to characterize and identify B.P.B. pathogen, including cultural approach, morphological technique, biochemical and molecular procedures(Bashir et al., 2024; Kawaradani et al., 2000; Mulaw et al., 2018; Zhou-qi et al., 2016). Despite the considerable efforts, these detection methods have their respective limitations (Mulaw et al., 2018).

3.2.1. Cultural, Morphological, and Biochemical technique

B. glumae can be grown in a wide range of culture media, including Luria-Bertani (L.B.) broth, SP-G medium, King's B Agar, and CCNT, among others (Ham et al., 2011; Kawaradani et al., 2000; Deepali et al., 2015). Kawaradani et al., (2000), developed and reported CCNT as a novel selective medium for *B. glumae* from rice seeds. Culture conducted at 41°C for 2 to 4 days produced several yellowish-white colonies of *B.glumae* alongside diffusible yellow pigments on the CCNT medium. Furthermore, they confirmed that the CCNT medium produces a comparable result as the S-PG medium. Noticeably, the CCNT cultural medium seems favorable for *B. glumae*. Other potential microorganisms were completely inhibited because of the selectivity medium, attributed to the symmetric composition of the grown colonies on the CCNT medium, thus testifying to its suitability for the *B. glumae*. Similarly, Mulaw et al., (2018) demonstrated the usefulness of artificial culture media to characterize B.P.B. in rice plants. They analyzed 178 rice panicles samples with CCNT semi-selective media, out of which 73 samples were reported to produce yellow colonies with symmetrical morphology. Based on the comparable color and the morphological appearance of an existing strain, the culture was attributed to *B. glumae*, causing the infection in the rice panicles. In another study, Deepali and Vishunavat (2015) used King's B agar medium for cultural identification of *B.glumae* in rice seeds. Similarly, whitish-grey or yellow colonies were predominantly observed on the medium. More so, Coenye and Vandamme (2003) noticed a similar grayish-white or yellow coloration around the grown colonies in the medium. It was substantiated that the yellow soluble pigments were due to the toxoflavin produced by *B.glumae*, as shown in Figure 6. From this figure, it is obvious that the yellowish toxoflavin is mostly found around the colonies. On the contrary, the portions devoid of colonies are free of the yellow pigments (toxoflavin). Similarly, Jeong et al., (2003) reported toxin (toxoflavin) production by *B.glumae*, evident by the yellow pigment on King's B agar plate. Morphologically, a scanning electron microscope examination revealed *B. glumea* as non-spore-forming, Gram-negative, a rod-shaped bacteria with cell-sized 0.5 to 0.7- 1.5 to 2.0 μm with rounded ends(Bashir et al., 2024).

Another method used to detect the causal pathogen of B.P.B. disease is a biochemical test. This method was used by Deepali and Vishunavat (2015) to identify the seed-borne bacterium, *B. glumae*. They reported that the bacterium tested positive in gelatin liquefaction, nitrate reduction, and K.O.H. solubility. At the same time, it gave a negative test in starch hydrolysis, oxidase reaction levan production, and arginine dihydrolase test. Similarly, Cottyn et al. (1996) reported that *B. glumea* tested positive in gelatin liquefaction, K.O.H., and nitrate reduction while negative in starch hydrolysis, oxidase reaction levan production, and arginine dihydrolase test.
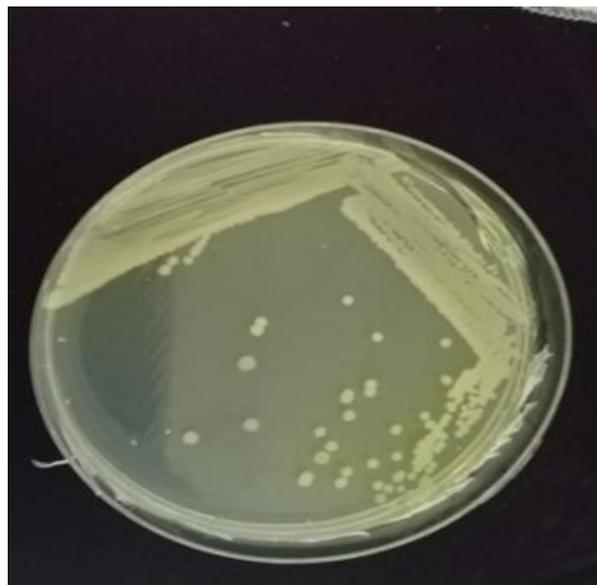

Figure 6. Colonies of *Burkholderia glumae* and production of yellow pigment (toxoflavin) by *B.glumae* on King's B agar. The photo was taken 48 hours after inoculation at 37^0 C. (**Bashir *et al*., 2024).**

However, it is noteworthy that identifying bacterial pathogen *B.glumae* based on colony morphology or disease symptoms is difficult and time-consuming because of the high similarity among *Burkholderia* spp.(Pinson et al., 2010).

3.2.2. Molecular procedures for detecting B.P.B. pathogen

In the effort to advance the existing B.P.B. pathogen detection technique, researchers have also attempted using molecular procedures with the sole aim to ensure accurate and rapid identification (Sayler et al., 2006). Comparably, identifying pathogens based on colony morphology, biochemical test, and disease symptoms are often difficult, unreliable, and time-consuming. This is due to the similar symptoms and features exhibited by other microorganisms, such as fungi (Maeda et al., 2006; Mulaw et al., 2018). Other *Burkholderia* species may exhibit similar symptoms and features; therefore, using only cultural, morphological, or biochemical tests could result in a false diagnosis of the actual causative pathogen responsible for the infection (Zhou-qi et al., 2016). For instance, Maeda et al. (2006) used a cultural method to investigate the presence of *B.glumae*, *B.plantarii*, and *B.gladioli*. At the end of the culture, all the *Burkholderia* species exhibited similar symptoms, thus making it very difficult to identify the pathogen responsible for the infection. However, using an alternative method with more reliability for pathogen identification became indispensable. A PCR technique was used to identify the constituted species and the particular pathogen responsible for the infection.

Besides, the use of PCR for detection has considerably gained attention, and it can perform specific amplification, which assists in diagnosing the pathogen accurately (Maeda et al., 2006; Mulaw et al., 2018; Ontoy et al., 2023). Salles et al., (2002), conducted a genetic identification of *Burkholderia* species using the 16S rRNA sequence by the PCR technique. Similarly, Hu et al., (2001), successfully identified the presence of *Burkholderia* species using this same PCR technique, but in this study, larger samples were considered. However, Yamamoto and Harayama (1998) observed that the discriminatory power of 16S rRNA was only able to detect the phylogenetic of the closely related pathogens. This necessitates the use of specific primers developed from gyrB sequences, and it was able to identify *B.glumae* and *B.gladioli* as the major causative pathogens in the rice materials. Other researchers have also attempted using these specific primers for pathogen detection, and remarkable results were reported. For instance, both Maeda et al., (2006) and

Mulaw et al., (2018) applied gyrB nucleotide sequences with PCR to identify the presence of *B.glumae*. In this investigation, a paired primer was used to detect the specific D.N.A. fragments corresponding to the gyrB nucleotide sequences, glu-FW (5'-GAAGTGTCGCCGATGGAG-3') and glu-R.V. (5'-CCTTCACCGACAGCACGCAT-3'). More so, another specific D.N.A. sequence was derived from gyrB and subsequently applied to identify the presence of *B. gladioli* (Mulaw et al., 2018). The paired primer used in this study is comparably distinct to the former, especially in the nucleotide sequence (gli- F.W. 5'- CTGCGCCTGGTGGTGAAG-3' and gli-RV (5'-CCGTCCCGCTGCGGATA-3'), and it successfully amplified the D.N.A. fragments confirming the presence of the *B.gladioli*. Based on these, it is evident that using a specific primer developed from the gyrB sequence for pathogen detection is more reliable than the 16S rRNA sequence. Besides the conventional PCR technique, Sayler et al. (2006) developed a real-time PCR (RT-PCR) technique to detect *B. glumae* isolates using specific primers. Similarly, Fang et al. (2009) developed an RT-PCR method to detect *B. glumae* using specific primers designed for ITS sequence. PCR detection technique distinguishes the constituted *Burkholderia* species based on their respective specific D.N.A. sequence. This shows that the PCR method uses the exhibited symptoms and the unique genetic properties of the pathogens for more accurate identification of the pathogen responsible for the infection.

3.3. Geographical Distribution and Economic Implication

B. glumae can induce up to 75% yield loss in severely infested fields (Honsho et al., 2017). The disease was first reported in Japan, which affected more than 69,000 ha in 2013 and 30 000 ha in 2015. Thus causing significant yield losses (JPPA., 2014, 2016). A survey conducted in Louisiana found the disease affecting about 60% of the rice fields, which made the disease one of the most important diseases economically in the United States (Groth, 2004). Similarly, in 1995, 1996, 1998, 2000, 2010, and 2011, significant yield losses from B.P.B. were reported in the Southern U.S.A. (Mulaw et al., 2018; Nandakumar et al., 2009; Zhou et al., 2011). Susceptible cultivars planted in Arkansas experienced severe B.P.B. disease resulting in yield losses of

almost 50% in 2010 (Mulaw et al., 2018). A yield loss estimate of about 10 -20% was also reported in 2010 in the rice belt of Texas as a result of the B.P.B. outbreak; a similar outbreak also occurred on rice produced under the organic production system in the same year (Zhou et al., 2011). In their study of disease-yield loss quantification, Zhou and Liu (2012) reported yield loss to B.P.B. ranging from 1-59% (83 to 4, 883kg/ha) at two locations in Texas. They also noted a yield loss increase of approximately 5% (453kg/ha) for each unit increase in disease severity using a rating scale of 0-9. (0 represents no symptoms, and 9 represents 100% affected kernels). In 2003-2013, an estimated yield loss of $61 million due to B.P.B. damage which could feed 1.1 million individuals annually, was recorded in the Mid-South U.S.A. (Honsho et al., 2017). Based on its pest risk analysis (P.R.A.), the People's Republic of China has listed *B.glumae* in its entry plant quarantine list because of its quarantine importance (Luo et al., 2007). In Malaysia, the Department of Agriculture of peninsular Malaysia (JPSM) reported 12 286.6 ha of paddy infested with B.P.B. across eleven states in the 2018 farming season, which necessitated the inauguration of a task force committee to proffer ways to manage the disease (Ramachandran et al., 2021). In Nigeria, Bashir et al., reported the incidence of BPB disease in Zamfara state, where a significant yield loss was recorded in the 2023 farming season (Bashir et al., 2024).

4. Control Measures

Current trends in the development of management techniques for B.P.B. have been reviewed, including exclusion, genetic resistance, use of chemicals, biological control, and cultural practices. Thus, not all the options above are available to control the disease in a given geographical area. Even so, to effectively manage the disease, the combination of two more of these options may be combined to reduce the damage caused by the disease.

4.1. Exclusion

In countries and regions where the B.P.B. pathogen is not present, plant quarantine and the use of pathogen-free seeds may serve as an important method to exclude the B.P.B. pathogen. This is always the first management option because the disease is not present in all rice-growing countries and regions. In California, the U.S.A., and China, plant quarantine laws were developed and implemented to prevent imports of the B.P.B. pathogen from foreign countries (Zhou-qi et al., 2016). Certified seeds free of B.P.B. pathogen are another effective method to exclude the disease from disease-free geographical areas. Besides, several molecular detection methods, including PCR, were developed to help certify B.P.B. pathogen-free seeds(Maeda 2004; Syahri et al., 2019). In most countries where PCR identification of B.P.B. pathogens in certified seeds has not been achieved, farmers are discouraged from using seeds harvested from fields that have been affected with the disease the previous year. This could help in the disease management process.

4.2. Genetic Resistance

In almost all the countries afflicted by the disease, there have been several attempts to develop resistant cultivars as a long-lasting response to the B.P.B. disease. However, no single Quantitative Trait Loci (QTLs) or genes for complete resistance have been found to date (Mirghasempour et al., 2018; Mizobuchi et al., 2016; Pinson, 2010; Protic et al., 2024) Thus, cultivars showing partial resistance are commercially available in some countries. Three cultivars showing partial resistance were found out of nine screened in a field study conducted in 1975 when the B.P.B. resistant breeding research effort began (Goto, 1975). In 1983, 292 cultivars and lines were tested in a greenhouse inoculation trial, none of which were confirmed to be B.P.B. resistant (Tabei et al., 1983). Of the 798 cultivars and breeding lines screened from 1985 to 2013, only 28 cultivars and lines exhibited partial B.P.B. resistance (Imbe et al., 1986; Mizobuchi et al., 2016, 2013; Mogi, 1985; Yasunaga et al., 2002). In their recent paper titled "Evaluation of major Japanese rice cultivars for resistance to bacterial grain rot caused by *Burkholderia glumae* and identification of standard cultivars for resistance," Mizobuchi et al. (Mizobuchi et al., 2020) found Kale and Jaguary as the two tropical japonica cultivars, showing a high level of resistance and numerous indica cultivars showing a moderate resistance level. They recommended using these cultivars as a good source of resistance to B.P.B. for cooperation into the B.P.B. resistance breeding program in Japan.

Similarly, from the southern United States, a large number of cultivars and elite breeding lines are annually evaluated through a joint research initiative in a Uniform Rice Research Nursery (URRN) in search of cultivars with high yield potential and novel resistant sources to B.P.B. and other diseases

affecting rice crop(Marsjan et al., 2007; Nandakumar et al., 2007). Even then, most cultivars and lines tested in the multi-year trials were susceptible to B.P.B. Except for certain hybrids and a, few inbreed lines showing partial resistance(Ontoy et al., 2023).

4.2.1. Quantitative trait Loci (QTL) mapping for B.P.B. resistance

The first study of B.P.B. rice resistance QTLs was first reported by Pinson et al., (2010). In this study, 12 QTLs were identified using a bi-parental population of 300 recombinant inbred lines (R.I.L.s). These QTLs were identified on seven chromosomes, out of which eight of the resistant alleles were originated from TeQing, a resistant cultivar from China, and the remaining four alleles from the susceptible American cultivar "Lemont." Similarly, another QTL for bacterial grain rot resistance (RBG2) was detected on chromosome one using backcross inbred lines (B.I.L.s) originating from a cross between Kele (resistant to B.P.B.) and Hitomebore (Susceptible) cultivars all from Japan (Mizobuchi et al., 2016, 2013). Also, RBG1 was identified on chromosome 10 using chromosome segment substitution lines (CSSLs) which were reported to be the first and the only QTL associated with seedling rot resistance caused by B .glumea (Mizobuchi et al., 2013).

4.3. Chemical control

Oxolinic acid (5-ethyl-5,8-dihydro-8-oxo-[1,3]dioxolo[4,5-g]quinoline-7-carboxylic acid, Starner ®), which is a quinolone derivative, is the first chemical recorded to be very effective for managing rice B.P.B. disease. This bactericide was first used in Japan in 1989 to combat rice seedling rot and grain rot (Hikichi et al., 1989). At the heading stage, foliar sprays with oxolinic acid have been reported as the best method for successfully managing seedling and grain rot (Hikichi, 2001). Similarly, it has been reported that oxolinic acid can inhibit *B.glumae* multiplication on spikelets by applying it at the rice heading stage, thereby combatting B.P.B. disease. In the multi-year trials conducted in Louisiana, reports showed that oxolinic acid could minimize B.P.B. severity by up to 88 percent when applied during boot to the heading stage(Groth et al., 2011). In Japan, oxolinic acid has been used for BPB management for more than two decades (Maeda et al., 2007), leading to *B.glumae* populations being resistant to oxolinic acid (Hikichi et al., 2001).

Oxolinic acid-resistant bacterial species have also been reported to be cross-resistant to other quinolone derivatives (Hikichi et al., 1998). Remarkably, one of the reasons affecting its use for the control of B.P.B. in rice is the rise in the occurrence of resistant *B.glumae* strains to oxolinic acid. Moreover, in many rice-growing countries, including Nigeria, the bactericide is not available for use. In addition to Oxolinic acid, bactericides containing copper compounds have also been found useful for B.P.B. management in rice (Groth et al., 2001).

4.4. Biological control

Another management option for controlling B.P.B. of rice is biological control methods. Bacterial antagonists for the control were first conducted in Japan under field conditions (Tsushima, 1991). Similarly, rice-associated bacteria (R.A.B.s), mostly *Bacillus* spp. showing a varying degree of antagonistic activities against *R. solani* and *B.glumae* was also reported by Shrestha et al. (Shrestha, 2016). A strain of *Pseudomonas* sp. was also effective in suppressing seedling rot when pretreated onto rice seeds before planting (Inoue et al., 2001). Treatment of rice seed with *B. glumae* avirulent strains was also found to significantly suppress the incidence of bacterial seedling rot of rice caused by the virulent strains (Furuya, 1989). Similarly, Miyagawa and Takaya reported the application of the *B. gladioli* avirulent strains on rice panicles to be very effective in reducing B.P.B. severity (Miyagawa and Takaya, 1987). Besides, the application of two strains *of Bacillus* sp. with antibacterial activities were found to reduce B.P.B. severity by up to 50 percent and increase yield by more than 11 percent when applied to rice at the flowering stage (Pedraza, 2021; Zhou et al., 2011). In addition to the bacterial antagonist, Bacteriophages (viruses infecting bacteria) that are particularly selective to the target bacteria have been regarded as possible biocontrol agents. Three strains of phages that lyse *B. glumae* and *B. plantarii* have been isolated and shown to inhibit both seedling rot and seedling blight even better than the existing pesticide and bactericide (Adachi et al., 2012).

4.5. Cultural practice

To date, little research has been carried out to search and establish cultural activities that could decrease the occurrence and severity of B.P.B. in rice (Honsho et al., 2017). Thus the susceptibility of the rice plant to B.P.B. is increased due to a high level of nitrogen

fertility and excessive seeding rate. It has also been shown that avoiding excessive nitrogen use can limit the damage caused by B.P.B. disease (Wamishe, 2014). Studies conducted in Arkansas show that the use of a high rate of nitrogen, for example (247kg/ha), is capable of increasing B.P.B. severity 1.6 times compared to a low application rate, say (168kg/ha) when applied during booting to heading stage of rice. Another way to reduce the damage caused by the disease is to use early maturing rice cultivars or to plant early to avoid the hottest times of the growing season (Honsho et al., 2017).

5. Concluding remarks and future perspectives

The majority of the recent findings collectively indicated *B. glumea* and *B. gladioli* are the most common pathogens responsible for B.P.B. disease in rice crops. *B. plantarii* is also considered a causative pathogen of the B.P.B. disease but rarely occurs compared to the previous two. Among these pathogens, *B. glumea* is highly destructive due to the secretion of toxic substances such as toxoflavin and lipase. Warm night temperature and high relative humidity are the environmental conditions that predispose rice to the disease outbreak. The B.P.B. disease is expected to be more widespread globally with global warming and do more harm in the future. B.P.B. presents some difficulties in diagnosing based on symptoms on the panicles, unlike other rice diseases, such as sheath blight and blast. Abiotic and biotic factors, such as heat, insect disruption, and secondary microorganisms, may cause similar symptoms. More so, the disease frequently progresses after the heading stage, and no symptoms or signs can be detected until heading, making it impossible to establish some form of scouting to diagnose and forecast the progression of the disease. There is currently no commercially standardized seed treatment method to eradicate or reduce the pathogen population in rice crops. In most rice-producing countries, including Nigeria, chemical remedies for managing the disease are not available for use.

Moreover, the excessive use of oxolinic acid (a bactericide) in Japan and a few other rice-growing countries has led to the unfortunate emergence of *the B. glumae* population that is chemical resistant. The use of avirulent strains of *B. glumae* and bacteriophage in B.P.B. control is demonstrated for scientific purposes only. It is not commercially available for use by farmers. To date, there are no commercially available BPB-resistant rice cultivars for use in most rice-producing countries, and only partially resistant cultivars have been reported. The coordinated use of the available control measures for the management of B.P.B. disease is thus, for the time being, the only alternative available. Plant quarantine, to exclude B.P.B. pathogens from disease-free countries and use of pathogen-free certified seeds can also be another successful method to contain the disease.

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