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Efficacy of a peracetic acid liquid formulation for controlling bacterial grain rot and bakanae disease and the underlying mechanisms

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Abstract

The peracetic acid (PAA) liquid formulation consists of 10% peracetic acid, 21% acetic acid, 17% hydrogen peroxide, and 52% water; these components are derived from food additives. Its broad antimicrobial spectrum and strong bactericidal effects make the PAA liquid formulation suitable for use as a seed disinfectant. In this study, PAA treatment methods and the efficacy of each component for controlling bacterial grain rot were evaluated. Soaking seeds for 24 h in a 100-fold diluted solution before seed treatments and soaking seeds in a 300-fold diluted solution prior to germination effectively controlled disease, with minimal phytotoxicity. Notably, hydrogen peroxide alone was ineffective. Acetic acid and a mixture of acetic acid and hydrogen peroxide were less effective than a mixture comprising peracetic acid and acetic acid. The study results revealed that the efficacy of treatments for controlling bacterial grain rot was due to peracetic acid. Additionally, treatment effects on bakanae disease were examined using the same methods. Treatments were effective for seeds with low disease incidence, but this efficacy was unstable when the disease incidence was high. Subsequently, the combined effects of treatment concentrations and durations were analyzed. Soaking seeds in a 100-fold diluted solution for 24 h the day before germination was highly effective, with minimal phytotoxicity. This treatment also high control efficacy against bacterial grain rot. However, application at advanced germination stages induce phytotoxicity.

Introduction

There are multiple seed-borne rice diseases. Seed disinfection is crucial for controlling bacterial grain rot caused by *Burkholderia glumae* and bakanae disease caused by *Fusarium fujikuroi*. Seed disinfection methods for controlling these seed-borne diseases include treatments with hot water, microbial pesticides, and synthetic chemical pesticides. When applied individually, these treatments are inconsistent in terms of their efficacy for controlling seed-borne diseases (Nakajima 2023). Although synthetic fungicides are relatively simple to apply and highly effective, demethylation inhibitors are usually necessary to control bakanae disease. Recently, resistance to prochloraz and pefloxate has been reported (Li et al., 2023; Nakajima et al., 2025). Furthermore, to mitigate their harmful effects on the environment, synthetic disinfectants require careful liquid waste management and disposal, which can be costly. Thus, rice seed disinfectants should ideally be suitable for organic cultivation in addition to being highly effective, simple to use, and inexpensive to apply.

Peracetic acid (PAA) is a compound (CH_3COOOH) formed in an equilibrium reaction involving hydrogen peroxide (H_2O_2) and acetic acid (CH_3COOH) ($CH_3COOH + H_2O_2 \rightleftharpoons CH_3COOOH + H_2O$). Therefore, PAA does not exist in isolation. More specifically, a PAA solution comprises 10% PAA, 21% acetic acid, and 17% hydrogen peroxide. Although derived from food additives, PAA has potent bactericidal activity and is used as a sterilant for PET bottles and food products (Zoellner et al. 2018). Its primary mechanism of action involves multiple targets, resulting in oxidative damage to cellular enzymes and disruption of cell membranes (Kataria et al. 2020; Tutumi et al. 1973). Considering this mechanism, there is likely a low risk of fungi developing resistance to PAA. Additionally, its origin as a food additive implies that it is safe

for humans and the environment. According to earlier research, PAA solutions can effectively control post-harvest diseases in blueberries (Saito et al. 2021). In terms of seed-borne diseases, research has been conducted on the efficacy of PAA solutions for managing bacterial fruit spot disease in cucurbit via seed treatments (Hara et al. 2006). In a previous study on rice, PAA was applied as a foliar treatment to evaluate its utility for managing bacterial leaf blight and sheath blight under field conditions (Jo et al. 2019). However, the efficacy of PAA for controlling seed-borne rice diseases remains to be investigated. Moreover, preliminary *in vitro* tests showed that PAA can effectively control *B. glumae* and *F. fujikuroi*. However, the optimal treatment method and efficacy of PAA solutions for disinfecting rice seeds are unclear. As mentioned above, a PAA solution consists of PAA, acetic acid, and hydrogen peroxide, but the efficacy of each solution component is unknown. PAA is a compound formed by an equilibrium reaction between hydrogen peroxide and acetic acid, and does not exist independently. Notably, catalase added to a PAA solution can decompose hydrogen peroxide (Chance 1949). Acetic acid and hydrogen peroxide exist independently. This study was conducted to develop the most effective PAA treatment protocol for controlling bacterial grain rot and bakanae disease and to clarify the contribution of each chemical component.

Materials and methods

1. Bacterial grain rot

Using infected rice seeds, we evaluated treatment conditions as well as the efficacy of a PAA solution (Hodogaya Chemical Industry Co., Ltd., Tokyo, Japan) for controlling bacterial grain rot. A total of 19 tests were conducted in a glasshouse at the Nagano Prefectural Agricultural Experiment Station from 2014 to 2020. Treatment concentrations, timings, and soaking durations are provided in Table 1a. Ipconazole-copper wettable powder (IpCu) (Kumiai Chemical Industry Co., Ltd., Tokyo, Japan) in a 200-fold diluted solution was used for a 24-h pre-soaking treatment and *Trichoderma atroviride* wettable powder (DJ) (Kumiai Chemical Industry) in a 200-fold diluted solution was used for a 24-h soaking treatment during the germination stage (control treatments).

Infected seeds were obtained by spraying spikelets during the flowering stage with a bacterial suspension (1×10^8 cfu/ml, 50 l/10 a) prepared using the Iiyama11 strain isolated from diseased spikelets collected in Nagano prefecture. For tests, infected seeds were mixed with healthy seeds at a weight ratio of 3%–30%. The rice varieties used for tests were ‘Koshihikari’ or ‘Akita Komachi’.

(1) Seedling test

Each test was performed using conventional seedling trays with 1/25-sized plastic cups (87 × 87 × 45 mm), with three replicates per treatment. In each cup, 8 g seeds were sown.

(2) Pre-soaking treatment and germination treatment

Seeds were placed in 50 ml beakers for the pre-soaking treatment and germination treatment. The ratio was 8 g seeds to 15 ml chemical solution for the pre-soaking treatment and 30 ml for the germination treatment. Seeds were then soaked in 30 ml distilled water for 5 days at 15°C in a constant-temperature chamber, which was followed by a 24-h germination or germination treatment at 32°C.

(3) Seedling cultivation

Each cup was filled with 160 ml granular potting soil (Shinano Potting Soil Co., Ltd., Nagano, Japan), which was moistened with 40 ml water. After sowing, seeds were covered with 40 ml granular potting soil. Cups were incubated at 32°C for 2 days to allow seeds to germinate. They were then transferred to an incubator (25°C; 5,000 lx under metal halide lamps) for 1 day for greening. Cups were subsequently placed in a concrete water tank (320 × 70 × 10 cm) in a glasshouse and bottom-irrigated twice daily. The glasshouse was set to open its skylights when the indoor temperature reached 30°C and to heat when the indoor temperature decreased below 15°C.

(4) Disease assessment

At 2 weeks after sowing, the disease index of all seedlings in each cup was determined using the following scale: 0: healthy; 1: mildly diseased (height of diseased seedlings ≥ 1/3 that of healthy seedlings); 2: severely diseased seedlings (height of diseased seedlings < 1/3 that of healthy seedlings); 3: dead or wilted seedlings.

Disease severity was calculated as follows: ∑(number of seedlings for a disease index × disease index)/(total number of analyzed seedlings × 3) × 100. Disease severity was averaged for three replicates. To integrate and compare results across tests, the control value was calculated using the following formula: 100 − (disease severity of each treatment/disease severity of the untreated control) × 100.

2. Mechanism of action

To identify the active components of the PAA solution, efficacy tests were conducted using the compositions listed in Table 2. Tests involved either a 24-h pre-soaking treatment in a 100-fold diluted PAA solution or a 24-h soaking treatment in a 300-fold diluted PAA solution during the germination stage. A PAA + acetic acid solution was prepared by adding 0.5 ml catalase (Hodogaya Chemical Industry) to 100 ml 100-fold diluted PAA solution to decompose hydrogen peroxide. An acetic acid solution, hydrogen peroxide solution, and acetic acid + hydrogen peroxide solution were prepared using 80% acetic acid (Hodogaya Chemical Industry) and 35% hydrogen peroxide (Hodogaya Chemical Industry), with concentrations adjusted as required. The pre-soaking test was conducted in 2017 under the conditions described above. Test seeds were prepared by mixing ‘Akita Komachi’ seeds inoculated at the booting stage in 2013 with healthy seeds at a 30% weight ratio. Disease assessments were conducted at 16 days after sowing using the criteria described above to compare the effects of each treatment on disease severity and seedling disease percentage. The seedling disease percentage was

analyzed by Tukey's Honest Significant Difference (HSD) test after root square transformation. The germination-stage test was similarly conducted in 2018. Test seeds were prepared by mixing 'Koshihikari' seeds inoculated at the booting stage in 2012 with healthy seeds at a 10% weight ratio. Disease assessments were conducted at 19–20 days after sowing and evaluated as described above for the pre-soaking treatment.

3. Bakanae disease

Infected seeds were used to assess the effectiveness of treatments with PAA solutions for controlling bakanae disease. A total of 22 tests were conducted between 2015 and 2020 in a glasshouse at the Nagano Prefectural Agricultural Experiment Station. PAA solution concentrations, treatment timings, soaking times, and number of tests for each test condition are provided in Table 1b. The control treatment was as described above. Naturally infected seeds were produced by transplanting naturally infected seedlings into fields and then cultivating them using conventional practices. Flowering stage-inoculated seeds were produced by spraying 'Koshihikari' plants at the flowering stage with a conidial suspension (1×10^6 conidia/ml, 40 l/10 a) prepared using the GF2017-45 strain isolated from diseased plants. Flowering stage-inoculated seeds were mixed with healthy seeds at a weight ratio of 5%–50%. Naturally infected seeds alone or mixed with healthy seeds at a weight ratio of 5%–50% were used for the test.

The temperature was set at 28°C for germination or the germination treatment. After sowing, seeds were allowed to germinate during a 3-day incubation in a constant-temperature chamber set at 28°C. To prevent seedling elongation under low-light conditions during the test period, a 20-fold diluted solution containing the dwarfing agent prohexadione calcium salt hydrate (Kumiai Chemical Industry) was applied (20 ml per cup) during the greening process. Seedlings were incubated for 1–2 days at 25°C under constant light to promote greening. Other seedling cultivation conditions were identical to those used in the aforementioned bacterial grain rot test.

At 19–21 days after sowing, each cup was examined to determine the number of dead seedlings, elongated seedlings, and healthy seedlings. The total number of dead and elongated seedlings was used as the number of diseased seedlings to determine the diseased seedling percentage (i.e., number of diseased seedlings/all seedlings in a cup × 100) for three replicates, after which the average was calculated.

To integrate and compare the results of each test, the control value was calculated using the following formula: 100 − (diseased seedling percentage of each treatment/diseased seedling percentage of the untreated control) × 100.

4. Evaluation of PAA treatment effects on bakanae disease using severely infected seeds

We analyzed PAA solution treatment conditions in terms of their effects on bakanae disease using highly infected seeds. Generally, high concentrations and long soaking times increased treatment efficacy, but they also increased the risk of phytotoxicity. Considering that rice seeds are not highly susceptible to phytotoxicity before germination, we evaluated methods involving a high-concentration treatment either before seed soaking or immediately before germination (period from the end of the soaking period to immediately before germination was induced). PAA solution concentrations, timings, and soaking durations are listed in Table 3. For the soaking treatment involving a 10-fold diluted PAA solution, seeds were placed in non-woven fabric for processing. For the water-rinsing treatment, seeds in the non-woven fabric were placed under running water for 10 min. For the pre-germination treatment, seeds were soaked at 15°C for 5 days and then treated before they germinated. Subsequently, germination was induced by incubating seeds at 28°C for 24 h. Test rice seeds were naturally infected 'Koshihikari' seeds harvested in 2018. Tests were conducted in 2021 under the conditions described above.

5. Repeated analysis of pre-germination treatments

After soaking seeds for 5–6 days, they were used for a pre-germination treatment, which involved soaking them in a 100- or 200-fold diluted PAA solution for 24 h 1 day before germination. Tests were conducted four times from 2022 to 2023. Test rice seeds were naturally infected 'Koshihikari' seeds harvested in 2021. Other test conditions were as described above.

6. Relationship between the germination percentage and phytotoxicity during the pre-germination treatment

At the famer, not always possible to properly control the seed soaking temperature. Therefore, the relationship between the germination percentage before the pre-germination treatment with the PAA solution and phytotoxicity was investigated in 2024 using healthy 'Koshihikari' seeds. The temperature during the 6-day seed-soaking period was set at 12, 14, 16, 18, and 20°C. The accumulated temperature during the soaking period was 72, 84, 96, 108, and 120°C. The untreated control group underwent the same soaking treatment at 15°C. After 6 days of soaking, seeds were used for a pre-germination treatment, during which they were soaked in a 100-fold diluted PAA solution for 24 h at 15°C. Subsequently, seeds were germinated at 28°C for 24 h. After sowing, seeds were placed in a constant-temperature chamber and emerged for 3 days at 28°C. Seedlings were then grown in a greenhouse. Each treatment was completed using three replicates of 200 seeds. Before the pre-germination treatment, all seeds were examined to determine the number of germinated seeds. The germination percentage was calculated as follows: number of germinated seeds/total number of seeds × 100. At 17 days after sowing, the number of emerged seedlings in each cup was recorded. The emerged seedling percentage was calculated as follows: number of emerged seedlings/total number of seeds sown × 100. Additionally, for each treatment, seedlings shorter than one-third of the average seedling height in the untreated control group were considered as stunted seedlings. The stunted seedling percentage was calculated as follows: number of stunted seedlings/total number of seedlings × 100.

7. Relationship between the seed-soaking temperature before the pre-germination treatment and the effective control of bakanae disease

The relationship between the seed-soaking temperature before the pre-germination treatment involving the PAA solution and the effective control of bakanae disease was investigated. Test conditions were the same as those used for the aforementioned phytotoxicity tests, which were conducted simultaneously. Test rice seeds were naturally infected 'Koshihikari' seeds harvested in 2023. The diseased seedling percentage was calculated at 25 days after sowing (as described in Section 3. Bakanae disease).

Results

1. Bacterial grain rot

The integrated results of all tests, expressed relative to untreated control values, are shown in Fig. 1. Disease severity for the untreated control ranged from 18.2 to 89.4, indicating that all tests were conducted under moderate-to-severe disease conditions. For the pre-soaking treatment, the 100-fold diluted solution was highly effective, which was in contrast to the unstable effects of the treatments with the 200- or 400-fold diluted solutions and the short-term soaking treatment in a 10-fold diluted solution. For the germination-stage treatment, soaking in 200- to 400-fold diluted solutions was highly effective. By contrast, the pre-soaking treatment in a 100-fold diluted solution and germination-stage treatment in a 200-fold diluted solution occasionally had minor phytotoxic effects (e.g., slight decrease in seedling emergence). The 4,000-fold diluted solution was relatively ineffective. Additionally, the effects of the pre-soaking treatment in a 100-fold diluted solution and the germination-stage treatment in a 300-fold diluted solution were nearly the same as the effects of DJ, but were superior to the effects of IpCu.

2. Mechanism of action

During the pre-soaking test, the untreated control was severely infected, with a disease severity of 63.0 and diseased seedling percentage of 80.5% (Fig. 2a). By contrast, the untreated control was less severely infected during the germination-stage test, with a disease severity of 38.2 and diseased seedling percentage of 59.8% (Fig. 2b). For both tests, treatments with the PAA solution or PAA + acetic acid solution were highly effective for controlling disease, with the effect of the PAA + acetic acid solution tending to be greater than that of the PAA solution. The acetic acid solution tended to be less effective than the PAA + acetic acid solution. The hydrogen peroxide solution had no detectable effect.

3. Bakanae disease

Considering that treatment efficacy varied depending on incidence conditions, results are presented using low-incidence conditions (diseased seedling percentage < 40%) and high-incidence conditions (diseased seedling percentage ≥ 40%) for the untreated control (Fig. 3a, b). For the untreated control, disease incidence ranged from 12.9% to 33.1% under low-incidence conditions, whereas it ranged from

43.6% to 98.9% under high-incidence conditions. Under low-incidence conditions, the pre-soaking treatment in a 100-fold diluted solution and the germination-stage treatments in 200- and 300-fold diluted solutions were highly effective for controlling disease, similar to effects of the control chemical. However, the pre-soaking treatment in a 100-fold diluted solution and the germination-stage treatments in 200- and 300-fold diluted solutions were less effective under high-incidence conditions than under low-incidence conditions. Moreover, under high-incidence conditions, these treatments were less effective than the treatment with the control chemical. Additionally, the pre-soaking treatment in a 100-fold diluted solution and the germination-stage treatment in a 200-fold diluted solution occasionally had minor phytotoxic effects (e.g., slight decrease in seedling emergence).

4. Evaluation of PAA treatment effects on bakanae disease using highly infected seeds

For the untreated control, the diseased seedling percentage was 99.2% (Fig. 4a). A 10-min pre-soaking treatment in a 10-fold diluted solution was relatively ineffective regardless of whether a rinsing step was performed. Notably, a 30-min pre-soaking treatment in a 10-fold diluted solution was relatively ineffective when a rinsing step was included, but it was highly effective when the rinsing step was excluded. However, this treatment resulted in a clarify decrease in the number of seedlings. Furthermore, 24-h pre-germination treatments using a 100- or 200-fold diluted solution were highly effective and had no phytotoxic effects. Pre-germination treatments were less effective than the IpCu treatment, but were more effective than the DJ treatment.

5. Repeated examination of pre-germination treatments

The diseased seedling percentage for the untreated control ranged from 85.8% to 91.7%, indicating high disease incidence under all conditions (Fig. 4b). Across the four pre-germination tests, the treatment with a 100-fold diluted solution was consistently highly effective, similar to the effects of the control agent. Although the treatment with a 200-fold diluted solution was also effective, its efficacy was less stable than that of the treatment with a 100-fold diluted solution. Phytotoxic effects were undetectable in all pre-germination treatments.

6. Relationship between the germination percentage and phytotoxicity during the pre-germination treatment

Increases in the seed-soaking temperature tended to increase the germination percentage during the pre-germination treatment, resulting in increases in the stunted seedling percentage and decreases in the emerged seedling percentage (Table 4). At soaking temperatures of 12 and 14°C, rice seed germination was undetectable. Increasing the temperature to 16°C had slightly germination was observed, but the emerged seedling percentage and stunted seedling percentage were nearly the same

as the corresponding percentages of the untreated control. Soaking temperatures of 18 and 20°C clearly induced germination. A comparison with the untreated control revealed increases and decreases in the stunted seedling percentage and emerged seedling percentage, respectively.

7. Relationship between the seed-soaking temperature before the pre-germination treatment and the effective control of bakanae disease

For the untreated control, the diseased seedling percentage was 92.8% (Table 4). A high soaking temperature before the pre-germination treatment resulted in a low diseased seedling percentage (i.e., increased efficacy).

Discussion

In this study, we first optimized PAA treatment conditions for controlling bacterial grain rot. A 24-h pre-soaking treatment in a 100-fold diluted solution and a 24-h germination-stage soaking treatment in a 200-, 300-, or 400-fold diluted solution were highly effective. However, minor phytotoxic effects, including a slight decrease in seedling emergence, were occasionally observed following the pre-soaking treatment in a 100-fold diluted solution and the germination-stage soaking treatment in a 200-fold diluted solution. Although this phytotoxicity may be acceptable, a pre-soaking treatment in a 150-fold diluted solution and a germination-stage soaking treatment in a 300-fold diluted solution may be more appropriate. However, considering the unstable efficacy of the pre-soaking treatment in a 200-fold diluted solution, the effectiveness of a pre-soaking treatment in a 150-fold diluted solution should be verified.

The contributions of the individual components of the PAA solution (10% PAA, 21% acetic acid, and 17% hydrogen peroxide) to the observed treatment effects were unclear. Therefore, on the basis of the effects of the pre-soaking treatment in a 100-fold diluted solution and the germination-stage soaking treatment in a 300-fold diluted solution, we examined the effects of each component on bacterial grain rot. According to two tests (pre-soaking and germination-stage treatments), the PAA solution and PAA + acetic acid solution were highly effective, but the PAA + acetic acid solution tended to be more effective. The acetic acid solution was somewhat effective, but tended to be less effective than the PAA + acetic acid solution. The hydrogen peroxide solution was ineffective. According to these results, the efficacy of the PAA solution may be largely attributable to PAA. Furthermore, removing hydrogen peroxide from the PAA solution may enhance efficacy, but this would require the addition of catalase, which is impractical for routine use. Alternatively, the composition ratio of the PAA solution (10% PAA, 21% acetic acid, and 17% hydrogen peroxide) may be modified relatively easily. Therefore, increasing and decreasing the PAA and hydrogen peroxide contents, respectively, may increase efficacy, but this will need to be experimentally verified.

The efficacy of the PAA solution for controlling bakanae disease varied depending on the degree of seed contamination. The pre-soaking treatment in a 100-fold diluted solution and the germination-stage soaking treatments in 200- or 300-fold diluted solutions, which effectively controlled seedling rot, were

highly effective under low-incidence conditions, with an efficacy that was nearly equivalent to that of the control chemical. Notably, under high-incidence conditions, efficacy decreased for all treatments, and was lower than that of the control chemical. Bakanae disease incidence is influenced by various factors, including seed-borne infection load, seedling-raising equipment contamination levels, and temperature management during the seed soaking-to-seedling raising period (Fuji et al. 2015, Sasahara 2013). Considering that sterile equipment was used in this study, inoculum sources other than seeds were unlikely. The temperature until greening was constant in the incubators used in this study, thereby minimizing temperature effects. Therefore, seed infection levels likely significantly influenced disease incidence. Under low-incidence conditions, naturally infected seeds produced before 2008–2016 were used in 11 of 16 tests. Under high-incidence conditions, naturally infected seeds or flowering stage-inoculated seeds produced in 2018 and 2019 were used in 15 of 17 tests. Generally, seed-borne infection levels decrease over time after seeds are collected, with disease incidence lower for naturally infected seeds than for flowering stage-inoculated seeds. Thus, the seed infection status may influence treatment efficacy.

PAA gradually decomposes upon contact with organic matter. In preliminary tests, the PAA residual percentage after a 24-h germination-stage soaking treatment in a 300-fold diluted solution at 32°C was 8.3%. Therefore, residual efficacy after a PAA treatment is unlikely, thereby necessitating high efficacy during the treatment. Bakanae disease can spread when *F. fujikuroi* spores from infected seeds are transmitted to healthy seeds during the seed soaking-to-sowing period (Sasahara 2013). Several factors contributed to the decreased efficacy under high-incidence conditions. For the pre-soaking treatment in a 100-fold diluted solution, the fungal load in the seed interior, which is relatively inaccessible to the PAA solution, gradually increased during the soaking period. The concentration of the 300-fold diluted solution during the germination-stage treatment was insufficient to protect against fungi in the seed interior. Accordingly, the treatment concentration may need to be increased. However, minor phytotoxic effects, such as inhibited germination, were observed for the pre-soaking treatment in a 100-fold diluted solution and the germination-stage treatment in a 200-fold diluted solution. Thus, the treatment timing and duration needed to be adjusted. Accordingly, we tested two methods: pre-soaking treatment at a higher concentration combined with rice hull washing and treatment with a higher concentration at the end of the soaking period (immediately before germination), when phytotoxicity is less likely to occur.

In tests using highly infected seeds, a 10-min pre-soaking treatment in a 10-fold diluted solution was ineffective regardless of whether seeds were rinsed. A 30-min pre-soaking treatment in a 10-fold diluted solution was highly effective, but only if seeds were not rinsed. However, this treatment significantly decreased the number of seedlings. A 24-h pre-germination soaking treatment in a 100- or 200-fold diluted solution was highly effective, with no phytotoxic effects. According to these results, short-term pre-soaking treatments at high concentrations are not practical, but pre-germination treatments are effective. Therefore, pre-germination treatments were repeatedly tested. The disease seedling percentage for the untreated control was 85.8%–91.7%. In all four tests under severe disease conditions, the pre-germination treatment with a 100-fold diluted solution was highly effective, similar to the treatment with the control agent. The pre-germination treatment with a 200-fold diluted solution was

effective, but its efficacy was more unstable than that of the pre-germination treatment with a 100-fold diluted solution. No phytotoxicity was observed for any of the pre-germination treatments. Furthermore, the pre-germination treatment with a 100-fold diluted solution was highly effective against bacterial grain rot (data not shown). However, the utility of pre-germination treatments may be limited by the fact that seeds may sprout depending on the soaking temperature, potentially leading to phytotoxicity. Therefore, the relationship between the sprouting status of seeds treated before germination and phytotoxicity was examined by gradually varying the seed-soaking temperature. Increasing the seed-soaking temperature tended to increase the germination percentage during the pre-germination treatment, leading to increases in the stunted seedling percentage and decreases in the emerged seedling percentage. At seed-soaking temperatures up to 16°C, treatments were completed when most seeds had not germinated, with the stunted seedling percentage and emerged seedling percentage nearly equivalent to those of the untreated control. By contrast, at seed-soaking temperatures of 18 and 20°C, germination was observed during the treatment period. According to a comparison with the untreated control, the stunted seedling percentage increased, while the emerged seedling percentage decreased. Rice seeds are soaked for a cumulative temperature of approximately 100°C (daily average temperature) (Hirai et al. 2008). Therefore, under the test conditions in the current study, a 6-day soaking treatment at 16°C (96°C cumulative temperature) is most similar to the standard rice seed soaking method. However, rice growers may not possess temperature-controlled incubators for precise management; sometimes soaking occurs in shaded areas of warehouses under ambient temperature conditions. When low-temperature management is not possible, rice growers must carefully monitor water temperature changes and the germination status before initiating pre-germination treatments.

During tests using bakanae-infected seeds, when the seed-soaking temperature was varied stepwise to examine the relationship between the germination status and efficacy after a pre-germination treatment, high soaking temperatures enhanced germination and treatment efficacy. Considering that the PAA solution temperature during the treatment was 15°C, differences in the germination status due to changes in the soaking temperature were considered to influence treatment efficacy. Most pathogens causing bakanae disease are thought to attach and infect the outer or inner surface of the hull or near the surface of brown rice in the form of conidia or hyphae (Naito et al. 2002; Tateishi et al. 1998). A gap exists between the outer glume and the embryo. Before water is absorbed, this gap is closed, but germination causes the outer glume to rupture, thereby opening the gap (Hoshikawa 1990). Therefore, during treatments, the PAA solution can likely easily reach the seed interior, enabling it to exert its fungicidal effect. On the basis of the active ingredient translocation properties and residual efficacy of synthetic pesticides, the germination status has a limited effect on treatment efficacy. However, the germination status may have a greater effect on the efficacy of treatments involving PAA solutions. This possibility should be thoroughly investigated in future studies.

Declarations

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Conflict of interest

The authors declare that they have no conflict of interest.

Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

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Tables

Tables 1 to 4 are available in the Supplementary Files section.

Figures

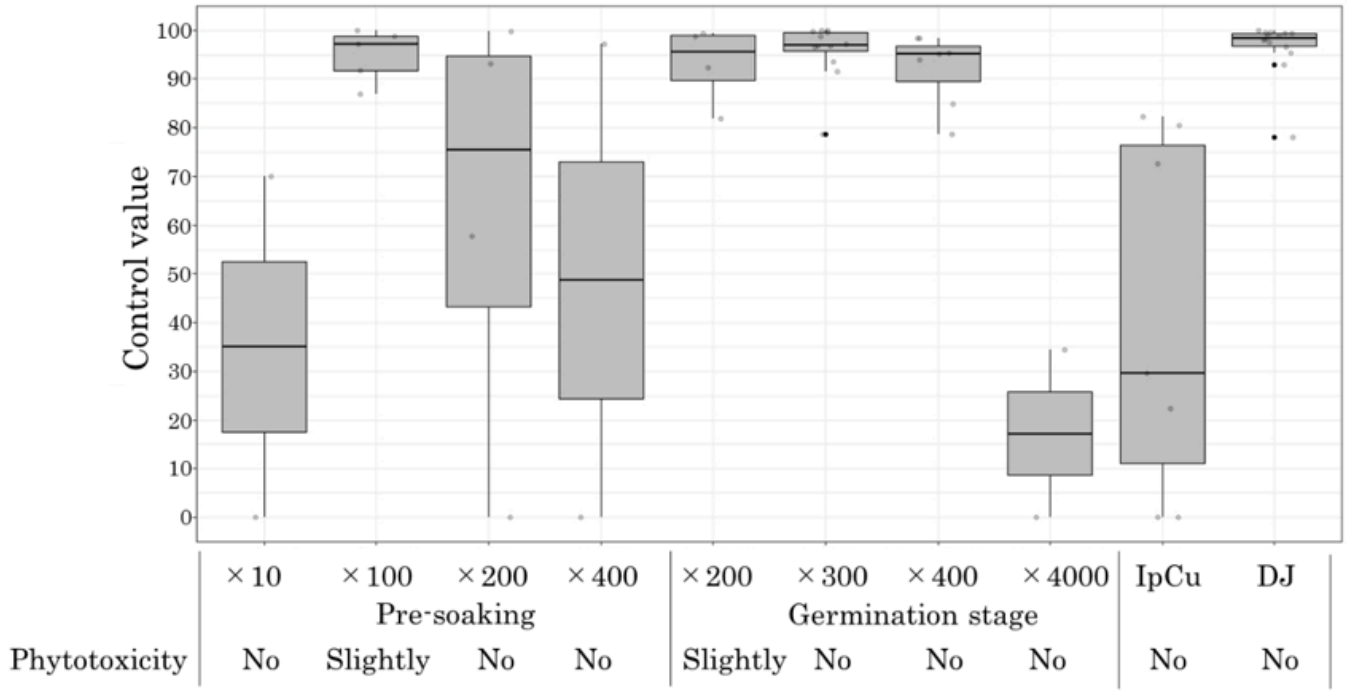


Fig.1

Figure 1

Efficacy and phytotoxicity of treatments with peracetic acid solutions to control bacterial grain rot

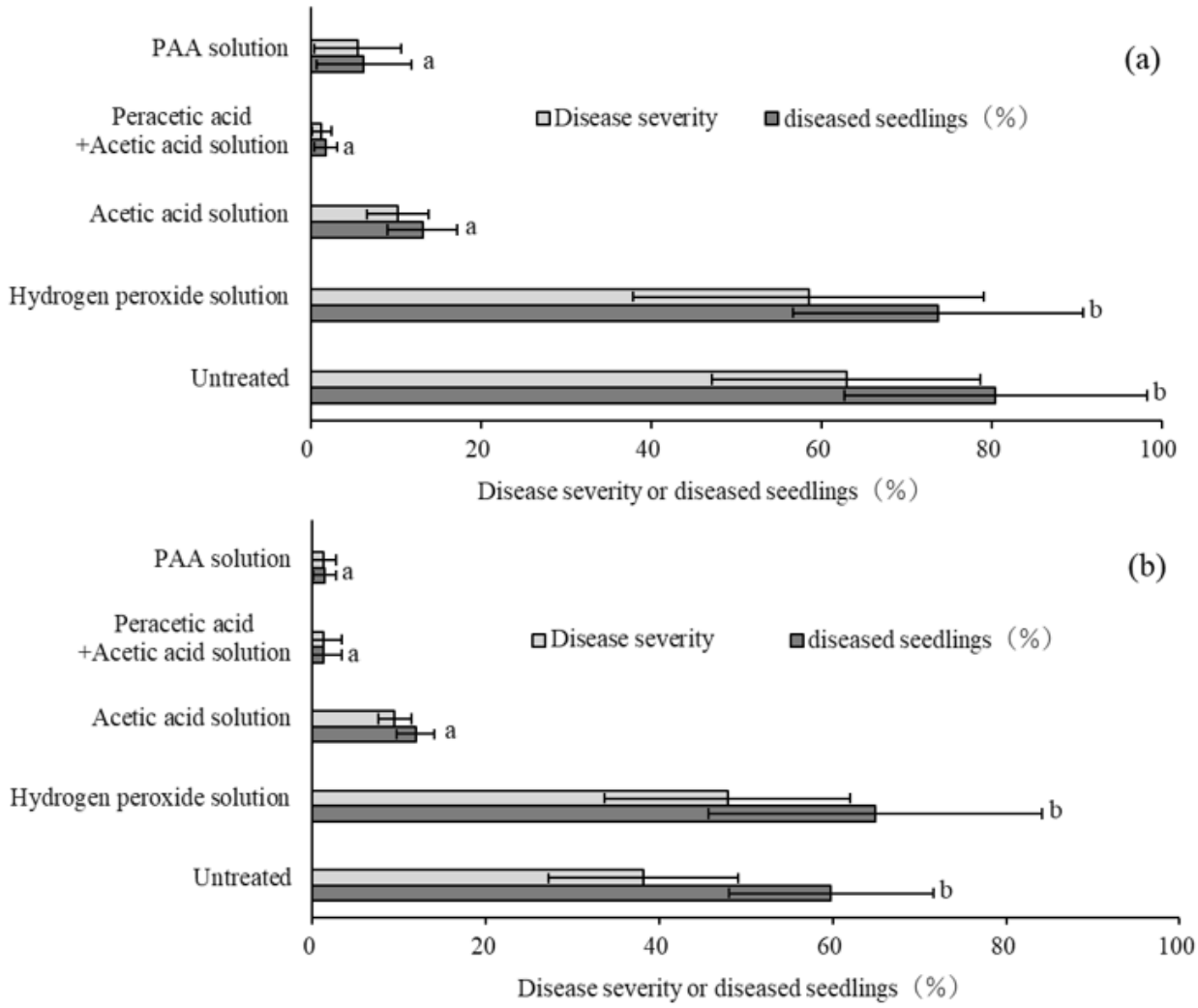


Fig.2

Figure 2

Effects of individual solutions on bacterial grain rot

(a) Pre-soaking treatment. (b) Germination-stage treatment. Different lowercase letters indicate significant differences in the seedling disease percentage according to Tukey's HSD test after square root transformation. Error bars indicate the standard deviation.

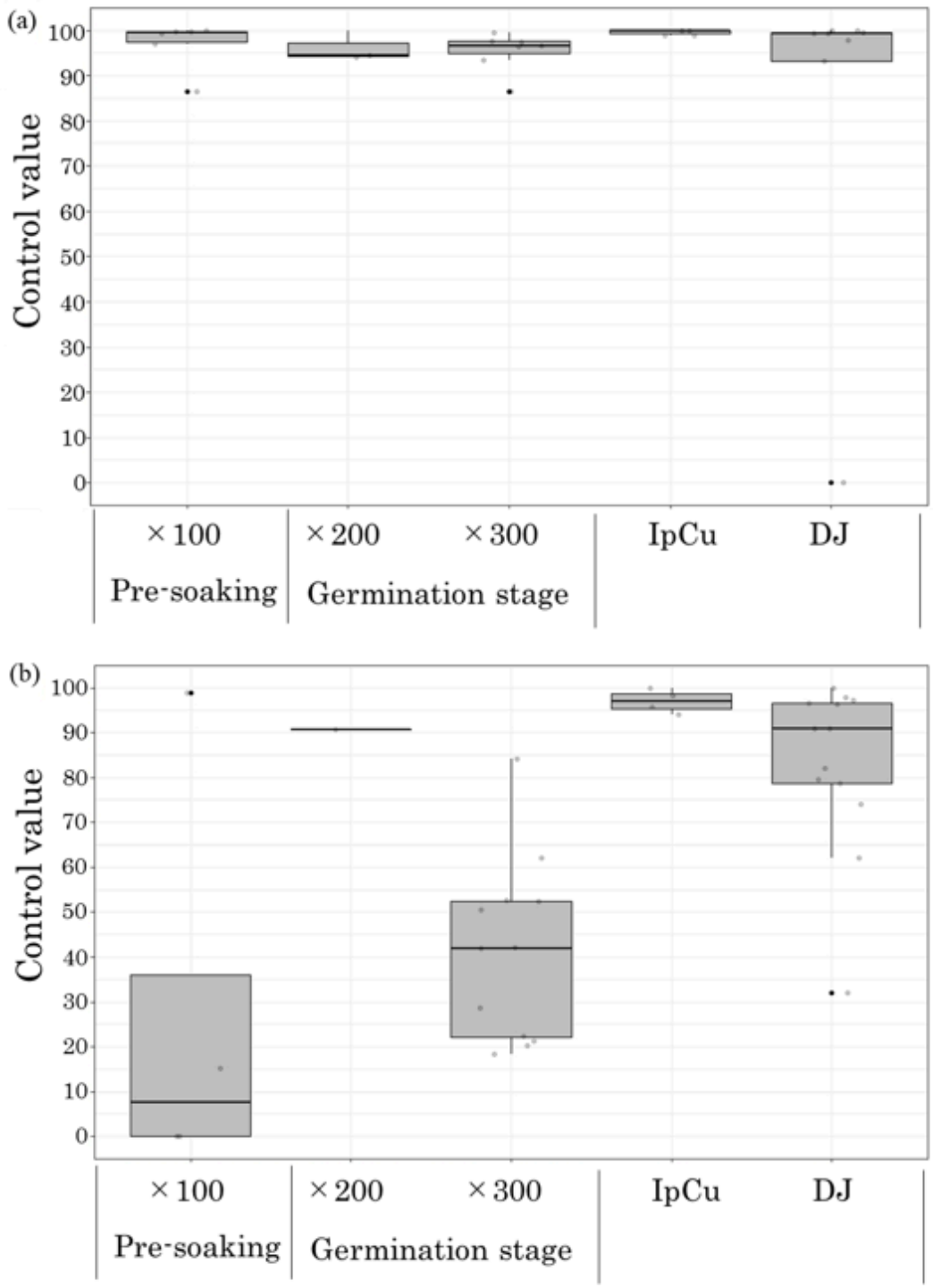


Fig.3

Figure 3

Efficacy of peracetic acid solutions for controlling bakanae disease

(a) Low-incidence conditions. (b) High-incidence conditions.

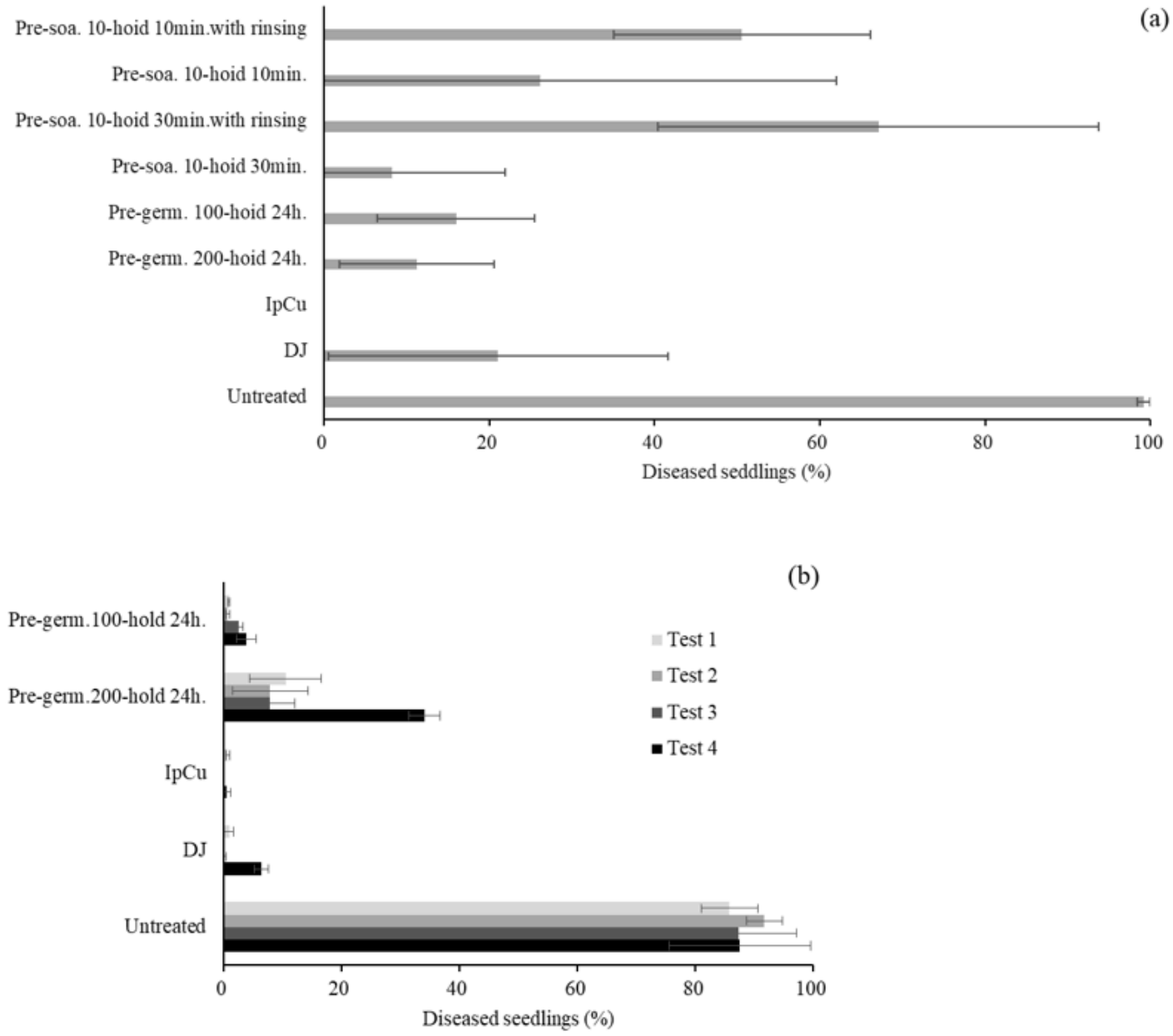


Fig.4

Figure 4

Evaluation of PAA treatment methods for controlling bakanae disease using highly infected seeds

(a) Tested treatments. (b) Repeated analysis of pre-germination treatments. Error bars indicate the standard deviation.

Supplementary Files

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- Tables.docx