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Research Article
Screening of Japanese Rice Cultivars for Seeds with Heat Stress Tolerance under Hot Water Disinfection Method

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Abstract

Background and Objective: Hot water disinfection is an easy and yet effective seed disinfection technique that does not require the use of agricultural chemicals. The typical condition of this method (60°C for 10 min) is not always sufficient to kill certain pathogens; however, the application of higher temperatures represses the germination rates of several rice cultivars, especially indica. This study was conducted to obtain applicable breeding materials that are extremely tolerant under treatment of hot water disinfection to facilitate the use of this useful technique in Japan and other countries in Asia. **Materials and Methods:** Four modern Japanese cultivars and thirty-nine cultivars of the Japanese rice landrace mini core collection were screened under hot water treatment at several high temperature conditions (60, 65 and 70°C) for 10 min. One-way analysis of variance followed by the Tukey-kramer multiple rang test and a Student's t-test were used for statistical analysis. **Results:** Germination rate results showed that 'Koshihikari' was the most tolerant among the four modern Japanese cultivars. Meanwhile, among the Japanese core collection, 'Aikoku' and 'Nagoya shiro' cultivated in 2013 and 2015 showed the highest germination and seedling emergence rates, which were constantly higher than 'Koshihikari'. **Conclusion:** The results from this study indicate that in light of their high tolerance under the hot water disinfection method, 'Aikoku' and 'Nagoya shiro' are potential breeding materials that are fruitful for the trait introduction to other rice cultivars.

Key words: Breeding materials, heat tolerance, hot water disinfection, *Oryza sativa*, seed disinfection

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rice is one of the most important staple foods for more than half of the world's population. The trend in rice consumption worldwide is increasing sharply along with the escalating world population, specifically in Asia and African areas that generally depend on rice as the main daily calorie source[1]. Thus, massive intensification of rice cultivation is needed in order to ensure the world's food security in the future.

In rice cultivation, seed disinfection prior to seeding is an important and indispensable process to prevent infection with seed-borne pathogens, which cause serious damage to yields[2,3]. Seed disinfection is usually carried out using chemicals containing bactericides, insecticides and fungicides[4,5]. However, these effective chemicals are costly and require a waste pre-treatment process prior to disposal that is also expensive. Therefore, the introduction of a chemically based disinfection method to developing countries is challenging. Moreover, it has been shown that long-term chemical use can result in the emergence of drug-resistant pathogens[4,6].

Hot water disinfection is a clean technique used to disinfect rice seeds by a physical process, i.e., using the heat from water, without the need for expensive and environmentally damaging chemicals. This method has recently received a great deal of attention as a replacement for the use of agricultural chemicals. Moreover, the process is easy, requiring only the soaking of the rice seeds for 10 min in 60°C water and 5 min in tepid water. After disinfection, the rice production cycle continues to the seeding process and plant establishment in the rice field as with the chemically based disinfection method[6]. Because this disinfection process is simple and low-cost, the potential for this technique to spread to developing countries is greater than for seed disinfection using chemicals.

Although the basic method of hot water disinfection has been confirmed to be as effective as disinfection using agricultural chemicals in eliminating pests[7], treatment at a higher temperature or for a longer time is sometimes necessary to completely eliminate certain pathogens[8]. However, the use of higher temperatures and longer treatment times may negatively impact germination, since tolerance to heat-stress from hot water treatment varies between rice cultivars[6,9,10]. Specifically, it was reported that glutinous and indica cultivars show higher heat sensitivity. Notably, indica cultivars are the most cultivated rice in many Southeast Asian countries, where the human population is increasing rapidly[11-13]. On the other hand, the seeds of japonica cultivars show greater heat tolerance compared to indica cultivars. For example, the germination rate of the japonica cultivar 'Hitomebore' was >90% when the seeds were treated with hot water at 67.5°C for 10 min[6]. Moreover, using the NIAS (National Institute of Agrobiological Science) world rice core collection[14], Kashiwagi *et al.*[9] reported that the average germination rate of japonica rice cultivars was 52.2%, which is higher than that of indica cultivars (31.5%) under hot water disinfection at 69°C for 10 min. These reports indicate that japonica cultivars contain genes conferring heat tolerance under hot water disinfection. Thus, it is important to introduce such tolerance traits to indica cultivars to spread the application of hot water disinfection throughout Southeast Asia. Consequently, the screening of japonica rice cultivars as a genetic source of heat tolerance traits under extreme hot water conditions is crucial.

The Japanese rice landrace mini core collection consists of fifty original Japanese cultivars and was established as a result of a genome-wide SSR polymorphism survey of 236 accessions cultivated traditionally in Japan[15]. The collection contains not only japonica rice but also several indica and tropical japonica cultivars (Table 1), which originate from all rice producing areas in Japan. These fifty cultivars were selected due to their coverage of the genetic diversity of Japanese landraces. Therefore, this collection is suitable for the screening of breeding materials containing genes conferring heat tolerance to seeds under hot water treatment.

In this study, the Japanese rice landrace mini core collection were screened to identify rice cultivars tolerant to heat stress under hot water disinfection, by evaluating seed germination and emergence rates after hot water treatment. Prior to the heat-tolerance investigation of the mini core collection, the germinability of 'Hitomebore', which is known to be tolerant[6,9] was evaluated by comparing its germination rate with three selected modern cultivars,'Koshihikari', 'Sasanishiki' and 'Habataki', commonly used as parental lines for QTL (Quantitative Trait Locus) analysis to detect genes of interest in genetic studies[16,17]. This study provides the useful breeding materials information for creating new tolerant rice cultivars under severe hot water disinfection method. Moreover, this study was also conducted to facilitate the application of this environmentally-friendly technique not only in Japan but also across the countries in Asia.

MATERIALS AND METHODS

Seed material: Seeds of the Japanese rice landrace mini core collection developed by Ebana *et al.*[15] were obtained from the

Table 1: List of the Japanese rice landrace mini core collection used in the present study

ID	Name	Origin	Japonica/Indica
JRC 01	Gaisen mochi	Unknown	Tropical japonica
JRC 03	Hinode	Kinki	Tropical japonica
JRC 04	Senshou	Tokyo	Tropical japonica
JRC 05	Yamada bake	Kagoshima	Tropical japonica
JRC 06	Kaneko	Kanto Higashiyama	Tropical japonica
JRC 07	Irima Nishiki	Saitama	Tropical japonica
JRC 08	Okkamodoshi	Unknown	Tropical japonica
JRC 10	Hirayama	Tokyo	Tropical japonica
JRC 11	Kahei	Kagoshima	Tropical japonica
JRC 12	Oiran	Kumamoto	Tropical japonica
JRC 17	Akage	Akita	Japonica
JRC 19	Wata burine	Shiga	Japonica
JRC 20	Hosogara	Aomori	Japonica
JRC 21	Akamai	Kochi	Japonica
JRC 22	Mansaku	Fukuoka	Japonica
JRC 23	Ishijiro	Toyama	Japonica
JRC 24	Joushuu	Yamagata	Japonica
JRC 25	Dango	Unknown	Japonica
JRC 26	Aikoku	Fukui	Japonica
JRC 27	Ginbouzu	Ishikawa	Japonica
JRC 28	Shinrikimochi	Kumamoto	Japonica
JRC 29	Shinchimenchou mochi	Unknown	Japonica
JRC 30	Morita wase	Yamagata	Japonica
JRC 31	Kameji	Shimane	Japonica
JRC 34	Kyoutoasahi	Kyoto	Japonica
JRC 35	Kabashiko	Miyazaki	Japonica
JRC 36	Sekiyama	Aomori	Japonica
JRC 37	Shinyamadaho 2	Hyogo	Japonica
JRC 38	Nagoya Shiro	Akita	Japonica
JRC 39	Shiroine (Kemomi)	Tokushima	Japonica
JRC 40	Akamai Nagasaki	Nagasaki	Indica
JRC 41	Akamai Tokushima	Tokushima	Indica
JRC 42	Touboshi	Kagoshima	Indica
JRC 43	Akamai Kanto	Kanto Higashiyama	Indica
JRC 44	Karahoushi	Kagoshima	Indica
JRC 45	Hiyadachitou	Yamagata	Japonica
JRC 47	Okabo	Unknown	Japonica
JRC 48	Hakamuri (Yokoyama)	Kagoshima	Japonica
JRC 50	Himenomochi	Akita	Japonica

NIAS Japan. All seeds of the collection and four Japanese modern cultivars ('Koshihikari', 'Hitomebore', 'Sasanishiki' and 'Habataki') were harvested during the rice cultivation season (May-September) under natural conditions at the Toyama Prefectural Agricultural Forestry and Fisheries Research Centre in 2013 and 2015. The four modern cultivars and thirty-nine cultivars of the core collection with sufficient seed numbers were used in the present study.

Hot water disinfection and germination assay: Fifty dry seeds per cultivar were wrapped using 10×10 cm straining bag prior to hot water treatment in a temperature-controlled water bath (NTT-2000, Eyela, Japan) for 10 min and then transferred to tepid water (~20-25°C) for 5 min. Hot water treatments were conducted at 60, 65 and 70°C. Seeds without hot water treatment were used as controls. The germination assay was conducted after hot water treatment and the seeds were transferred to a petri dish (90×15 mm) containing water and incubated in a 28°C controlled temperature incubator (Incubation box M-2ten FN, Taitec, Japan) for 10 days under darkness. The water in the petri dish was changed every day and the number of germinated seeds was recorded on 10 days.

Seedling emergence analysis: Seeds after hot water treatment at 60, 65 and 70°C were soaked under flow-through water (23-25°C) for 3 days. Incubation at 30°C for 2 days was performed prior to seeding on a 30×60 cm seeding tray (Kubota, Japan) in Shinano soil (Ohata Seed, Japan). Sowed seeds were incubated under natural conditions inside a greenhouse for 10 days with temperature range 25-33°C. Seedling emergence (%) was shown as the number of seedlings emerged from the soil.

Statistical analysis: Data were analyzed using one-way analysis of variance followed by the Tukey-kramer multiple range test (screening of modern Japanese cultivars) and a Student's t-test for comparisons among means (n = 3), with a significance level of 5 and 1%.

RESULTS

Seed germinability of four modern Japanese cultivars under hot water treatment: To confirm that 'Hitomebore' seeds are tolerant to hot water treatment, evaluation of 'Hitomebore' seed germination was carried out and compared to the three other modern Japanese cultivars ('Koshihikari', 'Sasanishiki' and 'Habataki'). All cultivars cultivated in 2013 showed germination rates of nearly 100% without hot water treatment, confirming normal germination of the seeds under normal conditions (Fig. 1). As mentioned previously, the typical method for hot water treatment of seeds is soaking in 60°C water for 10 min. To confirm the germinability of these four cultivars under this general treatment condition, the seeds were treated at 60°C for 10 min. After the following 10 days incubation, 'Hitomebore' and the other three cultivars showed seed germination rates >90%, similar to without hot water treatment (Fig. 1).

The treatment temperature was then increased to 65°C, resulting in a slightly decreased germination rate of 84% for 'Sasanishiki' but not 'Hitomebore' (97%). Because the germination rates of 'Koshihikari' and 'Habataki' were also not significantly affected by 65°C hot water treatment (93 and 95.3%, respectively), a more severe condition (70°C) was carried out. Treatment at 70°C for 10 min resulted in significantly reduced germination rates for 'Sasanishiki' and 'Habataki', 19.3 and 4.0%, respectively ($p<0.01$). On the other hand, 'Hitomebore' and 'Koshihikari' maintained high germination rates, 76% for both cultivars. This result confirmed the high tolerance of 'Hitomebore' seeds to severe hot water treatment as previously reported. Furthermore, 'Koshihikari' showed equivalent tolerance to 'Hitomebore'.

It has been reported that seeds from the same cultivar but harvested in different cultivation years can show distinct heat-stress tolerance under hot water treatment. This is attributable to differences in cultivation conditions, which is dependent on a number of factors including the dynamic climate[6,18]. To confirm the screening result of seeds cultivated in 2013, seeds from a different cultivation year (2015) were evaluated without and with hot water treatment under the same temperature conditions (60, 65 and 70°C) as seeds cultivated in 2013. The germination rates without and with hot water treatment at 60 and 65°C showed a similar tendency compared to the 2013 results. After 70°C treatment, the germination rate of 'Hitomebore' decreased and was significantly lower ($p<0.05$) than the 2013 result. Meanwhile, 'Koshihikari' showed a consistently high germination rate, the highest compared to the other cultivars (64.7%) under this condition. Thus, in this study, the seeds of 'Koshihikari' exhibited consistent heat-stress tolerance under hot water treatment, a trait that if introduced would be useful in sensitive cultivars. However, the germination rates of 'Koshihikari' remained <80% for both cultivation years. Consequently, in order to identify cultivars having higher germinability than 'Koshihikari' at the 70°C condition, we evaluated the germination rates of the Japanese rice landrace mini core collection treated with 70°C hot water for 10 min.

Screening of seed germinability of the Japanese rice landrace mini core collection under hot water treatment: To evaluate the seed germinability of cultivars belonging to the Japanese rice landrace mini core collection (Table 1), a germination test without hot water treatment using seeds cultivated in 2013 was carried out. Results showed that the seed germination of all cultivars was nearly 100% (Fig. 2). Screening after 70°C hot water treatment for 10 min clearly showed the cultivars that were extremely tolerant or sensitive to heat stress under this severe condition. The three most tolerant cultivars were 'Nagoya shiro', 'Akamai Nagasaki' and 'Hirayama', which exhibited germination rates of 97.3, 85.3 and 80.0%, respectively. However, only 'Nagoya shiro' was significantly different from 'Koshihikari' ($p<0.01$). On the other hand, 'Joushuu', 'Himenomochi' and the indica cultivar 'Touboshi' were the most sensitive cultivars, with germination rates <4% ($p<0.01$, Fig. 2).

A similar result was obtained for the seeds cultivated in 2015 without hot water treatment, in which all cultivars showed >90% germination rates (Fig. 3). Hot water treatment at 70°C for 10 min divided the extremely tolerant cultivars from the sensitive ones. Compared to the 2013 result, 'Nagoya shiro' was consistently tolerant, with an 87% germination rate. The other cultivars with significantly higher germination rates were 'Aikoku' (89.3%), 'Sekiyama' (89.3%) and 'Ishijiro (88.7%) ($p<0.01$, Fig. 3). The 2013 results under the same treatment condition revealed that 'Aikoku' had high germinability (72.7% germination), while 'Ishijiro' and 'Sekiyama' showed the slightly lower germination rates of 63.3 and 31.3%, respectively. On the other hand, the germinability of two indica cultivars, 'Akamai Nagasaki' and 'Akamai Tokushima', which showed high tolerance in 2013 samples

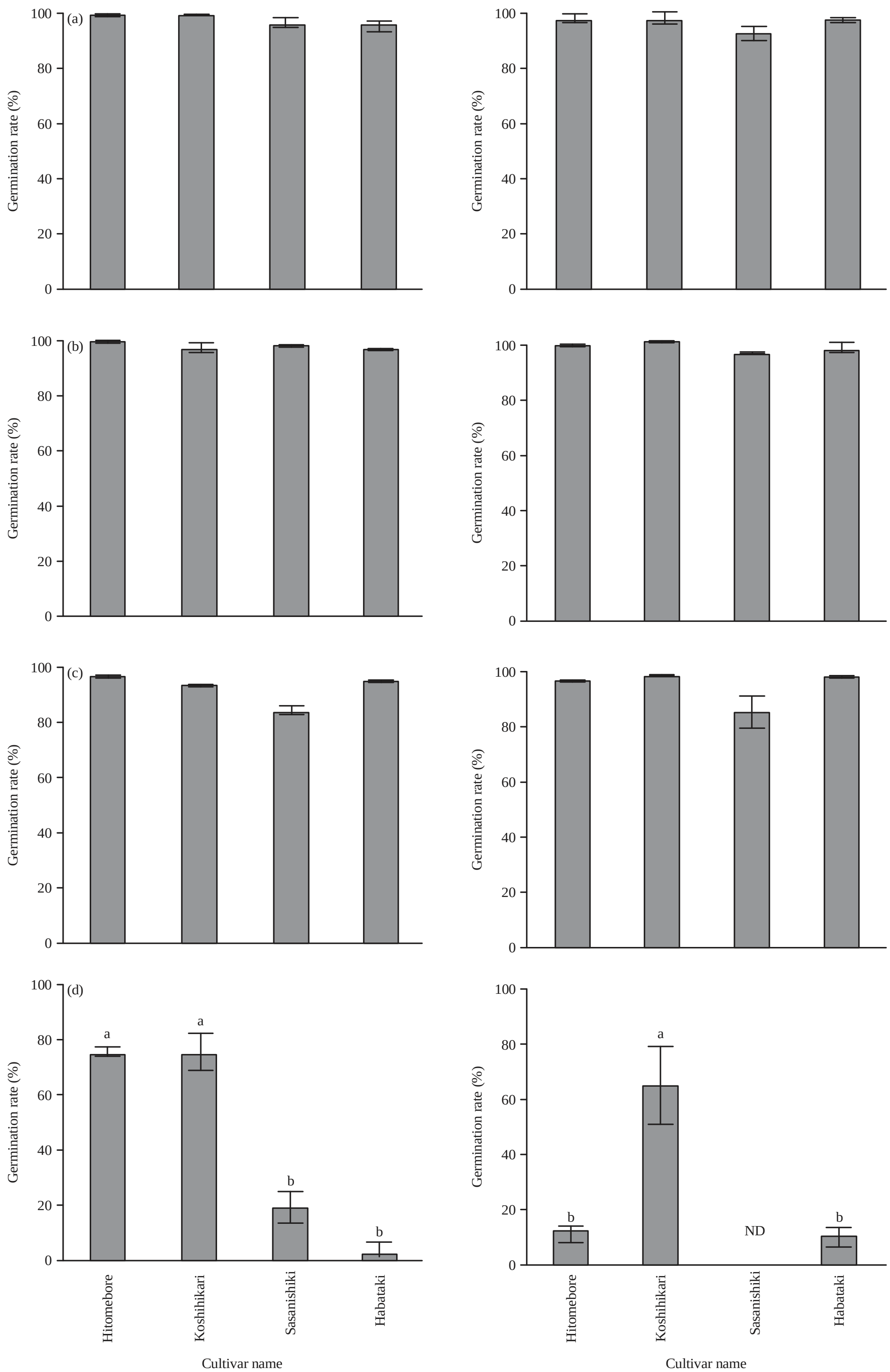

Fig. 1(a-d): Seed germination rates of 'Koshihikari', 'Hitomebore', 'Sasanishiki' and 'Habataki' cultivated in 2013 (left) and 2015 (right) (a) Without hot water treatment and with hot water treatment at (b) 60, (c) 65 and (d) 70°C for 10 min prior to incubation at 28°C for 10 days

Different letters above bars represent significant differences ($p<0.01$) among samples. N.D: No data

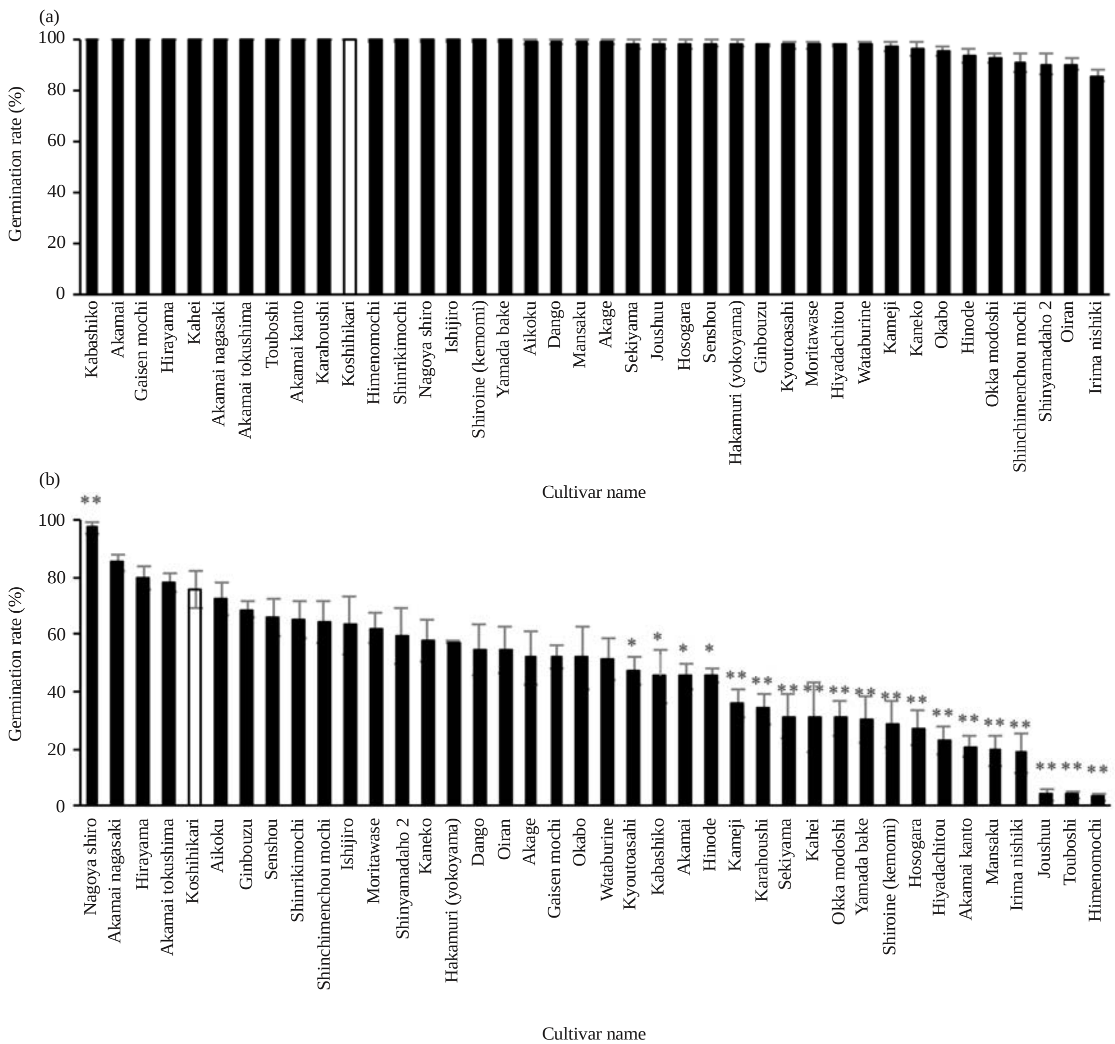

Fig. 2(a-b): Seed germination rates of 'Koshihikari' and thirty-nine cultivars in the Japanese rice landrace mini core collection cultivated in 2013 (a) Without hot water treatment and (b) With hot water treatment at 70°C for 10 min. The seeds of all cultivars were incubated in petri dishes with water at 28°C for 10 days. Black and white bars represent the Japanese rice landrace mini core collection and 'Koshihikari' germination rates, respectively

Asterisks indicate significant differences at p<0.05 (*) and p<0.01 (**) compared to 'Koshihikari'

Table 2: Summary of germination rates of 'Aikoku' and 'Nagoya shiro' in comparison to 'Koshihikari' under 70°C water treatment

	Germination rate					
	2013		2015			
Cultivar name	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Aikoku	72.7	5.7	89.3**	4.4	81**	4.9
Nagoya shiro	97.3**	1.8	86.6*	3.5	92**	3.0
Koshihikari	76.0	6.4	64.7	14.3	70.3	7.5

Asterisks indicate significant differences at p<0.05 (*) and p<0.01 (**) compared to 'Koshihikari'

(85.3 and 78%, respectively) was reduced to 58.6 and 12.7%, respectively. Considering these results, it was confirmed that the germinability of seeds from several cultivars and cultivated in different years varied following hot water disinfection as shown by the results of 'Hitomebore'. However, the germination rates of 'Nagoya shiro' and 'Aikoku' were consistently high between the two cultivation years and significantly higher (p<0.05) than 'Koshihikari' (Table 2),

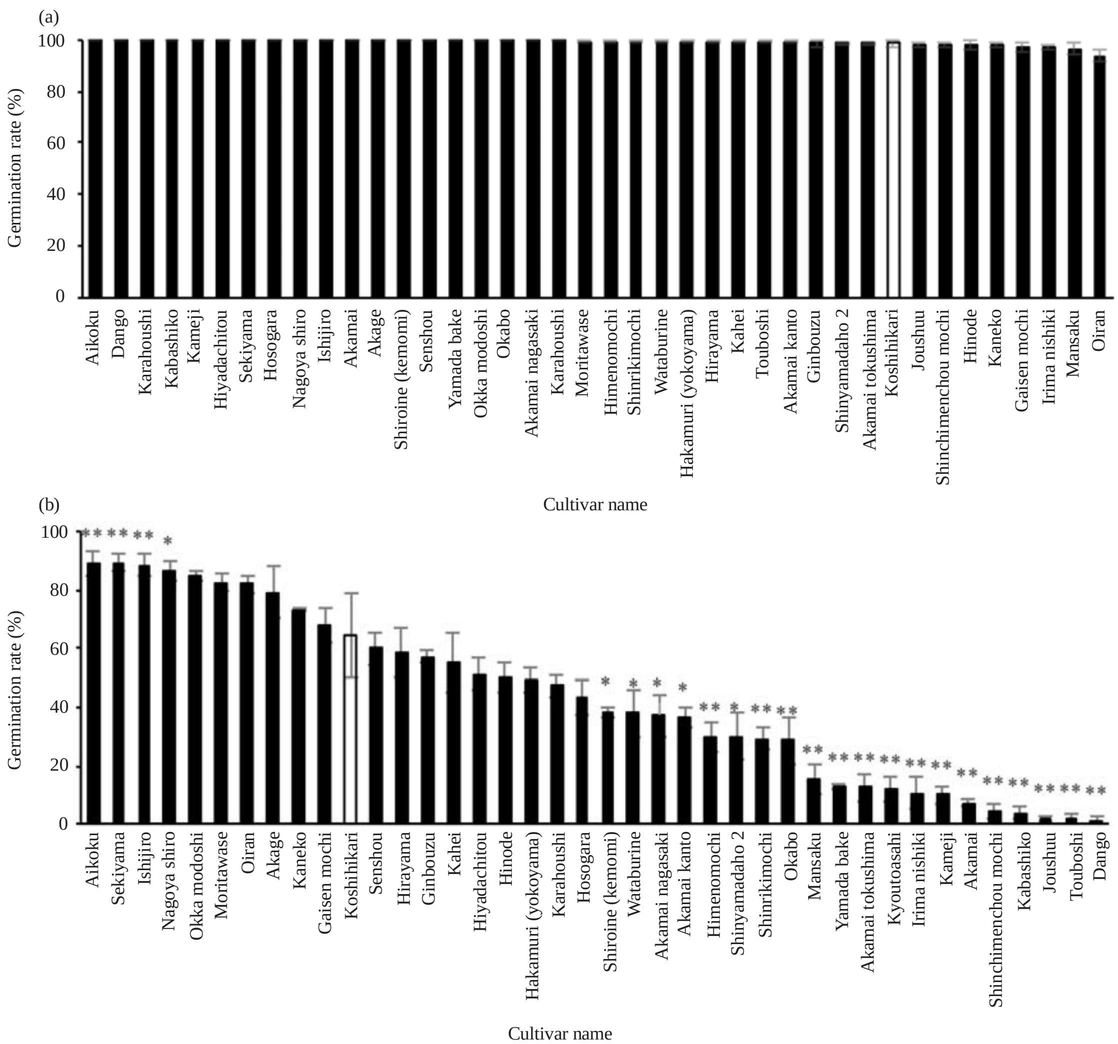

Fig. 3(a-b): Seed germination rates of ‘Koshihikari’ and thirty-nine cultivars in the Japanese rice landrace mini core collection cultivated in 2015 (a) Without hot water treatment and (b) With hot water treatment at 70°C for 10 min. The seeds of all cultivars were incubated in petri dishes with water at 28°C for 10 days. Black and white bars represent the Japanese rice landrace mini core collection and ‘Koshihikari’ germination rates, respectively

Asterisks indicate significant differences at p<0.05 (*) and p<0.01 (**) compared to ‘Koshihikari’

suggesting that the trait responsible for heat stress tolerance under severe hot water treatment exists in the seeds of these two cultivars.

Five indica rice cultivars are included in the mini core collection: ‘Akamai Kanto’, ‘Akamai Nagasaki’, ‘Akamai Tokushima’, ‘Karahoushi’ and ‘Touboshi’. Even though the heat tolerance of ‘Akamai Nagasaki’ and ‘Akamai Tokushima’ cultivated in 2013 was similar to ‘Koshihikari’ as mentioned previously in this study, the tolerance of seeds cultivated in 2015 was reduced (p<0.05) significantly. Furthermore, the germination rates of ‘Akamai Kanto’, ‘Karahoushi’ and ‘Touboshi’ were consistently and significantly (p<0.05) lower than ‘Koshihikari’ over the two cultivation years and they were grouped as sensitive cultivars. By these results, it was confirmed that indica cultivars are generally more sensitive to severe hot water treatment than japonica cultivars.

Effect of hot water disinfection on seedling emergence: To investigate whether hot water treatment also affects the emergence of seedlings, seeds after hot water treatment at 60, 65 and 70°C were sowed in soil in seeding trays and then grown for 10 days under natural conditions. Twelve cultivars for the analysis were selected, which included ten core collection cultivars (‘Aikoku’, ‘Nagoya shiro’, ‘Ginbouzu’, ‘Senshou’, ‘Kaneko’, ‘Mansaku’, ‘Irimanishiki’, ‘Joushu’, ‘Touboushi’ and ‘Kameji’) and ‘Koshihikari’ and ‘Habataki’.

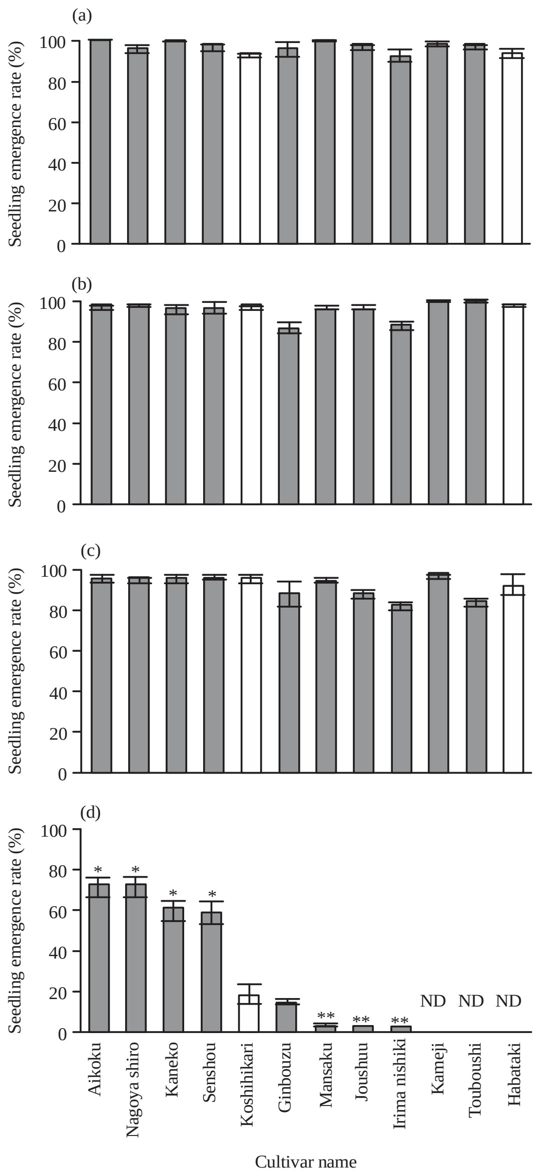

Fig. 4(a-d): Seedling emergence rates of the seeds of twelve selected cultivars (a) Without hot water treatment and with hot water treatment at (b) 60, (c) 65 and (d) 70°C for 10 min prior to seeding for 10 days under natural conditions. Grey and white bars represent the Japanese rice landrace mini core collection and modern cultivars germination rates, respectively.

Asterisks indicate significant differences at $p<0.05$ (*) and $p<0.01$ (**) compared to 'Koshihikari'. N.D: No data

'Aikoku' and 'Nagoya shiro' are representative of significantly tolerant cultivars compared to 'Koshihikari', while 'Ginbouzu', 'Senshou' and 'Kaneko' are cultivars with similar germinability to 'Koshihikari' under 70°C hot water treatment. On the other hand, 'Mansaku', 'Irimanishiki', 'Joushu', 'Touboushi', 'Kameji' and 'Habataki' were selected as consistently sensitive cultivars.

The results in Fig. 4 show that there were no significant differences in seedling emergence rate for both heat tolerant and intolerant cultivars after hot water treatment at 60 and 65°C for 10 min compared to without hot water treatment. On the other hand, the seedling emergence rate of sensitive cultivars after 70°C treatment was reduced significantly ($p<0.01$). After treatment at this temperature, even the emergence rate of 'Koshihikari' was reduced to 17.3%, grouping it as a sensitive cultivar. However, the emergence rate of 'Aikoku' and 'Nagoya shiro' as tolerant cultivars reached >50%, significantly greater than 'Koshihikari' at $p<0.01$ (Fig. 4). From this evaluation, it was shown that hot water treatment at 60 and 65°C did not significantly affect seedling emergence compared to control but treatment at an extreme temperature (70°C) decreased the seedling emergence rate of 'Koshihikari' and selected sensitive cultivars, except for 'Aikoku' and 'Nagoya shiro'.

DISCUSSION

This study was conducted to identify cultivars exhibiting high tolerance to heat-stress, achieved by severe hot water treatment for use as a source of genetic material that would facilitate the application of hot water disinfection in other countries. The results from this study and the previous study[9] revealed that indica cultivars are generally more heat sensitive than japonica cultivars, indicating that the trait of heat tolerance under hot water treatment is conserved among japonica cultivars. One cultivar that is known to be highly heat tolerant is 'Hitomebore', a modern japonica cultivar from Miyagi Prefecture. In this study also confirmed that the seeds of 'Hitomebore' cultivated in 2013 showed high germination even after 70°C treatment for 10 min. However, this trait was not observed in seeds cultivated in 2015 subjected to the same treatment conditions. 'Koshihikari' is the only cultivar that showed consistently high germination rates both in 2013 and 2015. As 'Hitomebore' is the progeny of 'Koshihikari' crossed with 'Hatsuboshi', another Japanese modern cultivar[19], the observed equivalent heat tolerance in 'Hitomebore' seeds cultivated in 2013 under 70°C water treatment might be attributable to the same genetic factor that exists in 'Koshihikari'. However, the germination rate of 'Hitomebore' seeds cultivated in 2015 was reduced ($p<0.05$) significantly, indicating that the trait is not consistently present in 'Hitomebore', due to an unknown mechanism and triggered by the different cultivation conditions between 2013 and 2015. Therefore, 'Koshihikari' shows greater potential for use as a genetic material than 'Hitomebore', since its seeds exhibit consistent heat-stress tolerance under hot water treatment. Since 'Koshihikari' is the most cultivated rice cultivar in Japan[20], the results from this study increase the prospect of expanding the application of the hot water disinfection method throughout Japan.

Even though the germination rate of 'Koshihikari' among the other three cultivars was the highest under 70°C hot water treatment for 10 min, the germination rate was still lower than 80% (76 and 64% for seeds cultivated in 2013 and 2015, respectively), which is the percentage required for seeds to be considered of "good quality"[21]. Since the heat tolerance trait under hot water treatment shows a tendency to be conserved among japonica cultivars, the mini core collection consisting of thirty-nine Japanese landraces developed by Ebana *et al.*[15] was used for screening with 'Koshihikari' as a standard cultivar. From both the germination and seedling emergence evaluations of mini core collection seeds cultivated in 2013 and 2015, 'Aikoku' and 'Nagoya shiro' were proposed as prospective breeding materials for the heat tolerance trait under severe hot water treatment, since the two cultivars exhibited significantly ($p<0.05$) higher germination and seedling emergence rates compared to 'Koshihikari'.

'Aikoku' is a japonica landrace from Fukui Prefecture, whose progeny comprise many modern cultivars commonly cultivated in Japan, such as 'Norin No. 1' and 'Norin No. 8' and cultivars used in this study ('Koshihikari', 'Hitomebore' and 'Sasanishiki')[22]. The tolerance trait 'Koshihikari' seeds may be inherited from 'Aikoku' and then transferred to 'Hitomebore'. The observation that the germination and seedling emergence rates of 'Aikoku' were higher than 'Koshihikari' and that 'Koshihikari' was more tolerant than 'Hitomebore' suggested that the trait may be altered by domestication and breeding[23]. Therefore, in this case, 'Aikoku' shows greater potential as a genetic material than 'Koshihikari'.

'Nagoya shiro' is a japonica landrace from Akita Prefecture. As mentioned previously, the Japanese rice landrace mini core collection used in this study was developed from 236 accessions, based on a genome-wide SSR polymorphism survey covering the genetic diversity of Japanese landraces. In other words, each cultivar of this core collection contains diverse genetic materials. Therefore, the tolerance trait of 'Nagoya shiro' is most likely derived from a different allele than that in 'Aikoku'. However, in order to demonstrate whether the tolerance trait of 'Aikoku' and 'Nagoya shiro' is on the same locus, further genetic studies, such as QTL identification, should be conducted.

CONCLUSION AND RECOMMENDATIONS

According to our study it was concluded that 'Aikoku' and 'Nagoya shiro' are useful breeding materials that possess the trait of heat-stress tolerance under severe hot water treatment. In the future, we aim to develop mapping populations derived from 'Aikoku' or 'Nagoya shiro' as a donor parent crossed with sensitive cultivars, particularly indica cultivars, in order to detect the responsible QTLs and continue to facilitate the application of hot water disinfection to Southeast Asian countries.

SIGNIFICANCE STATEMENTS

This study discovers the existence of varietal difference in terms of seed germination and seedling emergence abilities among Japanese rice cultivars after several hot water treatment conditions that can be beneficial for rice cultivar improvement and hot water disinfection broader introduction across the countries in Asia. This study will help the researcher to uncover the critical areas of heat tolerance ability inside the rice seeds after the treatment of hot water, not during the rice cultivation period that many researchers were not able to explore.

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