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REVIEW

Development of Barley Cultivars with Resistance to Scald (*Rhynchosporium secalis* (Oud.) Davis) in Japan

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Abstract

Hokuriku district in Japan is a major producing area of six-rowed barley, which is mainly consumed as pearled grain. However, the barley cultivated in this region has been damaged by scald, and commercial scald-resistant cultivars need to be developed. The influence of scald on yield reduction (previously addressed in many reports) and on pearled-grain quality was investigated. The whiteness of pearled grain, which is the most important quality in pearled barley, was not affected, but the pearling time tended to increase with the incidence of disease. Appropriate methods to evaluate resistance or susceptibility of cultivars in breeding programs were considered. Artificial inoculation in seedlings is not adequate to determine the resistance of cultivars because an outbreak of the disease has at times occurred after the heading stage. A field test with a source of infection, i.e., scattering leaves that were badly infected by prior inoculation of scald, accelerated the incidence and prevalence of the disease. When the source of infection was applied before the winter snows, it was especially effective in spreading the disease. The field test helped to determine resistant cultivars and breeding lines that inhibited the incidence of scald after the heading stage, although the occurrence of scald fluctuated according to weather conditions. Most Japanese commercial cultivars are highly susceptible in the field, whereas some foreign cultivars exhibit resistance to the dominant race of scald in Hokuriku district. We analyzed the resistance of “Brier” and showed that it was possibly controlled by one dominant gene at the heading stage, an observation that was in agreement with previous reports. On the other hand, three recessive genes are expected to confer resistance at the grain-filling stage. The heritability of resistance at the grain-filling stage was high, which means it is relatively easy to select resistant individuals in a breeding program even if it is controlled by several genes.

Discipline: Plant breeding

Additional key words: pearled barley, pearled-grain quality, resistant genes, yield

Introduction

A quantity of 56,000 tons of six-rowed barley is produced annually in Japan. It is consumed mainly as pearled barley, and cooked with rice. Domestic pearled barley used for human food accounts for more than 90% of its consumption in Japan, particularly in terms of pearled-grain quality. Barley grain contains more fiber than other cereals, and the demand for pearled barley has recently increased, thus reflecting a growing interest in healthy food.

Hokuriku district is a major producing area of six-rowed barley, accounting for 53.0% of the total cultivated acreage[13], and the barley produced in Hokuriku district is priced higher by barley-processing companies because of its high quality, in particular, the whiteness of the pearled grain. However, the yield in Hokuriku district is relatively low and fluctuates each year. One of

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the reasons for this low yield is excess moisture injury during the vegetative stage caused by high precipitation in autumn and snowmelt in early spring. Another factor for the low and unstable yield is scald, which is an important disease causing yield decline in Hokuriku. Controlling this disease is necessary to achieve a high and stable barley yield.

Scald, caused by *Rhynchosporium secalis* (Oud.) Davis, is particularly common in cool and semi-humid barley-growing areas. Yield losses caused by scald have been reported widely[9, 11, 17]. Epidemic studies, pathogenic differentiation, and fungicide applications that are effective against scald were extensively investigated after an outbreak in the 1950s in Japan[10, 14, 19], and some resistant resources have been found[6, 7, 22]. However, appropriate methods for determining and selecting resistant lines in breeding programs have not been studied in depth. Methods that require further investigation include inoculation tests in a greenhouse, which are useful for confirming resistance, and effective ways to spread the disease to form infected fields. Thus, breeding lines that were regarded as resistant in seedling tests or mildly affected fields are sometimes severely attacked by scald in advanced generations. Commercial scald-resistant cultivars have not yet been developed in Japan and the two barley cultivars cultivated in Hokuriku district, viz., “Fiber-Snow” and “Minorimugi,” are susceptible to scald. Since the application of fungicide is costly, it is critical to develop resistant cultivars with high yields and pearled-grain quality.

We review the recent studies conducted at the Hokuriku Research Center, a unique research center in Japan, aimed at breeding scald-resistant barley cultivars. In this study, we assessed the influence of scald on barley yield components and pearled-grain quality. We also present effective evaluation methods for resistant lines bred in the field and promising resistant genetic resources found using this method, and analyze the resistance of one cultivar in particular, “Brier.”

Influence of scald on barley yield and pearled-grain quality

The main barley product in Hokuriku district is pearled grain, and the effect of scald on pearled-grain quality has not been investigated, while yield loss due to scald is well recognized[9, 11, 17]. Therefore, we compared the yield and pearled-grain quality of plants with various severities of infection for two growing seasons with the scald-susceptible cultivar “Minorimugi”[15].

At the Hokuriku Research Center, in an average year, barley is grown from early October to early June, and its heading stage is in late April. In this study, barley plants were grown under different conditions to facilitate the spread or prevention of infection, e.g., sprinkling of water, scattering of infected barley leaves, or spraying of fungicide (triadimefon). Water was sprinkled for two weeks before heading, based on the observation that scald is spread by rain. Seedling leaves that were severely infected with scald were scattered in some experimental plots in early December or in mid-March as a source of infection. These seedlings had been grown in a greenhouse and inoculated at the two-leaf stage with a spore suspension of scald adjusted to 2×10^5 spores/ml. The isolate was classified as the dominant race “J-4a” in Hokuriku district[2]. Fungicide was applied to plants three times during the growing season. The severity of infection was scored from 0 to 5 (Table 1, Fig. 1).

The severity of scald infection differed among treatments and years, but lesions became clearly visible about 20 days before heading, and the infection spread during the grain-filling stage (duration from heading to maturity) (Fig. 2). There was a significant negative correlation between the severity of infection and plump grain yield in both years, and plump grain percentage (> 2.2 mm grain thickness) declined with the spread of scald infection (Fig. 3). The development of the disease during the grain-filling stage had no impact on the number of panicles. The whiteness of the pearled grain was not obviously affected by the incidence of scald. The pearling time tended to increase with the severity of infection, which may be attributed to a reduction in the size of grains due to the infection[12].

Development of scald-resistance evaluation effective for breeding resistant lines in the field

Simpler methods to distinguish clearly between resistant and susceptible cultivars and select resistant ge-

Table 1. Disease scoring by scald in mature plants in a field test

Severity of infection	Symptoms
0	no spots or lesions.
1	few small spots were observed.
2	lesions occupied about 1/4 of all leaves.
3	lesions occupied about half of all leaves.
4	lesions occupied about 3/4 of all leaves.
5	most leaves were withered.

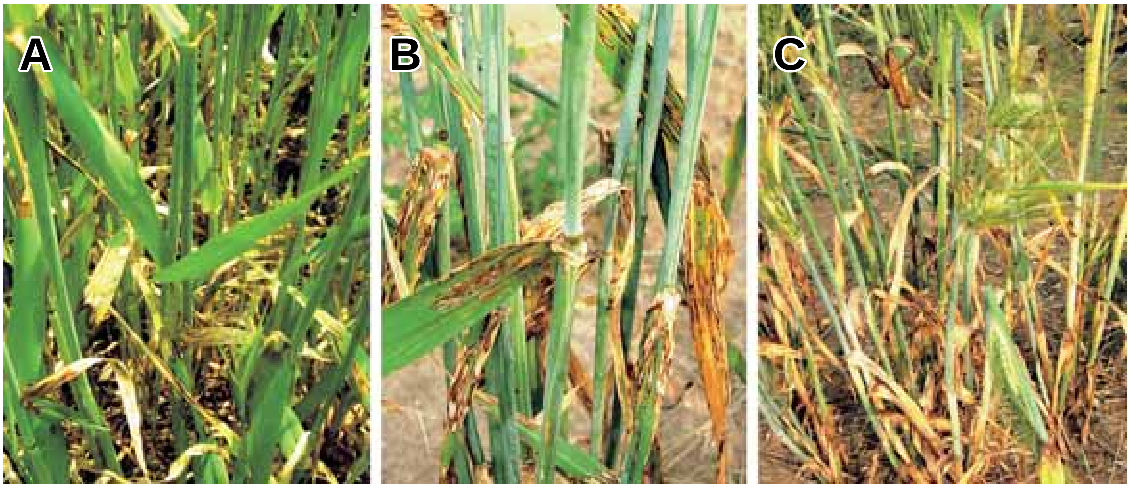

Fig. 1. Barley damage by scald

Lower leaves have withered and spots with brown edges have formed on the middle nodes of the leaf blades, as seen in Fig. 1A (severity of infection = 2, as shown in Table 1). Spots coalesced on the middle nodes of the leaf blades in Fig. 1B (severity = 3) and most of the leaves are dead, Fig. 1C (severity = 5). The shooting period was in the grain-filling stage of barley.

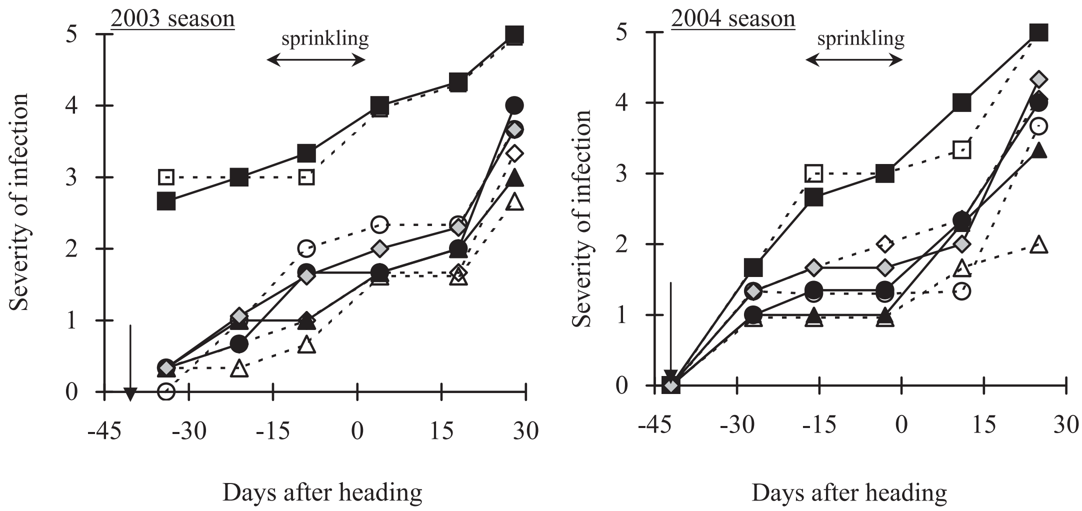

Fig. 2. Changes in severity of infection of "Minorimugi" by scald in various growing environments

Severity of infection by scald was scored from 0 to 5 as shown in Table 1.
■□: Infected leaves were scattered in a field in December, ●○: Infected leaves were scattered in March (indicated by arrows), ▲△: Fungicide was applied, ◆◇: no treatment. Plots shown with closed symbols were sprinkled with water. The data represent the means of three replications. The season refers to the sowing year.

netic resources are necessary for breeding programs. "Minorimugi," a susceptible cultivar, in the plot where infected leaves were scattered in early December was severely infected (Fig. 2). We then observed the severity of damage due to scald in other cultivars for several years to ascertain whether the above treatment was a useful method for determining scald resistance (Table 2). "Oomugi Sabikei 2" and "Oomugi Sabikei 3," designated as resistant cultivars, exhibited few lesions at heading and maturity. The severity of infection in another six cultivars, designated as susceptible, was >3.0 at the heading stage, and the severity scores increased during the grain-filling stage. Accordingly, the scattering of infected leaves early in December could be effective in distinguishing resistant and susceptible cultivars in the field. However, the severity of infection varied from year to year. The score of the severity was high in 2002 when the winter was mild, while it was low in 2005

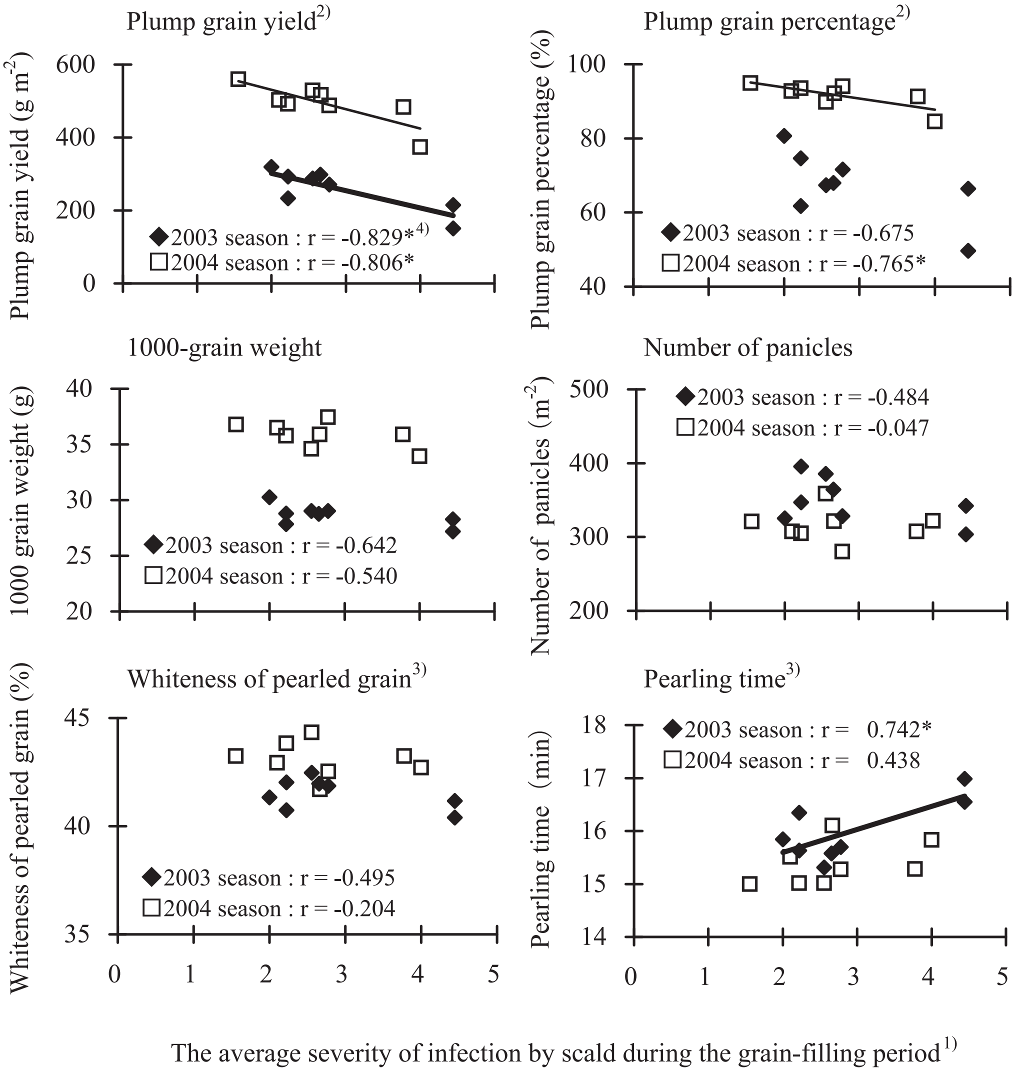

Fig. 3. Relation of severity of infection of "Minorimugi" during grain-filling to yield components and pearled-grain quality

1): The average values of severity of infection at heading, in the middle period of grain-filling, and in the late period of grain-filling, as shown in Fig. 2.
2): The thickness of plump grain was more than 2.2 mm.
3): Barley grains were pearled at the 55% pearled-grain rate and their whiteness was measured with a whiteness tester.
4): *shows significance at the level of 5% (n = 8 each year).

when there was heavy snow; this suggested that the evaluation of scald resistance in the field should be performed for several years with indicator cultivars. The examination of the severity of infection at maturity might be more important than that at heading, because severity scores at the heading stage fluctuated depending on the year, and the disease developed progressively during the grain-filling stage (Fig. 2).

The seedling test is sometimes useful to accelerate the process of crossing and generation advancement. The severity of scald on the seedlings with artificial inoculation has sometimes been compared with that seen in field tests, but the results and each author's interpretation of the results differs among papers[1, 21]. We also compared severity scores of infection on the seedlings to those in the field to assess whether the seedling test could be useful as part of a breeding program. Plants in the seedling test were inoculated at the two-leaf stage, grown at 20°C under a natural photoperiod in a greenhouse, and the severity scores of scald were assessed four weeks after inoculation according to the scores shown in Table 3. The evaluation of scald resistance in the seedling test with artificial inoculation was not always consistent with that observed in mature plants in the field. A large number of breeding lines were rated resistant in the seedling test (reaction types 0-2), but were found to be severely infected at maturity in the field test (Fig. 4). Consequently, a field test is recommended for breeding programs to select resistant lines and cultivars showing resistance at maturity.

Table 2. Analysis of variance in severity of infection by scald among seasons and cultivars

Source of variation	Severity of infection			
	Heading		Maturity[1]	
Season	**[2]		**	
2002	4.1	d[3]	−	
2003	3.4	c	4.2	c
2004	2.4	b	3.6	b
2005	0.9	a	2.1	a
Cultivars	**		**	
Hokuriku Kawa 35	3.0	b	3.2	c
Minorimugi	3.3	b	4.3	e
Fiber-Snow	3.1	b	4.0	d
Shinjuboshi	3.3	b	4.3	e
Shunrai	3.9	c	4.5	e
Kashima-mugi	4.0	c	4.8	f
Oomugi Sabikei 2	0.6	a	1.0	b
Oomugi Sabikei 3	0.4	a	0.3	a
Interaction	**		**	

[1]: During the grain-filling stage, severity was examined every 7 to 10 days. Severity of infection at maturity was rated before leaf death (at 23 to 32 days after their heading).
[2]: ** shows significance at 1% level.
[3]: Values with different letters are significantly different at the 5% level in their respective seasons, cultivars, and time of evaluation as determined by Tukey test.

Genetic resources showing scald resistance at maturity

The severity of infection in 519 cultivars was examined by scattering infected barley leaves in early December of the 2004 season (Table 4). The 429 cultivars, including Japanese commercial cultivars, were severely infected. Brier, 951/77 and Dea did not show any lesions. The high resistance of Japanese cultivars (severity of infection = 1) was assumed to originate from "Vogelsanger-Gold" and "Ager." Because the pedigrees of "Oomugi Sabikei 2" and "Oomugi Sabikei 3" are Asama mugi/Vogelsanger-Gold and Asama mugi/Ager, respectively, and "Oomugi Sabikei 2" and "Oomugi Sabikei 3" are the parents of "Hokuriku Kawa 34", "Hokuriku Kawa 38", and "Hokuriku Kawa 41". The severity scores of infection in cultivars that showed high resistance in the 2004 season were assessed over subsequent years to confirm their resistance.

Genetic analysis of scald resistance in "Brier"

"Brier" did not show any symptoms of scald for several years, including the 2004 season. In addition, it showed relatively early maturity and suffered little damage from snow. "Brier" was considered to be a prominent resistant resource and was analyzed genetically for its scald resistance[16] in the 2003 season, when scald was prevalent in the field making it suitable for analysis.

The severity of infection was investigated in F_1 and F_2 generations derived from two crosses that included "Brier" as a donor parent. The severity scores were observed approximately 10 days before heading and at every 10 days after heading. The severity at maturity represented the last score before leaf death.

At the heading stage, scald lesions were not observed (severity of infection = 0) in "Brier", F_1, and a large number of individuals in the F_2 populations in both crosses (Fig. 5). The severity of infection in the susceptible parents "Hokuriku Kawa 35 (hulled barley)"

Table 3. Disease scoring by scald in seedling test

Seedling reactions to infection	Symptoms
0	no visible lesion (resistant).
1	small lesions mainly at the tip or on the edge of the leaf blade (resistant).
2	somewhat larger lesions with a dark margin mainly on the edge of the leaf blade (resistant).
3	broad, well-developed lesions covering large areas across the leaf blade (susceptible).
4	greyish-green wilted areas in the leaf blade (susceptible).

and "Shikoku Hadaka 103 (naked barley)" were 2.6 and 3.7, respectively. Disease developed during the grain-filling stage, and the severity scores at maturity in "Hokuriku Kawa 35" and "Shikoku Hadaka 103" were 4.6 and 5.0, respectively. "Brier" did not show any lesions at maturity and the severity of infection in F_1 plants was found to be between the values of the parents in both cross combinations. The severity scores in F_2 generations varied from 0 to 5.0 in both combinations, suggesting that Brier's resistance is controlled by several genes.

The resistance in "Brier" is reported to be controlled by a single dominant gene (*Rh1*)[5], or a single dominant gene (*Rh1*) and a complementary recessive gene (*rh6*)[8]. In our study, genetic segregation of resistance in the F_2 generation of Brier/Hokuriku Kawa 35

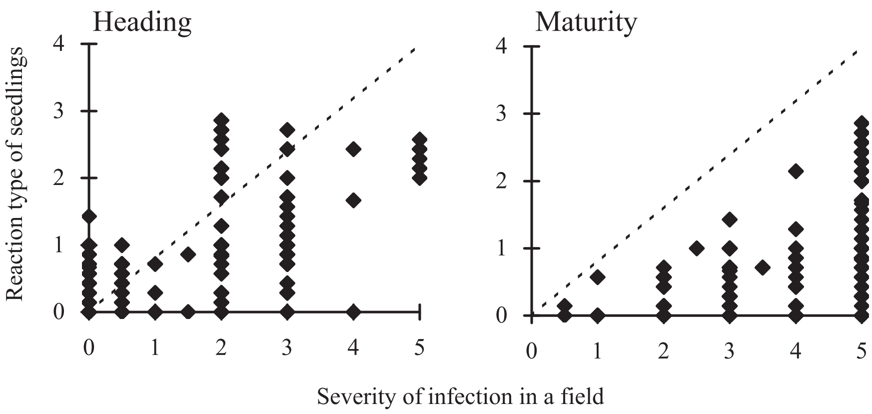

Fig. 4. Comparisons between severity of infection in a field at heading and maturity, and the reaction types of seedlings at four weeks after inoculation

The 161 breeding lines developed in the Hokuriku Research Center were examined.
The dotted lines show the ideal values where severity of infection in the field was fitted to the reaction type of seedlings.
The reactions of seedlings to infection are rated as shown in Table 3.

Table 4. Severity of infection in genetic resources in 2004 season

Severity of infection[1)]	Number of varieties	Representative cultivars[2)]
0	3	Brier (USA), 951/77 (unknown), Dea (unknown)
1	9	Turkey256 (Turkey), Turkey88 (Turkey), Turk (Turkey), Hudson (USA), Almerferder (Germany), Ager (France), Oomugi Sabikei 2 (Japan), Oomugi Sabikei 3 (Japan), Hokuriku Kawa 41 (Japan)
2	12	Turkey125 (Turkey), Turkey166 (Turkey), Carre26 (Algeria), Atlas57 (USA), Rivale (France), Volgensanger-Gold (Germany), Kalkreuther Fruhe (Germany), Hokuriku Kawa 34 (Japan), Hokuriku Kawa 38 (Japan)
3	15	Turkey22 (Turkey), Turkey123 (Turkey), Sultan (Turkey), Osiris (Algeria), Atlas68 (USA), Dajoukan (Japan)
4	51	Bey (Turkey), Debre Zeit31 (Ethiopia), Sudan (Spain), Kitchin (USA), Atlas46 (USA), West China (Argentina), Wong (China), Kumogatamugi (Japan), Ukawa Oomugi (Japan)
5	429	Nigrinudum (Ethiopia), Steudelli (Ethiopia), La Mesita (USA), Modoc (USA), Jet (USA), Atlas (France), Kashima-mugi (Japan), Fiber-Snow (Japan), Minorimugi (Japan), Shunrai (Japan), Ichibanboshi (Japan), Nishinohoshi (Japan), Misato Golden (Japan) , Asama mugi (Japan)

[1)]: Severity of infection in a field is the final score before leaf death.
[2)]: The origins of genetic resources are provided in parentheses.

fitted to 3 to 1 at the heading stage (Table 5) when individuals without any scald lesions (severity of infection = 0) were assumed to be resistant, because "Brier" plants exhibited no lesions. The result is in agreement with previous studies, despite different disease scoring methods and periods of examination. The segregation fitted to 1 to 63 in both crosses at maturity, and suggests the possibility that resistance in "Brier" at maturity is controlled by three recessive genes. These resistance genes, which exert control at different stages, remain to be elucidated; however, genes expressed at maturity could be different from those at heading or earlier stages. Another possibility is that resistance that functions at earlier stages could be mainly related to susceptibility to infection, while that at the mature stage is affected by both susceptibility to infection and spread, because the progress of scald was suppressed temporally around the heading stage[19].

The broad heritability of scald-resistance was calculated according to the infection severity of the parents, F_1 and F_2 generations at maturity (Table 6)[20]. The heritability in the crosses of Brier/Hokuriku Kawa 35 and Brier/Shikoku Hadaka 103 was high, i.e., 0.850 and 0.967, respectively, suggesting that it was relatively easy to select individuals and lines with high resistance in the early generation of a hybrid even if the resistance was controlled by several genes.

Concluding remarks

In our study, we confirmed that severe infection by scald decreased the barley yield principally by reducing the plump-grain percentage, which was followed by an increased pearling time. It was also shown that the effect of disease prevalence on the whiteness of the pearled grain, if any, was very small. Scald lesions spread progressively during the grain-filling stage in susceptible cultivars, and sometimes developed dramatically in individuals without lesions at the heading stage as shown for the F_2 generation in Fig. 5. Accordingly, it is important to breed cultivars that are resistant during the grain-filling stage.

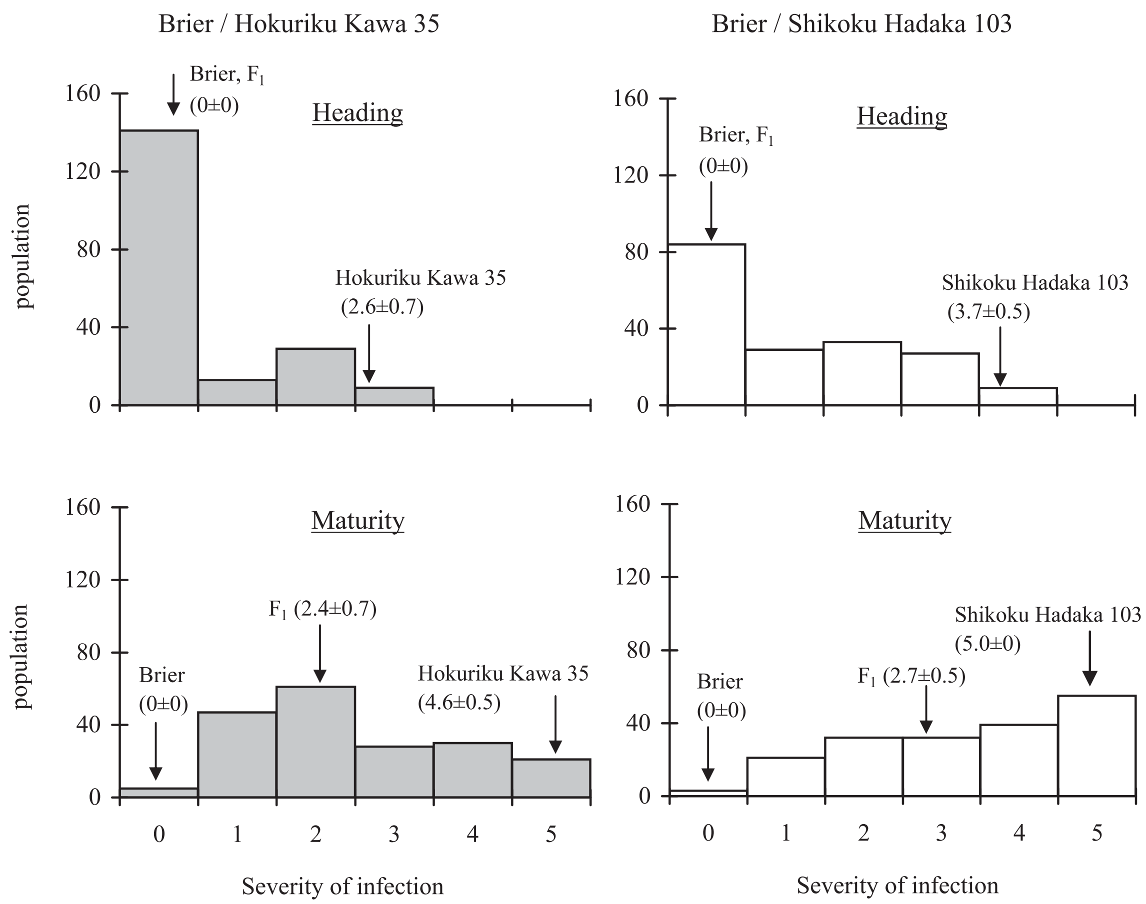

Fig. 5. Distribution of the severity of infection in F_2 populations in Brier/Hokuriku Kawa 35 and Brier/Shikoku Hadaka 103

The values in parentheses are the average ± standard deviation.

From Nakamura, E. et al. (2008)[16].

Table 5. Segregation of resistant and susceptible individuals in F_2 populations in cross of Brier/Hokuriku Kawa 35 and Brier/Shikoku Hadaka 103

Cross combination	Observed frequency in F_1		Observed frequency in F_2		Expected ratio in F_2		χ^2	P
	Resistant[1)]	Susceptible	Resistant	Susceptible	Resistant	Susceptible		
Heading					(3:1)			
Brier/Hokuriku Kawa 35	20	0	141	51	144	48	0.250	0.70>P>0.50
Brier/Shikoku Hadaka 103	18	0	84	98	136	46	80.769	P<0.0001
Maturity					(1:63)			
Brier/Hokuriku Kawa 35	0	20	5	187	3	189	1.354	0.30>P>0.20
Brier/Shikoku Hadaka 103	0	18	3	179	3	179	0.009	0.95>P>0.90

1): Only individuals showing a severity of infection of zero were considered to be resistant.

Table 6. Broad heritability of severity of infection at maturity

Cross combination	Genetic variance (V_{F2})	Environmental variance (V_E)	Broad heritability[1)] (h^2)
Brier/Hokuriku Kawa 35	1.874	0.282	0.850
Brier/Shikoku Hadaka 103	2.089	0.071	0.967

1): Broad heritability was calculated as shown below[20].
$h^2 = (V_{F2} - V_E) / V_{F2}$
V_E: environmental variance, V_{F2} : variance in F_2.
From Nakamura, E. et al. (2008)[16].

In the Hokuriku Research Center, methods for promoting the spread of scald in the field had not been established, and the difference in the severities of the disease between seedlings and mature plants had not previously been recognized. We therefore made incorrect decisions in attempts to develop resistance in breeding lines; breeding lines that were regarded as resistant in seedling tests or in mildly affected fields were sometimes severely damaged in conditions of high scald prevalence. In this study, we found that the assessment of scald resistance in an infected field (where highly infected leaves were scatted before snow) was useful for developing resistant cultivars, even though the severity of infection fluctuated over the years. Thus, we are currently selecting resistant individuals and lines in the F_4 and F_5 generations and confirming their resistance in advanced generations in an infected field, applying the source of infection before snow. Assessment of the severity of infection is performed at both the heading and grain-filling stages. This new approach contributes to the efficient selection and development of resistant lines with higher yields and better pearled-grain quality than current commercial cultivars.

The highly resistant genetic resources listed here represent the results of a one-year experiment with the dominant scald strain "J-4a" in Hokuriku district. The severity of their infection by scald has been observed for several years, and the effects of other races, which show more severely pathogenic than "J-4a," and growth traits of resistant resources have been investigated to determine whether they are appropriate to be used as parental stock.

In addition, the identification of the major resistant genes associated with scald resistance and quantitative trait loci analysis has been used to assist with breeding in recent years[4, 18]. The severity of infection fluctuated from year to year due to weather conditions, and it sometimes rapidly progressed during the grain-filling period, although lesions were not expressed at the heading stage. If marker-assisted selection is developed, resistant individuals and lines could be easily selected without observing their susceptibility over several years. Resistant cultivars determined by marker-assisted selection can be used as a crossing parent before their resistance is proved at the grain-filling stage in field tests, because their resistance could be evaluated before heading. The results of the field test described in this paper might contribute to the development of DNA markers for the selection of genes exhibiting resistance even at maturity.

References

1. Abott, D.C. et al. (1991) The relationship between seedling infection types and field reactions to leaf scald in Clipper barley backcross lines. Aust. J. Agric. Res., **42**, 801−809.
2. Arai, M. & Fujita, Y. (1997) The distribution of pathogenic races of barley scald fungus *(Rhynchosporium secalis*) in Japan. Nippon syokubutu byouri gakkaihou (*Jpn. J. Phytopathol.*), **63**, 214 [In Japanese].
3. Arai, M. et al. (1998) Evaluation of scald resistance in new breeding lines of barley at Hokuriku National Agricultural Experiment Station (1). *Hokuriku sakumotsu gakkaihou* (*Hokuriku Crop Sci*), **33**, 112−114 [In Japanese].
4. Barua, U.M. et al. (1993) Identification of RAPD markers linked to a *Rhynchosporium secalis* resistance locus in barley using near-isogenic lines and bulked segregant analysis. Heredity, **71**, 177−184.
5. Bryner, C.S. (1957) Inheritance of scald resistance in barley. Ph.D. thesis. ed. Pennsylvania State University, University Park.
6. Fukuyama, T. et al. (1990) Screening of resistant cultivars to barley scald. *Hokuriku sakumotsu gakkaihou* (*Hokuriku Crop Sci.*), **25**, 89−91 [In Japanese with English Summary].
7. Fukuyama, T. & Takeda, H. (1992) Survey of resistance to scald in world collections of cultivated barley. *Ikusyugaku zasshi* (*Japan. J. Breed.*), **42**, 761−768.
8. Habgood, R.M. & Hayes, J.D. (1971) The inheritance of resistance to *Rhynchosporium secalis in barley. Heredity*, **27**, 25−37.
9. James, W.C. et al. (1968) The relationship between leaf blotch caused by *Rhynchosporium secalis* and losses in grain yield of spring barley. *Ann. appl. Biol*., **62**, 273−288.
10. Kajiwara, T. & Iwata, Y. (1963) Studies on the strains of the barley scald fungus, *Rhynchosporium secalis* (Oud.) Davis. Nougyou gijutsu kenkyuujo houkoku (Bull. Natl. Inst. Afric. Sci.), Series C, **15**, 1−82 [In Japanese with English Summary].
11. Khan, T.N. (1986) Effects of fungicide treatments on scald [*Rhynchosporium secalis* (Oud.) J. Davis] infection and yield of barley in Western Australia. *Aust. J. Exp. Agric*., **26**, 231−235.
12. Kubota et al. (1991) Relation between pearled characteristics and 1000-kernel-weight, degree of glassyness, protein content and amylose content of barley. *Hokuriku Crop Sci.*, **26**, 89−92 [In Japanese].
13. MAFF: Statistics of Agriculture, Forestry, and Fisheries. http://www.maff.go.jp/toukei/ sokuhou/data/4baku 2008-11/4baku2008-11.pdf [In Japanese].
14. Matsuzawa, K. & Umehara, Y. (1986) Chemical control of scald and the relationship between the severity of scald and the loss in grain yield of winter barley. *Hokuriku byougaityuu kenkyuu kaihou* (*Proc. Assoc. Plant Prot. Hokuriku*), **34**, 52−55 [In Japanese].
15. Nakamura, E. et al. (2007) Effects of scattering infected leaves and sprinkling the field with water on the degree of scald infection, yield and pearled-grain quality of barley. *Nippon sakumotsu gakkai kiji* (*Jap. J. of Crop Sci.*), **76**, 383−393 [In Japanese with English summary].
16. Nakamura, E. et al. (2008) Genetic analysis of resistance to barley scald (*Rhynchosporium secalis*) in a field of the resistant cultivar Brier and its application to breeding. *Ikusyugaku kennkyuu* (*Breeding Res.*), **10**.101 −110 [In Japanese with English summary].
17. Ozoe S. (1953) Studies on the *Rhynchosporium secalis* scald of barley and its control. *Shimaneken nougyou shikennjou kennkyuu houkoku* (*Bull. of the Shimane Prefecture Agric. Inst.*), **1**, 1−122 [In Japanese with English summary].
18. Schweizer, G.F. et al. (1995) RFLP markers linked to scald (*Rhynchosporium secalis*) resistance gene *Rh2* in barley. *Theor. Appl. Genet*., **90**, 920−924.
19. Suzuki, H. & Arai, M. (1990) Occurrence and control of barley scald. Syokubutsu boueki (*Plant Prot.*), **44**, 214−219 [In Japanese].
20. Weber C.R. & Moorthy, B. R. (1952) Heritable and nonheritable relationships and variability of oil content and agronomic characters in the F_2 generation of soybean crosses. *Agron. J*., **44**, 202−209.
21. Xue A.G. et al. (1995) Variation in seedling and adult-plant resistance to *Rhynchosporium secalis* in barley. *Can. J. Plant Pathol*., **17**, 46−48.
22. Yukawa, T. et al. (1989) Varietal differences in *Rhynchosporium* scald of barley. *Hokuriku sakumotsu gakkaihou* (*Hokuriku Crop Sci.*), **24**, 55−56 [In Japanese].